

## SCIENTIFIC OPINION

### Safety evaluation of ractopamine<sup>1</sup>

#### Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-433)

Adopted on 2 April 2009

#### PANEL MEMBERS\*

Georges Bories, Paul Brantom, Joaquim Brufau de Barberà, Andrew Chesson, Pier Sandro Cocconcelli, Bogdan Debski, Noël Dierick, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, Joop de Knecht, Lubomir Leng, Anne-Katrine Lundebye Haldorsen, Sven Lindgren, Alberto Mantovani, Miklós Mézes, Carlo Nebbia, Walter Rambeck, Guido Rychen, Atte von Wright and Pieter Wester

#### SUMMARY

Ractopamine hydrochloride is pharmacologically classified as a phenethanolamine  $\beta$ -adrenoceptor agonist. The use of the substance as a feed additive is authorised in different countries (USA, Canada, Japan and Mexico) for growth promotion of fattening pigs and cattle. Ractopamine has not been assessed in the EU so far.

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to provide an opinion on the JECFA evaluation for ractopamine hydrochloride, having consulted and closely co-operated with other organisations such as EMEA and the Community Reference Laboratory responsible for  $\beta$ -agonists (BVL in Berlin).

The metabolic fate of ractopamine hydrochloride is similar in the target species (pig and cattle), laboratory animals and humans.

The FEEDAP Panel concluded from an acute study in dogs that tachycardia and peripheral vasodilatation observed are in line with the expected pharmacological action. From another acute study in dogs, with limited statistical power, a pharmacological NOAEL of 2  $\mu\text{g kg}^{-1}$  bw could be derived.

Comparing dog and monkey data it appeared that the dog could be considered as more sensitive to ractopamine ( $\beta$ -adrenergic substances). However, the FEEDAP Panel considered that there was not enough data to support this conclusion.

NOAEL's derived from pharmacological repeated dose studies should not be regarded as a meaningful basis for an ADI because of the observed down regulation of lung  $\beta$ -adrenergic

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\* One member of the Panel did not participate in the discussion on the subject referred to above.

receptors, at least as long as dose- and time-dependency and  $\beta$ -adrenoceptors speciation is not established. When evaluating hypothetical risks for the consumer, data from acute pharmacological studies would better reflect the consumer situation after intake of a single meal containing ractopamine residues.

The NOAELs derived from toxicological end points were considerably higher than those from pharmacological end points. Effects observed in toxicity studies were mostly related to the pharmacological action.

Although a series of mutation studies in prokaryotes and eukaryotic systems were negative, several *in vitro* tests were positive. The FEEDAP Panel considered that some positive genotoxicity studies *in vitro* are a possible cause of concern. However, these results have to be considered in conjunction with the carcinogenicity studies provided.

The FEEDAP Panel concluded that all treatment-related effects observed in the long-term studies in mice and rats were attributable to the  $\beta$ -adrenergic activity of ractopamine. It shares the JECFA and FDA opinion, considering that the induction of leiomyomas is a non-genotoxic event with a threshold and ractopamine is not a direct carcinogen. Considering all studies, the FEEDAP Panel concluded that ractopamine is not mutagenic and is not likely to present a carcinogenic risk to consumers.

Since data in laboratory animals gave a wide range of NOAELs, the available human data was considered pivotal by JECFA as it was by the FEEDAP Panel when assessing consumer safety.

On the basis of mean values from the study with 6 healthy volunteers the JECFA established an ADI for ractopamine of 0–1  $\mu\text{g kg}^{-1}$  bw per day based on the NOEL of 67  $\mu\text{g kg}^{-1}$  bw and the application of a safety factor of 50, rounded to one significant figure.

The human study was originally designed as a preliminary (open label) study intended to establish dose-effect responses to enable suitable doses to be selected for a larger (double-blinded) study. It was not intended to define a no effect level. Use of the data obtained for this purpose inevitably exposes experimental weaknesses and uncertainties and limits the conclusiveness of the study. The absence of a double-blinded study design to avoid placebo effects would introduce bias.

Significant subpopulations which may be at higher risk for adverse events after  $\beta$ -adrenergic stimulation require particular consideration when estimating the safety factor. The FEEDAP Panel concluded that the safety factor applied by JECFA to derive the ADI from the NOEL does not sufficiently take into account population subsets at higher risk.

Each evaluation of the human study based on a group mean value is handicapped by the poor statistical power. The FEEDAP Panel noted that an evaluation should be based on the individual response (pharmacodynamic effects). The FEEDAP Panel concluded that the 5 mg dose (the lowest administered dose) cannot be definitely considered a no-effect dose.

The FEEDAP Panel also examined the alternative of considering the 5 mg dose as a LOEL, and, because data for doses between 5 and 0 mg are not available, to apply the benchmark procedure for determining a NOEL. The benchmark procedure did not allow establishing a NOEL (to exclude a 10 % change in the electromechanical systole (QS2), a 20 % change in heart rate, and a 40 % change in cardiac output, the lower confidence limit of the benchmark dose would be 0 mg).

The FEEDAP Panel noted, that if an ADI would be derived from pharmacological studies, a NOEL must consider not only clinically relevant (“adverse”) effects in the consumer but also subjective discomfort even when occurring only for a short time.

The FEEDAP Panel was further of the opinion, that the uncertainties concerning the figure of a NOEL should not be balanced by a (higher) safety factor. All the uncertainties together would reach a dimension in which more or less arbitrary estimations prevail.

The FEEDAP Panel finally concluded that the human study can not be taken as a basis to derive an ADI, as proposed by JECFA, and consequently no proposal for MRLs could be made.

The CVMP fully supported the conclusions of the FEEDAP Panel with regard to the safety evaluation of ractopamine.

The FEEDAP Panel proposed to use the sum of free ractopamine and ractopamine glucuronoconjugates (sensitive analytical methods available, NRCP of the EU) instead of free ractopamine as marker substance, a view supported by CVMP.

**Key words:** Ractopamine hydrochloride, butopamine,  $\beta$ -agonist, feed additive, growth promoter, finishing pigs, cattle in confinement, cardiovascular effects, cardiac output, systolic blood pressure, diastolic blood pressure, electromechanical systole, heart rate, maximum fibre shortening, NOEL, NOAEL, safety factor, BMD, ADI, MRL, consumer safety, JECFA, CVMP

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## BACKGROUND AS PROVIDED BY EC

EC Directive 96/22/EC generally prohibits the use of  $\beta$ -agonists in food producing animals except for therapeutic use under direct veterinary supervision in calving cows, horses and pets.<sup>2</sup> This prohibition covers domestic production and imports from countries of meat from animals treated with  $\beta$ -agonists for growth promotion purposes.

Currently, different  $\beta$ -agonist substances are authorised for use as growth promoters in around 25 countries worldwide. As an example, ractopamine hydrochloride is authorised for use as feed additive in some third countries like USA, Canada, Japan and Mexico for growth promotion of fattening pigs and cattle.

A Codex Standard (maximum Residue Limit – MRL) for ractopamine has been advanced by the Codex Committee for Residues of Veterinary Drug in Foods for final adoption by the Codex Commission in July 2008. This standard takes as a basis a risk assessment carried out by the JECFA.<sup>3</sup> The EC did not support the advancement of MRLs to step 8 of the Codex procedure, based on the fact that Directive 96/22/EC does not allow the use of  $\beta$ -agonists for growth promotion. The Committee, noting that the justification for not supporting the advancements of MRL to step 8 was not based on scientific arguments, however decided to advance the draft MRL for ractopamine in cattle and pigs tissues to step 8.

The EU has not carried out a scientific evaluation of the whole group of  $\beta$ -agonists for use as a growth promoter. Only clenbuterol and isoxuprine have been evaluated by EMEA for veterinary medicinal use (Council Regulation (EEC) No 2377/90).

The European Commission is required to coordinate with the EU Member States the Community position. EFSA is therefore asked to provide an opinion on the JECFA evaluation for ractopamine hydrochloride, having consulted and closely co-operated with other organisations such as EMEA and the Community Reference Laboratory responsible for  $\beta$ -agonists (BVL in Berlin).

## TERMS OF REFERENCE AS PROVIDED BY EC

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to review the JECFA risk assessment leading to proposed Codex standard and other relevant scientific information, in order to establish if there are any scientific grounds for concern, in particular any information which would call into question the scientific grounds for the JECFA evaluation and/or on the safety of food and food products from animals treated with ractopamine.

## ACKNOWLEDGEMENTS

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<sup>2</sup> An MRL has been set for Clenbuterol in Regulation (EC) No 2377/90 for bovine animals (0.1  $\mu\text{g}/\text{kg}$  for muscle, 0.5  $\mu\text{g}/\text{kg}$  for liver, 0.5  $\mu\text{g}/\text{kg}$  for kidney and 0.05  $\mu\text{g}/\text{kg}$  for milk) and equidae (same MRLs for muscle, liver and kidney, no MRL for milk)

<sup>3</sup> The MRLs put forward for cattle and pigs are 10  $\mu\text{g}/\text{kg}$  for muscle and fat, 40  $\mu\text{g}/\text{kg}$  for liver and 90  $\mu\text{g}/\text{kg}$  for kidney (all expressed as Ractopamine), as well as an ADI of 0-1  $\mu\text{g}/\text{kg}$  bw.

## ASSESSMENT

### 1. Introduction

Ractopamine hydrochloride is a  $\beta$ -adrenoceptor agonist. The use of the substance as a feed additive is authorised in different countries. For example, in the USA, its use is authorised in pigs and cattle to increase the rate of weight gain, improve feed efficiency and increase carcass leanness, at a feed concentration of 5–20 mg kg<sup>-1</sup> feed for finishing pigs (68–109 kg body weight) and in dosages of 5–10 mg kg<sup>-1</sup> feed for finishing pigs heavier than 109 kg. For cattle in confinement, it is authorised at a feed concentration of 10–30 mg kg<sup>-1</sup> feed dry matter. If used in pigs, the substance has to be labelled with a caution statement (increased risk for exhibiting the downer pig syndrome, synonymous to fatigued pig syndrome).

Because EC Directive 96/22/EC<sup>4</sup> generally prohibits the use of  $\beta$ -agonists in food-producing animals, except for therapeutic use in some animal species, ractopamine has not been assessed in the EU so far.

The following assessment is limited to a review of the JECFA risk assessment (see Terms of Reference). The assessment of other relevant issues, particularly safety for the target species and product quality, is given in the appendices.

On request of EFSA, the producer of ractopamine (called later the ‘Notifier’) made available the original reports and studies already sent to EMEA when applying for setting MRL’s for ractopamine.

The FEEDAP Panel adopted in agreement with the Community Reference Laboratory responsible for  $\beta$ -agonists (BVL in Berlin) a preliminary opinion (4 February 2009) which was sent to EMEA for further consultation. The Committee for Medicinal Products for Veterinary Use (CVMP) commented during its meeting on 10–12 March 2009 on the FEEDAP Panel opinion.

### 2. Ractopamine hydrochloride

Ractopamine hydrochloride, an off-white to cream coloured solid, is the common name for benzenemethanol, 4-hydroxy- $\alpha$ -[3-(4-hydroxyphenyl)-1-methylpropylaminomethyl]- hydrochloride (CAS number 90274-24-1, molecular formula C<sub>18</sub>H<sub>23</sub>NO<sub>3</sub>HCl, molecular weight 337.85). It is pharmacologically classified as a phenethanolamine  $\beta$ -adrenoceptor agonist. The structural formula is shown in Figure 1. Ractopamine hydrochloride occurs in four stereoisomers (RR, SR, SS, RS).

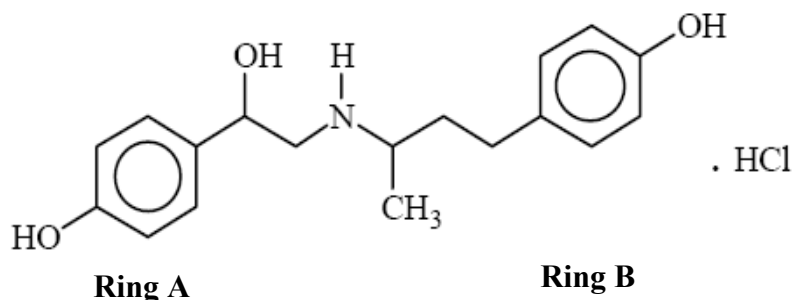


Figure 1. Structural formula of ractopamine hydrochloride

<sup>4</sup> Official Journal L 125 , 23/05/1996 P. 0003 - 0009

## 2.1. Metabolism

Radiolabelled ractopamine was used to study the metabolism of ractopamine hydrochloride in food-producing animals (pigs: 45 to 90 kg; cattle: 115 to 250 kg) and laboratory animals. The doses administered either in feedingstuffs (pig) or intra-uminally twice a day (cattle) were in the range of those proposed for use (x1 to x2.5).<sup>5</sup>

### 2.1.1. Target species (pig and cattle)

The summarised conclusions are based on three studies in pigs<sup>6,7,8</sup> and four in cattle.<sup>9,10,11,12</sup>

- (i) balance studies indicate that the compound is absorbed, distributed and eliminated rapidly; 95 % of the amount ingested is excreted the first three days; about 90 % and 55 % (for pigs and cattle, respectively) are excreted in the faeces, 10 % and 45 % in the urine; significant biliary excretion occurs indicating first-pass metabolism. Steady-state is reached after a four-day repeated administration in both species;
- (ii) metabolites representing more than 10 % (and even less) have been identified in the excreta and tissues of both species and correspond to ractopamine glucuronconjugates. Metabolites A and B, consisting of monoglucuronides of the diastereomeric pairs (RS,SR and RR,SS, respectively) conjugated to the ring A hydroxylic function, and metabolite C, corresponding to a mixture of stereomeric monoglucuronides conjugated to ring B hydroxylic function, are common to both species. Metabolite D, corresponding to stereomeric diglucuronides conjugated to rings A and B, is specific of the bovine; two very minor metabolites in pig were separated but not identified;
- (iii) metabolic profiles in tissues (zero-withdrawal) of pig and bovine indicate a different quantitative distribution of ractopamine and metabolites (ractopamine conjugates) in both species, the ratio-free ractopamine vs. conjugated ractopamine being lower in cattle (0.144 and 0.136 in the liver and kidney, respectively) when compared to pig (0.508 and 0.306); non-extractable residues are below 10 %.

### 2.1.2. Laboratory animals (rat and dog)

The summarised conclusions are based on four studies in rat,<sup>13,14,15,16</sup> plus comparative metabolic studies with pig<sup>17</sup> and cattle.<sup>18</sup>

<sup>5</sup> Metabolic studies of ractopamine hydrochloride in the pig, cattle, rat and dog, as well as residue studies in pig and cattle have been performed using either <sup>14</sup>C-ractopamine hydrochloride uniformly labelled on the hydroxyphenylethyl (ring A) portion of the molecule, or an equimixture of that labelled compound and <sup>14</sup>C-ractopamine hydrochloride uniformly labelled on the hydroxyphenylbutyl part (ring B). Both options proved to be satisfactory to isolate and identify most ractopamine metabolites and to follow the kinetics of residues in tissues. The analysis of both radiolabelled ractopamine hydrochloride indicated a mixture of about 47-53% of diastereomer couples, RS, SR and RR, SS, the same as in the active substance proposed for use. The radiochemical purity was checked and found acceptable (> 95%, < 5% corresponding to uniform background radioactivity).

<sup>6</sup> Original reports/Reference 13B

<sup>7</sup> Original reports/Reference 31B

<sup>8</sup> Original reports/Reference 32B

<sup>9</sup> Original reports/Reference 6B

<sup>10</sup> Original reports/Reference 10B

<sup>11</sup> Original reports/Reference 14B

<sup>12</sup> Original reports/Reference 16B

<sup>13</sup> Original reports/Reference 01B

<sup>14</sup> Original reports/Reference 02B

<sup>15</sup> Original reports/Reference 03B

<sup>16</sup> Original reports/Reference 08B

<sup>17</sup> Original reports/Reference 32A



- (i) the absorption, distribution and excretion are rapid; about 60 % of the amount ingested is excreted in the urine and 40 % in the faeces; considerable biliary excretion occurs, indicating first-pass metabolism;
- (ii) metabolites are essentially ractopamine conjugates: a sulphate ester, glucuronic acid diconjugate (conjugated to ring A and ring B, respectively), a monoglucuronide at ring B and a monosulphate ester at ring A.

One study concerns the identification of ractopamine metabolites in dog<sup>19</sup> which proved to be qualitatively identical to those found in pig and cattle.

### 2.1.3. Primates

Rhesus monkeys treated with a single dose by gavage excreted almost twice the radioactivity in the urine compared to faeces. The excretion pattern in monkeys is similar to the one in dogs. A similar pattern of excretion has been observed in humans.<sup>20</sup> Ractopamine was essentially excreted as conjugates to sulphate ester at rings A (mainly) and B.

### 2.1.4. Conclusion

The metabolic fate of ractopamine hydrochloride is similar in the target species (pig and cattle), laboratory animals and humans.

## 2.2. Analytical methods

The Community Reference Laboratory responsible for  $\beta$ -agonists (BVL in Berlin) prepared a report related to the methods used for the control of ractopamine in tissues within the National Residue Control Plans (NRCPs) of the EU. The report corresponding to the analysis of ractopamine residues in animal products, confirmatory methods and screening methods can be found in Appendix I.

## 3. Pharmacological and toxicological studies

All studies were performed with ractopamine hydrochloride referred to here as 'ractopamine'.

### 3.1. Genotoxicity including mutagenicity

Three mutation studies in prokaryotes (Ames tests using eight *Salmonella typhimurium* strains (G46, TA1535, TA1537, TA1538, TA98 and TA100, D3052, C3076) and two *E. coli* strains (WP2, WP2*uvr*), with and without metabolic activation<sup>21,22,23</sup> were conducted. The results of those tests were negative (non-mutagenic).

*In vitro tests* in eukaryotic systems (Unscheduled DNA synthesis in rat hepatocytes,<sup>24</sup> chromosome aberration tests in CHO cells<sup>25</sup> and several *in vivo* studies (mouse bone marrow cytogenetics and micronucleus tests,<sup>26,27</sup> sister chromatid exchange tests in Chinese hamster bone marrow<sup>28</sup>) were also negative. A bone marrow micronucleus test in rat<sup>29</sup> was equivocal (positive at low doses, negative in a follow-up study at higher doses). However, several *in vitro*

<sup>18</sup> Original reports/Reference 30A

<sup>19</sup> Original reports/Reference 07B

<sup>20</sup> Original reports/Reference 19B

<sup>21</sup> Original reports/Reference 56A

<sup>22</sup> Original reports/Reference 57A

<sup>23</sup> Original reports/Reference 58A

<sup>24</sup> Original reports/Reference 59A

<sup>25</sup> Original reports/Reference 62A

<sup>26</sup> Original reports/Reference 66A

<sup>27</sup> Original reports/Reference 68A

<sup>28</sup> Original reports/Reference 72A

<sup>29</sup> Original reports/Reference 69A

tests were positive, namely chromosome aberration tests in human lymphocytes<sup>30,31</sup> and two out of three forward mutation assays in mouse lymphoma cells.<sup>32,33</sup>

The Notifier (in the USA and other countries) has produced limited evidence that the genotoxicity found in some studies could be due to a secondary auto-oxidative mechanism from ractopamine-catechol producing reactive intermediates as a possible mechanistic explanation for the genotoxicity, as is the case for the natural similar catecholamine, epinephrine.<sup>34,35</sup> On the basis of those results, JECFA concluded that ractopamine was not intrinsically genotoxic *in vitro* or *in vivo*.

The FEEDAP Panel considers that some positive genotoxicity studies *in vitro* are a possible cause of concern. However, these results have to be considered in conjunction with the carcinogenicity studies provided (see Sections 3.2.4 and 3.2.6).

## 3.2. Studies on laboratory animals

### 3.2.1. Acute oral toxicity

Oral LD<sub>50</sub> were found to be in mice 3547–2545 mg kg<sup>-1</sup> bw (males and females)<sup>36</sup> and in rats 474–367 mg kg<sup>-1</sup> bw.<sup>37</sup>

### 3.2.2. Single dose studies

#### Intravenous administration in anaesthetised dogs (study A)

A GLP study was performed to evaluate the hemodynamic effects of intravenous administration of ractopamine in anaesthetised dogs.<sup>38</sup> The aim of this study was to check the appropriateness of a canine model for the safety assessment of ractopamine using a single intravenous dose of 35 µg kg<sup>-1</sup> bw. This dose was infused once for a period of ten minutes in four (two males, two females) pentobarbital-anaesthetised beagle dogs. Different haemodynamic parameters were recorded from five minutes pre-start to 39 minutes post-start of dosing. All four dogs survived. The main effects recorded were: tachycardia (65 % increase in heart rate, elevated by at least 50 % until the end of the observation), peripheral vasodilatation (mean arterial pressure fell to approximately half the control level), total peripheral resistance fell to approximately -65 % of control levels at infusion and subsequently returned to a level of -50 % for the next 30 minutes, cardiac output (increase by 40 %). Cardiac arrhythmia was observed in one dog.

#### Oral administration in conscious dogs (study B)

A further GLP-compliant study<sup>39</sup> was performed in conscious dogs to provide acute cardiovascular toxicity data of oral doses of ractopamine. Beagle dogs (four males and four females of 10 to 19 months of age) were administered orally 0, 2, 50 or 125 µg ractopamine kg<sup>-1</sup>. The study was designed as a double Latin square that allowed testing for residual effects. Left ventricular pressure, aortic blood pressure, heart rate and electrocardiograms were recorded to provide data on the effects of an oral dose of ractopamine on the left ventricular function and systemic blood pressure. The peak value of the first derivative of left ventricular pressure

<sup>30</sup> Original reports/Reference 60A

<sup>31</sup> Original reports/Reference 61A

<sup>32</sup> Original reports/Reference 63A

<sup>33</sup> Original reports/Reference 65A

<sup>34</sup> Original reports/Reference 70A

<sup>35</sup> Original reports/Reference 71A

<sup>36</sup> Original reports/Reference 39A

<sup>37</sup> Original reports/Reference 40A

<sup>38</sup> Original reports/Reference 8A

<sup>39</sup> Original reports/Reference 9A

(dP/dtmax) was used as an index of left ventricular inotropic state. Systolic, diastolic, mean aortic and aortic pulse pressures were derived by the data acquisition system from the aortic pressure signal. Heart rate and left ventricular end-diastolic pressure were measured from the ventricular pressure signals.

All dogs survived to the treatment. There was no residual carry-over effect from one treatment to the next in the Latin square design. Ractopamine caused statistically significant dose-dependent increases in heart rate and left ventricular inotropic state at the 50 and 125  $\mu\text{g kg}^{-1}$  bw doses. Maximum effects occurred approximately two hours after dosing with an increased heart rate of 40 and 80 beats per minute at the mid- and high-dose, respectively. Increases of the left ventricular inotropic effects were recorded during the six-hour period after dosing. No significant change in heart rate or left ventricular inotropic state was observed at the 2  $\mu\text{g kg}^{-1}$  dose. A drop in blood pressure was evident in both the systolic and diastolic (and therefore the mean) pressures in response to treatment with the 50 and 125  $\mu\text{g kg}^{-1}$  doses during the six-hour period immediately after dosing. The 125  $\mu\text{g kg}^{-1}$  dose caused a decrease in aortic pulse pressure. The analysis of electrocardiograms did not indicate any treatment-related effects in the dogs. Two dogs in the 50  $\mu\text{g kg}^{-1}$  dose group and seven dogs in the 125  $\mu\text{g kg}^{-1}$  dose group demonstrated a slight colouring of the abdominal skin (erythema). The 2  $\mu\text{g kg}^{-1}$  dose did not display any significant effect on any of the parameters measured.

Because of the high variability of all measured parameters – e.g. the minimum and maximum baseline measures (24.97 and 285.90, respectively) for systolic arterial pressure differed by a factor of more than 10, and effects (i.e. the differences between baseline and phase 1) in the placebo group ranged from -7.34 to 62.16 – at a sample size of eight, differences would have to be very large to be detected as statistically significant. Clinically relevant effects could have been missed due to the lack of statistical power, but since thresholds for clinical relevance have not been provided, the probability of a  $\beta$  error cannot be estimated.

In summary, ractopamine treatment at 50 and 125  $\mu\text{g kg}^{-1}$  bw caused tachycardia, an increased left ventricular inotropic state and a fall in systemic blood pressure in dogs. The increase in heart rate was consistent with a reflex tachycardia since the increased heart rate was always accompanied by a fall in systemic blood pressure. The results of this study concur with expected pharmacological effects associated with vascular  $\beta_2$ -adrenoceptor stimulation and subsequent vasodilatation. The increased heart rate and left ventricular inotropy are consistent with the known effect on cardiac  $\beta_1$  adrenoceptors predominant in cardiac tissue. The 2  $\mu\text{g kg}^{-1}$  dose was determined to be the NOAEL by the Notifier.

### **Intravenous administration in anaesthetised monkeys (study C)**

In a GLP-compliant study, four anaesthetised Rhesus monkeys (two males and two females) were used to study the hemodynamic effects of intravenous administration of ractopamine.<sup>40</sup> The objective of this study was to determine the appropriateness of a primate model for the safety assessment of ractopamine, especially to investigate the haemodynamic effects of ractopamine administered intravenously once to pentobarbital-anaesthetised monkeys at a dosage of 35  $\mu\text{g kg}^{-1}$ . The data were recorded from five minutes pre-start to 39 minutes post-start of dosing. The heart rate increased about 20 % during infusion and remained elevated throughout the monitoring period. Cardiac output, stroke volume and peak aortic flow increased by 35 %, 14 % and 80 %, respectively. The results may indicate that the Rhesus monkey is less sensitive than the conscious dog.

<sup>40</sup> Original reports/Reference 10A

### **Intravenous administration in anaesthetised and conscious monkeys (study D)**

In a GLP-compliant study,<sup>41</sup> the acute cardiovascular response in six Rhesus monkeys to ractopamine 35 µg kg<sup>-1</sup> bw i.v. in the conscious state was compared to the response under barbiturate-anaesthesia. Heart rates increased during infusion with approximately 40–50 % in both states and normalised quickly in conscious animals but remained elevated in anaesthetised animals, similar to findings in study C. Systolic pressure increased during infusion in both states; it declined over 90 minutes to control level in conscious animals, whereas systolic pressure in anaesthetised animals continued to increase throughout the observation period (40 minutes).

In anaesthetised monkeys, the diastolic pressure decreased slightly and the mean arterial pressure was maintained during infusion. Both parameters increased during the 30-minute observation period after withdrawal of the infusion. In contrast, in conscious monkeys both parameters increased during infusion with a peak during that time. Baseline values were reached during the 30-minute observation interval. The data demonstrated that anaesthesia slightly impacts the cardiovascular effects of ractopamine.

### **3.2.3. Repeated dose studies**

#### **Oral administration in monkeys for six weeks (study E)**

A six-week gavage study was conducted in Rhesus monkeys (two per sex and dose) at doses of 0, 250, 500 and 4000 µg ractopamine kg<sup>-1</sup> bw.<sup>42</sup> Routine toxicological end points were not affected. Heart rate was increased shortly after dosing, maximal at 0.5 hour in middle- and high-dose groups, but the electrocardiogram and heart histopathology were normal.

A statistically significant decrease in β-adrenergic receptor density occurred in membranes prepared from lung of monkeys (sexes combined) given 500 and 4000 µg ractopamine kg<sup>-1</sup> bw for six weeks. The number of β-adrenergic receptors was decreased from values observed in the control group monkeys (264 fmol mg<sup>-1</sup> protein) to values of 191 and 179 fmol in the mid- and high-dose group, respectively. Lung β-adrenergic receptor number for the low dose group of monkeys was 250 fmol mg<sup>-1</sup> protein and not different from controls. No effect of the affinity of receptors for ligand was observed at any dose. Heart β-adrenergic receptor density could not be determined in this study due to methodological difficulties.

The lowest dose, 250 µg kg<sup>-1</sup> bw, has been derived as NOAEL in this study based on cardiovascular effects and lung β-adrenergic receptor density.

#### **Oral administration in monkeys for 90 days (study F)**

A GLP-compliant repeated dose study was conducted to evaluate, in comparison to a control group, the effect of subchronic administration of 125 µg ractopamine kg<sup>-1</sup> bw on heart rate and electrocardiographic waveforms in conscious Rhesus monkeys.<sup>43</sup> Twelve animals (three per treatment and sex) were used and the duration of the test was 90 days. The test material was prepared as a solution in water, and animals received 1.0 mL kg<sup>-1</sup> of vehicle or ractopamine solution daily by gavage. The parameters included were survival, clinical and physical observations, body weight, food consumption, heart rate and electrocardiograms. No adverse effects were observed throughout the study period.

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<sup>41</sup> Original reports/Reference 11A

<sup>42</sup> Original reports/Reference 13A

<sup>43</sup> Original reports/Reference 47A

### Oral administration in monkeys for one year (study G)

A one-year gavage study was performed in Rhesus monkeys (four per sex and dose) at doses of 0, 125, 500 and 4000  $\mu\text{g}$  ractopamine  $\text{kg}^{-1}$  bw.<sup>44</sup> Routine toxicological parameters were not affected except for an increase in body weight in the highest-dose group. Pharmacological end points were affected in the middle- and high-dose groups, including an increased in heart rate that remained during the study, and a decrease in heart weight. In membranes prepared from heart (left ventricle) and lung tissues,  $\beta$ -adrenoceptor binding assays were performed. The affinity and number of heart  $\beta$ -adrenoceptors were not affected by prolonged ractopamine treatment at any dose. The lung  $\beta$ -adrenoceptors density significantly decreased only in the highest-dose group in both sexes, being reduced by 23 % when compared to controls (246 vs. 320  $\text{fmol mg}^{-1}$  protein), while receptor affinity remained unchanged. No significant changes were observed at lower doses (320 and 316  $\text{fmol mg}^{-1}$  protein for 125 and 500  $\mu\text{g}$  ractopamine  $\text{kg}^{-1}$ , respectively). On the basis of the observed effects, the lowest dose of 125  $\mu\text{g kg}^{-1}$  bw  $\text{day}^{-1}$  was the NOAEL in this study.

### Oral administration in mice for three months (study H)

A three-month feeding study with ractopamine was conducted in B6C3F1 mice at doses of 0, 25, 125 or 1250  $\text{mg kg}^{-1}$  bw (ten animals per sex and dose).<sup>45</sup> Treatment- and dose-related increases in the haematological parameters (increased erythrocyte count, haemoglobin concentration and packed cell volume) were observed in both sexes. A decrease in the thrombocytes number was observed in high-dose mice. Clinical chemistry parameters showed an increase in urea nitrogen and cholesterol in the high-dose male mice and in both middle- and high-dose female mice. A decrease in sodium serum in the female high-dose group was observed. Heart weight was increased, testis weight decreased and brown fat was altered both in gross and histopathology. Other organs were unaffected in histopathology. The described effects occurred in the high-dose group in both sexes, where applicable, but in many cases also in the middle-dose. Since testis weight was decreased in all treated males, no NOAEL could be established for males. The NOAEL for females was 25  $\text{mg ractopamine kg}^{-1}$  bw.

### Oral administration in rats for three months (study I)

A three-month feeding study was conducted in 344 Fischer rats, doses being 0, 1.3, 13.4 and 152.9  $\text{mg ractopamine kg}^{-1}$  bw in males (M) and 0, 1.4, 15.3 and 156.8  $\text{mg ractopamine kg}^{-1}$  bw in females (F).<sup>46</sup> Effects were found in the higher-dose groups on body weight (decreased gain) and food consumption (increase). Changes in haematology included increase in erythrocyte count, haemoglobin and packed cell volume (M), and a decrease in thrombocytes; in clinical chemistry, a decrease in serum triglyceride (M) and cholesterol (F), and an increase of serum urea nitrogen, potassium and alkaline phosphates were reported. A decrease in the weight of uterus, liver, testis and spleen was observed. An increase in kidney weight was recorded. In histopathology, the only finding was a microscopic change of brown fat tissue. The findings were observed in the high-dose group of both sexes (where applicable), except for the reduction in spleen weight and the observation on brown fat, which occurred also in the mid-dose group. A NOAEL of 1.3 (M) and 1.4 (F)  $\text{mg ractopamine kg}^{-1}$  bw could therefore be derived from this study.

<sup>44</sup> Original reports/Reference 12A

<sup>45</sup> Original reports/Reference 44A

<sup>46</sup> Original reports/Reference 45A

### Oral administration in dogs for one year (study J)

A one-year oral study (0, 0.112, 0.224 and 5.68 mg kg<sup>-1</sup> bw day<sup>-1</sup>, divided in three daily portions in gelatin capsules) was conducted in beagle dogs.<sup>47</sup> Changes were seen in haematology, clinical chemistry, gross and histopathology, including organ weights in the high-dose group only. Cutaneous erythema was found in the highest-dose group and transiently in the middle-dose group, whereas significant resting bradycardia (measured at 7.00 a.m., before the first daily capsule) occurred in all treated groups, more expressed during the first half of the study, and for the lowest-dose group returning near to normal until the end of the study. Therefore, a NOAEL cannot be derived from this study.

#### 3.2.4. Carcinogenicity studies in rats and mice

In a study<sup>48</sup> with CD-1 mice, 60 animals per sex and group were given ractopamine for 21 months at 0, 0.02, 0.1 and 0.6 % in the diet, resulting in intake on bw day<sup>-1</sup> basis of 0, 25, 130, 840 mg (males) and 0, 35, 175 or 1085 mg (females). Increased mortality was observed in the highest concentration associated with cardiomyopathy. Body weight (males) and feed efficiency were decreased. Hyperplasia and leiomyomas of the uterine muscle were found, even in the lowest dose; therefore, a NOAEL could not be derived. A benchmark dose was calculated as 201 mg kg<sup>-1</sup> bw based on an excess incidence of leiomyoma of 5 % above that in controls and of a 95 % confidence limit.

The rat study<sup>49</sup> comprised a two-year experiment with 60 Fischer rats per sex and group. Doses were 0, 2, 60, 200 or 400 (females only) mg kg<sup>-1</sup> bw. Survival was significantly increased for both sexes at the highest doses tested. The effects observed were consistent with the  $\beta$ -adrenergic activity of ractopamine, including decreased body weight and feed utilisation, which probably contributes to explaining the results on survival. Hypertrophy and leiomyomas of the uterine ligament and enhanced cardiomyopathy in males were observed. The NOAELs for the incidence of leiomyoma and of cardiomyopathy are 60 and 2 mg kg<sup>-1</sup> bw, respectively.

The JECFA 'noted that the induction of benign leiomyomas in mice and rats appears to be a general feature of  $\beta$ -adrenoceptor agonists, as shown by the prevention of the development of these tumours by co-administration of the  $\beta$ -adrenoceptor blocker propranolol in studies with other  $\beta$ -adrenoceptor agonists. The Committee considered, therefore, that ractopamine is not a direct carcinogen and the induction of leiomyomas is a non-genotoxic event with a threshold and concluded that all treatment-related effects observed in the long-term studies of toxicity in mice and rats were attributable to the  $\beta$ -adrenergic activity of ractopamine.'

#### 3.2.5. Reproductive toxicity, including teratogenicity

Reproductive toxicity was tested in a two-generation study<sup>50</sup> in rats (CrI:CD(SD)), with the inclusion of teratogenicity parameters in the F<sub>2</sub> generation. Dietary doses were 0, 2, 20, 200 or 2000 mg kg<sup>-1</sup>, equivalent to 0, 0.15, 1.4, 15 and 160 mg kg<sup>-1</sup> bw (males). Average pre-partum test article intake for females closely paralleled the values for the males of the respective groups. Average pre-partum test article intake by nursing dams was about 2.6 times the intake levels during late gestation. The parental group consisted of 25 animals per dose and sex. Treatment-related effects were observed in the highest-dose group, indicating both parental (increased mortality) and developmental toxicity (increased mortality, structural abnormalities, growth retardation). Depression in body weight and body weight gain was observed for F<sub>0</sub> and

<sup>47</sup> Original reports/Reference 46A

<sup>48</sup> Original reports/Reference 75A

<sup>49</sup> Original reports/Reference 77A

<sup>50</sup> Original reports/Reference 50A

F<sub>1</sub> males and for F<sub>1</sub> females at 2000 mg kg<sup>-1</sup> dietary level. Food consumption was significantly depressed on F<sub>1</sub> males, also in the highest dosage group.

The NOAEL for maternal and foetal effects was 200 mg kg<sup>-1</sup> diet, equivalent to approximately 15 mg ractopamine kg<sup>-1</sup> bw. Given those findings (evidence of teratogenicity observed), a teratogenicity study in a second species was not considered necessary.

### 3.2.6. Summary of the pharmacological and toxicological studies on laboratory animals

A set of pharmacological studies were performed to describe the pharmacodynamic properties of ractopamine in different animal species. The aim of most of the studies was to identify a pharmacological ‘no-observed-effect-level’. A summary of the main results deriving from these studies is reported in Tables 1 and 2.

The FEEDAP Panel concludes from study A that the results regarding tachycardia and peripheral vasodilatation are in line with the expected pharmacological action. It could also be shown that dog can serve as a model to demonstrate the pharmacodynamic properties of β-adrenoceptor agonists. Despite the limited statistical power, the FEEDAP Panel concludes from study B that a pharmacological NOAEL of 2 µg kg<sup>-1</sup> bw could be derived from this study.

Comparing dog and monkey data (Table 1, study B; Table 2, study G and Table 3, study J) it appears that dog could be considered as more sensitive to ractopamine (β-adrenergic substances). However, the critical acute dose in study B (NOAEL 2 µg kg<sup>-1</sup> bw) shows wide dose intervals (next higher dose 50 µg kg<sup>-1</sup> bw); a higher NOAEL could thus not be definitively excluded. But ractopamine-induced cardiostimulation in monkeys occurred in the absence of cutaneous erythema (study J) and of clinical signs indicative for cardiotoxicity as observed in dogs. On this basis, JECFA suggested ‘that the cardiovascular response in monkeys differs from that in dogs.’ The FEEDAP Panel considers that there is not enough data to support this conclusion.

Table 1. Single administration studies, pharmacological end points

Study	A		B		C		D	
Species	Dogs		Dogs		Monkeys		Monkeys	
Experimental condition	Anesthetised		Conscious		Anesthetised		Conscious/Anesthetised	
Number of animals	2 F, 2 M		4 F, 4 M		2 F, 2 M		3 F, 3 M	
Administration	Infusion		Oral		Infusion		Infusion	
Dose (µg kg <sup>-1</sup> bw)	35		0, 2, 50, 125		35		35	
End points affected (increase + decrease -)	Tachycardia	+	Heart rate	+	Tachycardia	+	Tachycardia	+
	Vasodilatation	+	Aortic pressure	-	Vasodilatation	-	Vasodilatation	-
	Peripheral resistance	-	Blood pressure	-	Peripheral resistance	-	Peripheral resistance	-
	Cardiac output	+			Cardiac output	+	Cardiac output	+
NOAEL (µg kg <sup>-1</sup> bw)	none		2		none		none	
Remarks	Expected pharmacodynamic action				Expected pharmacodynamic action		Slight impact of anesthesia on the cardiovascular effects	

NOAELs derived from repeated dose studies should not be regarded as a meaningful basis for an ADI because of the observed down regulation of lung β-adrenergic receptors (Table 2, study E and G), at least as long as dose- and time-dependency and β-adrenoceptors speciation is not established. It is considered indicative for a down regulation that 35 µg ractopamine kg<sup>-1</sup> bw in two monkey studies was shown as an effective dose after a single administration and that 125 µg ractopamine kg<sup>-1</sup> bw in the 90-day and one-year studies did not show effects on the same parameters, despite the different route of administration (i.v. infusion in the single administration studies, oral (gavage) in the repeated dose studies, considering the rapid and nearly complete intestinal absorption). The partial disappearance of resting bradycardia

observed for the second half of the one-year dog study (J) at 112  $\mu\text{g}$  ractopamine  $\text{kg}^{-1}$  bw can also be considered as a further demonstration of down regulation.

JECFA also ‘noted that the two studies in monkeys could have underestimated ractopamine-induced  $\beta$ -adrenoceptor desensitization.’ But JECFA concluded ‘that  $\beta$ -adrenoceptor desensitization would not be induced at a NOEL at which  $\beta$ -adrenergic activity was virtually absent.’ The FEEDAP Panel does not agree on this conclusion since the absence of reduced receptor density in the long-term study (G) at 500  $\mu\text{g}$  does not preclude a transient change in the short term, as was seen in the six-week study (E). This may have influenced NOAELs at which the reduction of receptor density has not been observed.

Table 2. Repeated administration studies, pharmacological end points

Study	E	F	G
Species	Monkeys	Monkeys	Monkeys
Number of animals	8 F, 8 M	6 F, 6 M	16 F, 16 M
Administration	Oral	Oral	Oral
Dose ( $\mu\text{g}$ $\text{kg}^{-1}$ bw)	0, 250, 500, 4000	0, 125	0,125, 500, 4000
Duration	Six weeks	90 days	One year
End points affected (increase + decrease -)	Heart rate + Lung receptor density At 500 and 4000 $\mu\text{g}$ -		Heart rate + Heart weight - At 4000 $\mu\text{g}$ $\text{kg}^{-1}$ bw lung $\beta$ receptors -
NOAEL ( $\mu\text{g}$ $\text{kg}^{-1}$ bw)	250	125	125

When evaluating hypothetical risks for the consumer, data from acute pharmacological studies would better reflect the consumer situation after intake of a single meal containing ractopamine residues. Effects were observed in acute studies (Table 1) at lower doses than in repeated dose studies (Table 2).

Toxicological studies are summarised in Table 3. The NOAELs derived from toxicological end points are considerably higher than those from pharmacological end points. Effects observed in toxicity studies are mostly related to the pharmacological action, such as changes in vital signs (e.g. heart rate, blood pressure),  $\beta$ -receptors density and related pathological findings.

Reduction of testes weight is a common effect after administration of  $\beta$ -agonist in food producing animals (Blanco et al., 2002). The FEEDAP Panel also notes that tocolytic effects, which are elicited by  $\beta$ -adrenergic drugs, have not been considered as an end point in toxicological studies.

Table 3. Repeated administration studies, toxicological end points

Study	H	I	J
Species	Mice	Rat	Dogs
Number of animals	10 per sex and dose	20 per sex and dose	4 per sex and dose
Administration	Oral	Oral	Oral
Dose ( $\text{mg}$ $\text{kg}^{-1}$ bw)	0, 25, 125, 1250	0, 1.3, 13.4, 152.9 in males 0, 1.4, 15.3, 156.8 in females	0, 0.112, 0.224, 5.68
Duration	Three months	Three months	One year
NOAEL ( $\text{mg}$ $\text{kg}^{-1}$ bw)	25 mg for females only	1.3 for males, 1.4 for females	None
Remarks	No NOAEL could be established for males because of decreased testis weight for all treated males.		Nocturnal bradycardia occurred in all treated groups during the first half of the study.

The FEEDAP Panel, sharing the JECFA and FDA opinion (FDA, 1999), considers that ractopamine is not a direct carcinogen and the induction of leiomyomas is a non-genotoxic event with a threshold; it also concludes that all treatment-related effects observed in the long-term studies of toxicity in mice and rats were attributable to the  $\beta$ -adrenergic activity of ractopamine. This conclusion is supported by studies on related adrenergic  $\beta$ -receptor agonists which have shown this response to be blocked by the co-administration of adrenergic  $\beta$ -receptor antagonists (Jack et al., 1983; Gibson et al., 1987; Gopinath et al., 1987). The NOAEL



in the two-year rat study is  $2 \text{ mg kg}^{-1} \text{ bw}$ , based on cardiomyopathy as end point. Although no NOAEL could be derived for female mice, it is concluded that a NOAEL of  $2 \text{ mg kg}^{-1} \text{ bw}$  for chronic toxicological studies could be accepted for both species because the benchmark dose calculated for female mice was substantially higher ( $201 \text{ mg kg}^{-1} \text{ bw}$ ).

Considering all studies, the FEEDAP Panel concludes that ractopamine is not mutagenic and is unlikely to present a carcinogenic risk to consumers.

Since data in laboratory animals gave such a wide range of NOAELs, the available human data was considered pivotal by JECFA, as it is by the FEEDAP Panel, when assessing consumer safety (see Section 3.3).

### 3.3. Observations in humans: cardiovascular effects of ractopamine

#### 3.3.1. Study design

The study was designed to characterise the dose-response relationships concerning the cardiovascular function which were expected to provide estimates of the NOEL. The study<sup>51</sup> was an open-label trial with each of six healthy male volunteers receiving placebo plus five oral doses of 5, 10, 15, 25 and 40 mg ractopamine with a washout period of 48 hours between doses.

The age of the subjects ranged from 19 to 26 years (average 23.5 years), the weight ranged from 66.4 (mean of two measurements) to 79.6 kg (average 75.3 kg). On a body weight basis, the doses ranged from 66 to  $529 \mu\text{g ractopamine kg}^{-1}$  (calculated on an individual basis from 63 to  $590 \mu\text{g kg}^{-1}$ ).

Following each dose, subjects were monitored using Echo-Doppler cardiography and measurements of vital signs (heart rate and blood pressure). Furthermore, pharmacokinetics after the application of the highest 40 mg dose were described. The following 14 pharmacodynamic parameters were assessed two hours and one hour before dosing and seven times after dosing, at hourly intervals (starting with a measurement immediately post-dose):

- Vital signs (systolic and diastolic blood pressure, heart rate);
- Systolic time interval (QS2): defined as total electromechanical systole, the period of systole from the beginning of the QRS complex of the ECG to the closure of the aortic valve as determined by Doppler aortic flow measurement;
- QS2(I), the corrected QS2; corrected for heart rate using the formula  $\text{QS2}+1.2\text{HR}$ ;
- Maximum fibre shortening: determined by M-mode echocardiography;
- Maximum velocity of circumferential fibre shortening (VcFc): determined by M-mode echocardiography;
- End systolic volume (ESV): determined by M-mode echocardiography;
- End diastolic volume (EDV): determined by M-mode echocardiography;
- Cardiac output (CO): determined by Echo-Doppler 2D evaluation of aortic valve area and Doppler aortic flow measurement;
- Left ventricular ejection time (LVET): the phase of systole during which the blood is pumped out of the ventricle into the arterial system; measured by time from beginning to the end of aortic flow, as determined by Echo Doppler;
- LVET(I): corrected for heart rate using the formula  $\text{LVET}+1.1\text{HR}$ ;
- Pre-ejection period (PEP): defined as the interval from ventricular depolarization to the beginning of left ventricular ejection as determined by Echo-Doppler measurement of aortic flow;

<sup>51</sup> Original reports/Reference 37A

- $QT_c$ : calculated from the computerized ECG or by using the formula of QT divided by the square root of the RR interval.

The subjects were continuously monitored for adverse events by the study staff.

For the pharmacokinetic assessment, blood was drawn for clinical laboratory studies on day 11 of the study (after application of the 40 mg dose). Ten 10 mL samples of blood were obtained prior to dosing with 40 mg ractopamine and at the following post-dose times: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours. Plasma from those samples was assayed for ractopamine concentration.

For each of the above-mentioned pharmacodynamic parameters, the maximum response (defined as the one-hour post-dose value) was expressed as a difference from baseline, computed as the average of pre-dose values.

### **3.3.2. Comments on the study design**

The study reported was originally designed as a preliminary (open label) study intended to establish dose-effect responses to enable suitable doses to be selected for a larger (double-blinded) study. It was not intended to define a no-effect level. The use of the data obtained for this purpose inevitably exposes experimental weaknesses and uncertainties and limits the conclusiveness of the study. The absence of a double-blinded study design to avoid placebo effects would introduce bias.

The sample size does not provide sufficient statistical power to detect a clinical relevant response that would be statistically significant. Power calculations by the FEEDAP Panel have shown that the statistical power to detect a 10 % change in cardiac output at a significance level of  $p=0.05$  is less than 20 %; to detect the same change with the usual power of 80 %, a sample size of about 60 would have been required. For the parameter heart rate, only an increase of 20 % could be detected as statistically significant, with an 80 % power, at the given sample size.

Among the 14 endpoints, many parameters were chosen which show changes secondary to a change in the heart rate (QS2, LVET, PEP). This is not considered the most sensible approach since changes in those parameters mainly account for a change in the heart rate and thus are not indicative of the direct chronotropic or inotropic action of ractopamine. Furthermore, it is unclear why  $QT_c$  was included in the end point assessment.

Additionally, the end points selected were restricted to the cardiovascular effects of ractopamine. Therefore, they do not cover all the effects which could be expected after stimulation of  $\beta$ -adrenoceptors. The only extra-cardiac effects that are accounted for by the parameters heart rate and blood pressure are the actions on the kidney mediated via  $\beta_1$ -stimulation (increase of renin causes increase in aldosterone as well as vasoconstriction which leads in turn to an increase in blood pressure) and on the blood vessels via  $\beta_2$  receptors (vasodilatation and decrease in blood pressure).

Other possible extracardiac effects mediated by  $\beta$ -adrenergic stimulation are not represented by the parameters selected. Those are, among others, effects on metabolic parameters (glucose, free fatty acids), effects on muscle tremor, effects on behaviour (restlessness, apprehension, anxiety) and effects on bronchial hyper-reactivity.

In the pharmacokinetic study (40 mg ractopamine), probably not enough measurements were performed within the first hour since the ascending part (and by this the absorption kinetics) of the plasma ractopamine concentration is only insufficiently described by measured values.

### 3.3.3. Results

The adverse event analysis showed no serious adverse events occurring during the study. Subject 5 was withdrawn from the study before receiving the 40 mg dose of ractopamine because of adverse events (sensation of an increase in heart rate and sensation of heart pounding). All non-serious adverse events reported were considered to be either mild or moderate in severity. No adverse events occurred after the 0 mg, 5 mg and 10 mg dose. After application of 15 mg, 25 mg and 40 mg, there were nine reports of sensation of increase in heart rate and five reports of sensation of heart pounding.

The principal findings are described in the original report as follows: dose-dependent changes of cardiac variables appeared within the first hour after administration of ractopamine and gradually returned to baseline values before treatment. At a dose of 5 mg, there was apparently no cardiovascular response, and at 10 mg only minor effects were reported. At 15, 25, and 40 mg, the heart rate was elevated to about 20, 30, and 50 beats per min above control and the cardiac output increased by approximately 35 %, 55 %, and 90 %, respectively. At the same doses, the electromechanical systole was shortened by about 10 %, 14 %, and 19 %, respectively. The systolic blood pressure increased in a dose-dependent manner. In contrast to the vasodilative effects recorded in monkeys and dogs, ractopamine did not change the diastolic pressure.

The NOELs for the study variables derived using a standard toxicological model independent approach are summarised in Table 4.

Table 4. **NOELs for cardiovascular effects in healthy human volunteers after oral administration of ractopamine (taken from the study report)\***

Parameter	NOEL (mg person <sup>-1</sup> )	NOEL (µg kg <sup>-1</sup> bw)**
Electromechanical systole (and HR corrected QS2)	5	71
Left ventricular ejection time (and HR corrected LVET)	5	71
Maximum velocity of circumferential fibre shortening	5	71
Heart rate	10	143
End systolic volume	10	143
Systolic blood pressure	10	143
Cardiac output	15	214
End diastolic volume	15	214
Maximum fibre shortening	15	214
Diastolic blood pressure	25	357
Corrected QT-interval	40	571
Pre-ejection period	40	571

\* From Hunt (1994)<sup>52</sup>

\*\* bw used for calculation, 70 kg

#### 3.3.3.1. Evaluation by the Notifier

The Notifier calculated the NOELs by a piecewise linear model of log-transformed data. The three primary variables and the overall composite including confidence intervals (95 %) are given in Table 5.

The composite NOEL derived from the study was 99 µg kg<sup>-1</sup> (6.9 mg for a 70 kg person).

<sup>52</sup> Original reports/Reference 37A

Table 5. NOEL and confidence intervals (95 %) calculated for three salient variables of cardiac function and for a composite over all variables

Variable	NOEL ( $\mu\text{g kg}^{-1}$ )	Confidence Interval	
		Lower	Upper
Electromechanical systole	96	35	156
Heart Rate	114	58	171
Cardiac Output	84	41	128
Composite	99	77	120

### 3.3.3.2. Evaluation by JECFA

JECFA noted that the ‘NOELs for the relevant cardiac variables were  $67 \mu\text{g kg}^{-1}$  bw for electromechanical systole, ventricular ejection time, and maximum velocity of circumferential fibre shortening,  $133 \mu\text{g kg}^{-1}$  bw for heart rate and  $200 \mu\text{g kg}^{-1}$  bw for cardiac output.’ These NOELs are derived from daily doses of 5, 10 and 15 mg ractopamine per 75 kg person.

JECFA concluded ‘that the acute cardiac responses to ractopamine in humans were the most appropriate endpoints for the calculation of an ADI. A combined NOEL of  $67 \mu\text{g kg}^{-1}$  was determined on the basis of changes in electromechanical systole, left ventricular ejection time, and maximum velocity of circumferential fibre shortening.’

### 3.3.3.3. Alternative

From the data given in Table 5 another option could have been proposed. The NOEL could be derived considering the small sample size and the large standard deviation as the lowest value of the lower confidence limit (95 %) of the relevant NOEL (here:  $35 \mu\text{g kg}^{-1}$  bw from electromechanical systole).

### 3.3.4. Comments on the data evaluation

The maximum response in this study was calculated by using the one-hour post-dose value. However, for many of the parameters assessed, the maximum effect was achieved at time points distinct from the two hours value (which corresponds to the one-hour post-dose). The maximum response for most of the parameters occurred at time points later than the two hours value. The  $E_{\text{max}}$  (the maximum pharmacodynamic effect) is the decisive value for NOEL calculation. Table 6 shows the time points post-dose at which the  $E_{\text{max}}$  was observed.

Table 6. Post-dose time (hours) for  $E_{\text{max}}$

Subject	QS2	Max fibre shortening	VcFc	Cardiac Output	Systolic BP	Diastolic BP	Heart rate
1	7	3	3	3	1	1	6
2	7	7	7	7	6	7	7
3	7	6	6	6	3	3	7
4	6	7	7	7	4	4	2
5	7	2	2	2	1	1	7
6	7	2	7	2	1	4	7

The Notifier derived a NOEL from a composite end point (end points which consists of two or more mono-components) which represents three out of 14 measured variables. Consequently, the resulting NOEL is higher than the lowest parameter-related NOEL – this contradicts the concept of the NOEL (no-effect level).

The parameters for the overall composite were selected post-hoc without any justification given in the protocol or in the report. This appears to be methodologically unacceptable. Heart rate and QS2, both taken for the overall composite, depend on each other and, as such, are not

eligible to be components of a composite end point. This is another reason why this composite end point is not acceptable. Mixing dependent and independent variables in one statistical procedure (overall composite) would be a source of bias.

The regression model used in the study is not considered appropriate. No reasons for performing a log-transformation of individual doses or a piecewise linear regression were given in the study report. Generally, the simplest method should be applied, unless otherwise indicated. Therefore, and since the usual preconditions for such an approach are fulfilled, a simple linear regression without log-transformation would be appropriate to analyse the data, as the FEEDAP Panel's comparisons based on the Akaike Information Criterion showed for the parameters QS2 and HR.

### 3.3.5. JECFA's ADI and the FEEDAP Panel's comments on the safety factor applied

JECFA established 'an ADI for ractopamine of 0–1  $\mu\text{g kg}^{-1}$  bw per day based on the NOEL of 67  $\mu\text{g kg}^{-1}$  bw in the study in human volunteers and the application of a safety factor of 50, rounded to one significant figure.'

For the safety factor of 50 applied by JECFA, 'a figure of 10 was used to account for individual variability and an additional safety factor of 5 was considered appropriate to protect sensitive individuals and in view of the small sample size in the human volunteer study.'

Significant subpopulations which may be at higher risk for adverse events after  $\beta$ -adrenergic stimulation require particular consideration when estimating the safety factor. The FEEDAP Panel identified subpopulations potentially at higher risk, e.g. individuals with cardiovascular disease, children and individuals with specific  $\beta$ -receptor polymorphisms.

Cardiovascular diseases are likely to enhance sensitivity to  $\beta$ -adrenergic substances. In patients with no cardiac disease,  $\beta$ -agonists rarely cause significant arrhythmias or myocardial ischemia. However, patients with underlying coronary artery diseases or pre-existing arrhythmias are at much greater risk. In view of the high prevalence (10–15 % of the population)<sup>53</sup> of cardiovascular diseases, this issue is of special relevance. The risk of adverse cardiovascular effects is also higher in patients receiving MAO inhibitors or tricyclic antidepressants.

Aging is associated with various changes in the cardiovascular function. With age, the cardiac response to  $\beta$ -adrenoceptor stimulation declines. Studies in human showed that in persons of less than 20 years old (mean age 13), the  $\text{EC}_{50}$  values for the positive inotropic effect of isoprenaline (measured on isolated electrically driven right atria) were about tenfold lower than those from elderly patients (over 50 years old) (Brodde et al., 1995). The ractopamine study was performed in young adult volunteers (mean age 23.5 years); children are more sensitive to  $\beta$ -adrenergic substances and this should be considered when selecting the safety factor. Neonates until 18 months of age could be particularly at risk due to the poor glucuronidating capacity resulting in the inability to inactivate the drug, which would maintain its full pharmacological activity for a long period of time (Miyagi and Collier, 2007).

The response to  $\beta$ -agonists may differ depending on the genetic polymorphism of the  $\beta$ -receptor. There are four polymorphisms in the coding block of the gene encoding the  $\beta_2$ -adrenoceptor, resulting in changes of amino acids: the most common polymorphism occurred at position 16, where arginine is replaced by glycine, and in the homozygous form it makes up to 50 % of the  $\beta_2$  adrenoceptor in the normal population. Studies in patients with asthma showed that the therapeutic response to salmeterol differed largely depending on the polymorphism

<sup>53</sup> WHO health data database (HFA-DB) 2006, the European hospital morbidity database (HBDM), [www.euro.who.int/information-sources](http://www.euro.who.int/information-sources)

(Wechsler et al., 2006). It is unlikely that the different polymorphisms are properly represented in the study group; no comment is given in the study report.

Altogether, the FEEDAP Panel concludes that the safety factor applied by JECFA to derive the ADI from the NOEL does not sufficiently take into account population subsets at higher risk of adverse events after  $\beta$ -adrenergic stimulation.

### 3.3.6. Evaluation of the data by the FEEDAP Panel

The NOELs derived from the study and applied by different bodies are, in the view of FEEDAP Panel, compromised by the reasons given above. Each evaluation based on a group mean value is handicapped by the poor statistical power (see also Appendix II). An evaluation should therefore be based on the individual response (pharmacodynamic effects). This has been done for the lowest administered dose (5 mg per subject).

Hence, seven parameters which are thought to best reflect the positive inotropic and chronotropic action of ractopamine were selected from the 14 parameters measured in the study. All frequency corrected parameters, except for systolic time interval (STI), were omitted, because changes in these parameters occur secondary to changes in heart rate and, as such, are not ideal to reflect the chronotropic action of ractopamine. Other parameters, like  $QT_c$ , were omitted since it is unclear in which direction a  $\beta$ -agonist would impact this parameter.

The following parameters were selected:

1. STI, QS2 (msec): expected to decrease following application of a  $\beta$ -agonist;
2. Maximum Fibre Shortening (%): expected to increase following application of a  $\beta$ -agonist;
3. Maximum velocity of circumferential fibre shortening (VcFc) (cir/sec): expected to increase following application of a  $\beta$ -agonist;
4. Cardiac output (L/min): expected to increase following application of a  $\beta$ -agonist;
5. Systolic BP (mmHg): expected to increase following application of a  $\beta$ -agonist;
6. Diastolic BP (mmHg): expected to increase following application of a  $\beta$ -agonist;
7. Heart rate: expected to increase following application of a  $\beta$ -agonist.

For those parameters, the effect was calculated as  $[E_{max} - (\text{average of the two pre-dose values})]$  for each of the six subjects for the 0 mg (placebo) and the 5 mg dose. The FEEDAP Panel notes again that this evaluation is of a pure descriptive nature and no conclusions with respect to statistical significance can be drawn.

Effects (5 mg ractopamine produces greater effect than placebo) were seen in more than three out of six subjects for the four parameters QS2, cardiac output, systolic and diastolic blood pressure.

Graphs describing the absolute values measured during the observation period (eight hours) for the 0, 5, and 10 mg ractopamine-dosed individuals and the individual effect values can be found in Appendix III.

#### 3.3.6.1. Cardiac output

Considering the time course of CO after treatment with 5 mg ractopamine (Figures AIII.1.1.1-1.1.6), CO was constantly higher for a longer period ( $\geq$  four hours) than after placebo in subjects 1 and 4, slightly higher for subject 3, not distinctly different from the placebo values for subjects 5 and 6, and lower than in the placebo period for subject 2.

Effects were seen in three out of six subjects (Figure AIII.1.2). The differences were above  $0.5 \text{ L min}^{-1}$  in two subjects. For cardiac output, a clinically relevant effect could be assumed at differences greater than  $0.5 \text{ L min}^{-1}$ , since this value has been chosen in clinical trials on

patients with heart failure as a clinically significant difference between treatments (Staier et al., 2008).

### 3.3.6.2. Systolic and diastolic blood pressure

Considering the time course of SBP (Figures AIII.2.1.1-2.1.6), subjects 1, 5 and 6 show a rather parallel course; only subject 4 shows a marked increase over time. However, the effects on SBP (Figure AIII.2.2) were seen in four out of six subjects.

The time course of diastolic blood pressure seems to be not evidently different after placebo and 5 mg ractopamine. However, effect calculation accounts for a response of five out of six subjects (Figure AIII.2.2).

Each increase in blood pressure which exceeds placebo is considered clinically relevant in epidemiological studies because of the increased cardiovascular risk (risk for myocardial infarction, stroke). However, a single exposure leading to an acute and transient change is not considered to pose the same risk.

### 3.3.6.3. Systolic time interval, total electromechanical systole

Changes in QS2 cannot be judged in terms of clinical relevance due to the lack of data.

The time course of QS2 of subjects 1, 2, 3, and 4 implies that QS2 after 5 mg ractopamine was lower than after placebo (Figures AIII.3.1.1-3.1.6).

Effects were shown in four out of six subjects (subjects 2, 3, 4, and 6, Figure AIII.3.2).

### 3.3.6.4. Heart rate, maximum fibre shortening and maximum velocity of circumferential fibre shortening

An effect on heart rate was calculated for three subjects; the other three subjects showed only small (1), none (1) or opposite effects.

The two parameters related to fibre shortening (maximum fibre shortening and VcFc), both expected to increase following application of a  $\beta$ -agonist, did not reveal any effects (the effect on fibre shortening was in four subjects higher after placebo administration, on VcFc on three subjects).

### 3.3.7. Conclusions

The FEEDAP Panel first considered whether the relevant effect which would later serve as basis for consumer safety must be a NOAEL or a NOEL.<sup>54</sup> The FEEDAP Panel notes, that if an ADI would be derived from pharmacological studies, a NOEL must be taken to consider not only clinically relevant (“adverse”) effects in the consumer but also subjective discomfort even when occurring only for a short time.

<sup>54</sup> WHO Environmental Health Criteria, No. 170, Assessing the human health risk of chemicals: Derivation of guidance values for health-based exposure limits:

**No-observed-adverse-effect level (NOAEL):** greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure.

**No-observed-effect level (NOEL):** greatest concentration or amount of a substance, found by experiment or observation, that causes no alteration of morphology, functional capacity, growth, development, or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

The FEEDAP Panel notes also that a pivotal study based on six test persons cannot only be evaluated by considering arithmetic means. The sample size does not provide sufficient statistical power to detect a clinically relevant response as statistically significant. Although being more descriptive by nature, individual response has to be considered.

Overall, the 5 mg dose cannot be definitely considered to be a no-effect dose, although within this descriptive evaluation random effects cannot be clearly distinguished from systematic effects. A parameter-free paired test (see Appendix II), the only statistical approach justified, does not have enough power to detect differences at a level of  $p < 0.05$ . Therefore, the same chance exists that, given the observed differences, no effect occurred at 5 mg dose. But this is considered not scientifically sound to conclude on a NOEL.

The FEEDAP Panel also examined the alternative of considering the 5 mg dose as a LOEL and, because data for doses between 5 and 0 mg are not available, to apply the benchmark procedure for determining a NOEL (see Appendix II).

Without specifying a critical response (clinical relevance) for the respective parameter, the lower confidence limit of the benchmark dose is 0 mg to exclude a 10 % change in QS2, a 20 % change in heart rate and a 40 % change in cardiac output. The benchmark procedure would consequently not allow either to establish a NOEL.

Furthermore, the FEEDAP Panel is of the opinion that the uncertainties concerning the figure of a NOEL should not be balanced by a (higher) safety factor. All the uncertainties together would reach a dimension in which more or less arbitrary estimations prevail.

The FEEDAP Panel finally concludes that the human study cannot be taken as a basis to derive an ADI.

#### **3.3.7.1. CVMP comments**

The CVMP agreed that the ADI of  $0\text{--}1 \mu\text{g kg}^{-1} \text{ bw day}^{-1}$  established by JECFA for ractopamine cannot be accepted.

The CVMP agrees with the FEEDAP Panel that the most relevant hazards would be those associated with acute pharmacological effects, and the information available is insufficient to establish an overall NOEL/NOAEL related to these.

As the CVMP, the FEEDAP Panel concluded that there is great uncertainty associated with this NOEL. The CVMP is in full agreement with the conclusion of the FEEDAP Panel that this study cannot be taken as a basis from which to calculate an ADI, due to the small sample size not providing sufficient statistical power and no non-zero confidence interval of the benchmark dose. The CVMP considered that in addition, due to the omission to determine cardiovascular effects in coincidence with  $C_{\text{max}}$  and  $t_{\text{max}}$  at 0.5 hours after dosing, the NOEL of  $67 \mu\text{g kg}^{-1} \text{ bw day}^{-1}$  may underestimate the sensitivity of cardiovascular end points.

#### **3.4. Additional data on cardiovascular effects of butopamine**

The RR isomer of ractopamine (butopamine) is considered the most active, binding to  $\beta_1$  and  $\beta_2$  adrenergic receptors (WHO, 2004, Mills et al., 2003). The other isomers showed a lower (SR) or no (RS, SS) affinity to  $\beta$ -adrenergic receptors.

A study in eight patients with congestive heart failure given butopamine intravenously was published in 1980 (Thompson et al., 1980). As the systemic systolic blood pressure, the most sensitive end point, increased at higher or equal to a dose of  $0.04 \mu\text{g kg}^{-1} \text{ min}^{-1}$ , the NOEL was  $0.02 \mu\text{g kg}^{-1} \text{ min}^{-1}$  (the lowest dose tested).

In a review paper, Smith (1998) proposed, 'for discussion purposes only', a NOEL for ractopamine based on the above data extrapolated from butopamine to ractopamine by a factor



of 4, assuming that the RR isomer is the only active isomer. Following the assumption of the author, the FEEDAP Panel calculated that the NOEL would be 4.8 µg ractopamine kg<sup>-1</sup> day<sup>-1</sup>.

This hypothetical NOEL was not considered further by the FEEDAP Panel because (i) the potential β-adrenergic activity of the non-RR isomers was not taken into consideration, and (ii) pharmacokinetic data that would allow comparisons between continuous intravenous and single oral administration were absent.

These data on butopamine were not assessed by JECFA.

#### 4. Consumer safety

##### 4.1. Review of JECFA assessment

The dataset on residues in pig and cattle tissues used by JECFA to assess consumer safety for ractopamine is the same as that submitted by the Notifier to EMEA when applying for setting MRLs for ractopamine. These were made fully available to EFSA (see Appendix IV).

In recommending MRLs for ractopamine, the JECFA took into consideration the following key factors:

- ADI rounded to 60 µg for a 60 kg person;
- Free ractopamine as the marker residue;
- MRL calculations based on tissue residues at a 12-hour withdrawal time, corresponding to practical zero withdrawal;
- MRLs for liver and kidney of pig and cattle based on the mean residue concentrations of free ractopamine plus three standard deviations, the mean being calculated from the pooled data for pigs in all studies at 12 hours after the last feeding at the maximum dose of 20 mg kg<sup>-1</sup> and from cattle data obtained with the maximum dose of 30 mg kg<sup>-1</sup>;
- Ratios free ractopamine vs. total residues derived at a 12-hours withdrawal in cattle used to convert free ractopamine residues to total residues in pig and cattle (more conservative);
- MRLs for muscle and fat based on twice the LOQ and ratio of 1 applied to convert marker to total residues.

For the assessment exercise, the FEEDAP Panel considered the first two pre-requisites and followed its own rationale (Regulation (EC) No 429/2008) for the evaluation of consumer exposure.

Five studies carried out in pigs with <sup>14</sup>C-ractopamine were submitted by the Notifier, of which only one<sup>55</sup> was performed using the maximum dose proposed for use, applying withdrawal periods of one, two and three days. In order to assess the zero-day withdrawal time, the FEEDAP Panel, as the JECFA, considered and pooled the results of two studies<sup>56,57</sup> aiming at measuring free ractopamine residues in tissues of pigs fed diets containing ractopamine hydrochloride at a dose of 20 mg kg<sup>-1</sup> and slaughtered 12 hours after the last administered dose. Total residues were back calculated from ractopamine residues using the ratios ractopamine vs. total residues established in the former study<sup>58</sup> at one-day withdrawal time (representing a worse case), e.g. 0.141 and 0.276 for the liver and kidney; the LOQ and a ratio of 1 were retained also for muscle and fat for which ractopamine levels are close to or below the LOQ. The average values plus 2SD (14 animals for liver and kidney, four for muscle and fat) were

<sup>55</sup> Original reports/Reference 24B

<sup>56</sup> Original reports/Reference 31B

<sup>57</sup> Original reports/Reference 33B

<sup>58</sup> Original reports/Reference 24B

used to calculate consumer exposure, based on the theoretical consumption figures established in Regulation (EC) No 429/2008. Total exposure amounted to 0.036 mg ractopamine equivalent person<sup>-1</sup>, which represents 60 % of JECFA ADI.

Four studies performed with cattle using <sup>14</sup>C-ractopamine have been submitted by the Notifier, of which only one<sup>59</sup> was carried out using the maximum dose proposed for use and applying a withdrawal period of 12 hours (practical zero-withdrawal). Only three animals were used per time point and therefore the highest individual values of total radioactivity in tissues (only liver and kidney were measured) were retained to calculate the theoretical consumer exposure. The muscle and fat contributions were taken from another study<sup>60</sup> where animals received feed supplemented with ractopamine hydrochloride at a dose of 45 mg kg<sup>-1</sup>, representing a worst case, and were slaughtered after a 12-hour withdrawal time. Total exposure amounted to 0.061 mg ractopamine equivalent person<sup>-1</sup> day<sup>-1</sup>.

#### 4.1.1. Conclusions on the JECFA assessment

If the pre-requisites of JECFA of an ADI value of 0.06 mg person<sup>-1</sup> and free ractopamine as the marker residue are taken as a basis, the FEEDAP Panel would reach a similar conclusion that consumer safety would be ensured without applying a withdrawal period to pig and cattle.

Using the same dataset as JECFA for ractopamine residue levels in the different tissues of pigs, the FEEDAP Panel would have reached similar MRLs. As the specific studies in cattle from which MRLs have been proposed by JECFA are not clearly documented, the FEEDAP Panel is not in a position to conclude on the pertinence of the JECFA proposal.

The FEEDAP Panel notes that the specific ratios free ractopamine vs. total residues (in liver and kidneys) for pig and cattle should have been derived and used instead of common ratios for both species.

#### 4.2. Assessment by the FEEDAP Panel

The FEEDAP Panel was not in a position to support the ADI based on the human study, as proposed by JECFA, and consequently no proposal for MRLs can be made.

The use of the only pharmacological NOAEL that could be derived from existing animal data (dog study) would finally lead to MRLs which are exceeded by all existing residue data regardless of the withdrawal period.

##### 4.2.1. CVMP comment

The CVMP is in agreement with the EFSA position which does not support the maximum residue limits based on the ADI set by JECFA.

#### 4.3. Marker residue

It can be anticipated that ractopamine glucuronoconjugates in edible tissues are extensively hydrolysed by bacterial  $\beta$ -glucuronidases in the intestinal tract of the consumer to release free ractopamine. Therefore, free ractopamine and ractopamine glucuronoconjugates represent the residues of toxicological/pharmacological concern for the consumer. Consequently, as free ractopamine and ractopamine conjugates represent the essential of ractopamine-derived residues in tissues, total residues (worst case) should first be retained for calculating consumer theoretical exposure.

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<sup>59</sup> Original reports/Reference 27B

<sup>60</sup> Original reports/Reference 26B

Considering that a very sensitive analytical method is available (NRCP of the EU), the FEEDAP Panel proposes to consider the sum of free ractopamine plus de-conjugated ractopamine as the marker residue. This would multiply by a factor of at least three the total amount of ractopamine measured when compared to free ractopamine only; it would also reduce the uncertainty related to the variability of the relative amounts of free and conjugated ractopamine in tissues from different individuals and species, and at different withdrawal time points. Moreover, as this marker residue represents most of the ractopamine-derived residues in tissues, it could be therefore assimilated to total residues, avoiding the use of converting ratios.

#### **4.3.1. CVMP comment**

The CVMP agrees with the recommendation of the FEEDAP Panel that the marker residue proposed by JECFA (free ractopamine) is not acceptable and that the glucuronides of ractopamine should be included in the definition of the marker residue.

However, the CVMP considers that, based on the information referred to in the draft opinion, the FEEDAP Panel's assumption that the parent compound plus its glucuronides would be identical to the total residue is not sufficiently justified.<sup>61</sup> However, given that the JECFA ADI and consequently the MRLs recommended by JECFA cannot be supported, this issue is of minor importance at present.

### **CONCLUSIONS AND REMARK**

#### **CONCLUSIONS**

The metabolic fate of ractopamine hydrochloride is similar in the target species (pigs and cattle), laboratory animals and humans.

The FEEDAP Panel concludes from an acute study in dogs that tachycardia and the peripheral vasodilatation observed are in line with the expected pharmacological action. From another acute study in dogs, with limited statistical power, a pharmacological NOAEL of 2 µg kg<sup>-1</sup> bw could be derived.

Comparing dog and monkey data, it appears that the dog could be considered as more sensitive to ractopamine (β-adrenergic substances). However, the FEEDAP Panel considers that there is not enough data to support this conclusion.

NOAELs derived from pharmacological repeated dose studies should not be regarded as a meaningful basis for an ADI because of the observed down regulation of lung β-adrenergic receptors, at least as long as dose- and time-dependency and β-adrenoceptors speciation is not established. When evaluating hypothetical risks for the consumer, data from acute pharmacological studies would better reflect the consumer situation after intake of a single meal containing ractopamine residues.

The NOAELs derived from toxicological endpoints are considerably higher than those from pharmacological end points. The effects observed in toxicity studies are mostly related to the pharmacological action.

Although a series of mutation studies in prokaryotes and eukaryotic systems were negative, several *in vitro* tests were positive, namely chromosome aberration tests in human lymphocytes and two out of three forward mutation assays in mouse lymphoma cells. The FEEDAP Panel considers that some positive genotoxicity studies *in vitro* are a possible cause of concern. However, these results have to be considered in conjunction with the carcinogenicity studies provided.

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<sup>61</sup> The FEEDAP Panel notes that the total residues should first be retained to calculate consumer theoretical exposure.

The FEEDAP Panel concludes that all treatment-related effects observed in the long-term studies in mice and rats were attributable to the  $\beta$ -adrenergic activity of ractopamine. It shares the JECFA and FDA opinion, that the induction of leiomyomas is a non-genotoxic event with a threshold and ractopamine is not a direct carcinogen. Considering all studies, the FEEDAP Panel concludes that ractopamine is not mutagenic and is unlikely to present a carcinogenic risk to consumers.

Since data in laboratory animals gave a wide range of NOAELs, the available human data was considered pivotal by JECFA as it is by the FEEDAP Panel when assessing consumer safety.

On the basis of mean values from the study with six healthy volunteers the JECFA established an ADI for ractopamine of 0–1  $\mu\text{g kg}^{-1}$  bw per day based on the NOEL of 67  $\mu\text{g kg}^{-1}$  bw and the application of a safety factor of 50, rounded to one significant figure.

The human study was originally designed as a preliminary (open label) study intended to establish dose-effect responses to enable suitable doses to be selected for a larger (double-blinded) study. It was not intended to define a no-effect level. The use of the data obtained for this purpose inevitably exposes experimental weaknesses and uncertainties and limits the conclusiveness of the study. The absence of a double-blinded study design to avoid placebo effects would introduce bias.

Significant subpopulations which may be at higher risk for adverse events after  $\beta$ -adrenergic stimulation require particular consideration when estimating the safety factor. The FEEDAP Panel concludes that the safety factor applied by JECFA to derive the ADI from the NOEL does not sufficiently take into account population subsets at higher risk.

Each evaluation of the human study based on a group mean value is handicapped by the poor statistical power. The FEEDAP Panel notes that an evaluation should be based on the individual response (pharmacodynamic effects). This has been done for the lowest administered dose (5 mg per subject). The FEEDAP Panel concludes that the 5 mg dose cannot be definitely considered a no-effect dose, although within this descriptive evaluation random effects cannot be clearly distinguished from systematic effects.

The FEEDAP Panel also examined the alternative of considering the 5 mg dose as a LOEL and, because data for doses between 5 and 0 mg are not available, to apply the benchmark procedure for determining a NOEL. The benchmark procedure did not allow establishing a NOEL (to exclude a 10 % change in the electromechanical systole (QS2), a 20 % change in heart rate and a 40 % change in cardiac output, the lower confidence limit of the benchmark dose would be 0 mg).

The FEEDAP Panel notes that if an ADI would be derived from a pharmacological study, a NOEL must be taken to consider not only clinically relevant ('adverse') effects in the consumer but also subjective discomfort even when occurring only for a short time.

Furthermore, the FEEDAP Panel is of the opinion that the uncertainties concerning the figure of a NOEL should not be balanced by a (higher) safety factor. All the uncertainties taken together would reach a dimension in which more or less arbitrary estimations prevail.

The FEEDAP Panel finally concludes that the human study cannot be taken as a basis to derive an ADI, as proposed by JECFA, and consequently no proposal for MRLs can be made.

The CVMP fully supported the conclusions of the FEEDAP Panel with regard to the safety evaluation of ractopamine.

The FEEDAP Panel proposes to use the sum of free ractopamine and ractopamine glucuronoconjugates (sensitive analytical methods available, NRCP of the EU), which is supported by CVMP, instead of free ractopamine as the marker substance.

## REMARK

The CVMP noted that no further discussion is provided on the only 'acute' NO(A)EL which seemed acceptable to the FEEDAP Panel of  $2 \mu\text{g kg}^{-1}$ , seen in a single dose/'acute' oral study in dogs. However, the FEEDAP Panel already noted that the available human data was considered pivotal when assessing consumer safety since data in laboratory animals gave such a wide range of NOAELs.

## DOCUMENTATION PROVIDED TO EFSA

1. Ractopamine hydrochloride. First Draft prepared by Dr. J.D. MacNeill and Dr. Stefa Soback. Supersedes the monograph prepared by the 40<sup>th</sup> Meeting of the Committee and Published in FAO and Nutrition Paper 41/5.
2. Ractopamine hydrochloride. First draft prepared by Dr. J.D. MacNeil, Dr. Pascal Sanders, Dr. D. Arnold. Addendum the ractopamine hydrochloride residue monographs prepared by the 62<sup>nd</sup> meeting of the Committee and published in FAO Food and Nutrition Paper 41/16, Rome 2004.
3. Original report. Application for a maximum residue limit for ractopamine hydrochloride. Part A (Safety documentation). Part B (Residues). Elanco Animal Health Ltd. 2005.
4. CVMP comments on the scientific opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on ractopamine. London 16 March 2009.
5. WHO Technical Report Series 925. Evaluation of certain veterinary drug residue in food. 62<sup>nd</sup> report of the Joint FAO/WHO Expert Committee on food additives. 2004
6. WHO Technical Report Series 939. Evaluation of certain veterinary drug residue in food. 66<sup>th</sup> report of the Joint FAO/WHO Expert Committee on food additives. 2006
7. Freedom of information summary. Original new animal drug application. NADA 140-863. Ractopamine hydrochloride (Paylean<sup>®</sup>). December, 1999

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## APPENDICES

### APPENDIX I: CRL REPORT

#### Analysis of ractopamine residues in animal products

According to the list of methods used by the NRLs, edited by the Community Reference Laboratories (CRL) (Bohm *et al.*, 2008), and according to the National Residue Control Plans (NRCP) for 2008 of the Member States (MS), ractopamine residue methods for muscle and liver are reported by 17 and 4, respectively, of the 27 National Reference Laboratories (NRL) within the EU.

The MS used different methods such as separation methods like liquid chromatography or gas chromatography coupled with a mass spectrometry detector (LC-MS, GC-MS) or tandem mass spectrometry (LC-MS/MS, GC-MS/MS) for screening and confirmatory purposes, the state of the art analytical methods being the LC-MS/MS test methods. Furthermore ELISA tests, RIA test kits and Biosensor methods are in use for screening purposes.

By virtue of the CRL's state-of-the-art test methods, recommended concentrations for NRCPs were established in order to improve and harmonise the performance of analytical methods used for substances for which maximum residue limits (MRLs) have not been established. It should be noted that the recommended concentrations do not have legal force. For ractopamine  $1 \mu\text{g kg}^{-1}$  in liver, muscle and urine and  $10 \mu\text{g kg}^{-1}$  in retina were established as recommended concentrations (CRLs, 2007).

#### Confirmatory methods

According to CD 2002/657/EC, chromatographic methods (GC or LC) coupled to mass-spectrometric detection are mandatory for group-A substances. For this reason only such methods are considered.

In 1993 Montrade *et al.* published a multi-residue method for the determination of 14  $\beta$ -agonists including ractopamine in urine. The samples were first treated with  $\beta$ -glucuronidase/arylsulphatase from *Helix pomatia* juice. Afterwards, the samples were purified on Clean Screen Dau cartridges (mixed mode C8/benzene sulphonic acid). Following purification the extracts were evaporated to dryness and TMS derivatives were prepared with the help of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The samples were measured by GC/MS, applying the EI mode for screening purposes and the PCI mode for confirmation. The detection level was given with  $< 0.5 \text{ ng/ml}$  in urine.

The principle of the sample preparation – enzymatic hydrolysis followed by clean-up on mixed-mode cartridges - was also adapted to more recent methods and is still in use.

GC/MS methods were equally applied for the determination of ractopamine in animal tissues (Wu *et al.*, 2008, CRL, 2001) and feed (He *et al.*, 2007).

A method for the determination of ractopamine in muscle was developed by the responsible CRL (CRL Berlin, BVL). In addition to the enzymatic hydrolysis and SPE on Clean Screen Dau cartridges, the method incorporated three further steps to achieve a better sample purification: 1) acidic precipitation, 2) defatting and 3) liquid-liquid extraction on diatomaceous earth. A derivatisation was performed using hexamethyldisilazane. Four ions ( $m/z$  250, 267, 179, 502) were monitored. Decision limit ( $CC\alpha$ ), detection capability ( $CC\beta$ ) and within-laboratory reproducibility  $SD_{\text{WIR}}$  were quoted as  $1.34 \mu\text{g/kg}$ ,  $1.58 \mu\text{g/kg}$  and 13.8 % respectively.

Bocca *et al.* (2003) cited a method based on GC-MS/MS, which had been used for measuring ractopamine among other  $\beta$ -agonists. In this method an acidic hydrolysis and a purification on

C18-cartridges were applied. Decision limit and detection capability were quoted as 69.8 and 78.1 ng/g respectively.

Recent publications used LC-MS/MS test methods for the determination of ractopamine in urine and animal tissues like muscle, liver and retina. In almost all methods two diagnostic ion mass transitions ( $m/z$  302 > 284, 302 > 164) were monitored, whereas the quantification trace was represented by the transition 302 > 164. Further transitions were 302 > 121 and 302 > 107 with a much lower intensity.

Doerge *et al.* (2001) published a single method for ractopamine in retina and liver. The enzymatic hydrolysis was carried out using  $\beta$ -glucuronidase/arylsulphatase. The samples were purified by solid-phase extraction (SPE) on C18 cartridges. Critical concentrations or reproducibility were not estimated.

Antignac *et al.* (2002) cited a single ractopamine method based on LC-MS/MS, which had been used for measuring ractopamine residues in tissue (liver, kidney meat, lung and retina) and in urine. Tissue extracts were treated with  $\beta$ -glucuronidase/arylsulphatase, followed by liquid-liquid extraction on diatomaceous earth and SPE on Clean Screen Dau cartridges. Isoxsuprine was used as internal standard. On the basis of the standard deviation, CC $\alpha$  and CC $\beta$  were quoted as 9 ppt and 28 ppt, respectively.

A further single method based on LC-MS/MS for ractopamine in porcine and bovine muscle was published by Shishani *et al.* (2003). Instead of mixed-mode cartridges, Alumina A SPE cartridges were applied. The lowest spike level was 1  $\mu$ g/kg.

Multi-residue methods based on LC-MS/MS for the determination of more than 22  $\beta$ -agonists including ractopamine in liver, urine, muscle and retina were validated by the responsible CRL (CRL Berlin, 2003, 2006). In all methods  $\beta$ -glucuronidase/arylsulphatase and protease, respectively, were applied. For SPE mixed-mode cartridges were used. The critical concentrations CC $\alpha$  and CC $\beta$  were quoted as follows: muscle 0.25, 0.28  $\mu$ g/kg, liver 0.38, 0.45  $\mu$ g/kg, retina 2.58, 3.03  $\mu$ g/kg, and urine 0.36, 0.50  $\mu$ g/kg. The validations were performed with the help of the validation software "Interval" based on an experimental design. As internal standard ractopamine – d5 was used.

Williams *et al.* (2004) cited an LC-MS/MS multi-residue method for nine  $\beta$ -agonists including ractopamine in bovine retina and liver. As in other methods  $\beta$ -glucuronidase/arylsulphatase was used for enzymatic hydrolysis. The SPE was performed by means of mixed-mode HXC-90-well array cartridges. The limit of quantitation (LOQ) and limit of confirmation (LOQ) were estimated as 0.8 and 0.1  $\mu$ g/kg, respectively.

### Screening methods

In general the methods based on mass spectrometric detection coupled with chromatographic separation can also be used as screening methods for ractopamine. In 20 MS these methods are applied for screening purposes.

Apart from this some other screening methods like ELISA-, RIA- and Biosensor methods have been developed.

An ELISA method for ractopamine in liver and urine was developed (Elliot *et al.*, 1998). The cross-reactivity for ractopamine was estimated as 100 %, whereas the cross-reactivities for some other  $\beta$ -agonists were below < 0.01 %. The LOD was quoted as 0.53  $\mu$ g/kg. As in the confirmatory methods an enzymatic hydrolysis step using glucuronidase/sulfatase was applied as the first step of sample preparation.



In a more recent publication Thompson et al. (2008) described a method based on SPR biosensor for the monitoring of ractopamine residues in urine and liver. The LODs for urine and liver were quoted as 0.34 µg/l and 0.19 µg/kg, respectively.

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**APPENDIX II: FURTHER STATISTICAL ANALYSES BY THE FEEDAP PANEL**

**Parameter-Free Paired Wilcoxon Test.** At a sample size of 6, the strongest significance one can obtain by a single Wilcoxon test is 0.03125 (this p-value is obtained if all subjects show a change in the same direction). The lowest doses for which this p-value is obtained are 10 mg for each of the three parameters QS2, HR and CO. However, since multiple tests (one for each dose and each parameter) are performed, one has to correct the p-values for multiple testing. Irrespective of the correction method, p-values below 0.05 are not obtainable – thus, once more charging the small sample size, there is not sufficient statistical power to detect effects using the Wilcoxon test.

**Benchmark Dose Approach.** One can determine so-called benchmark doses (BMD)<sup>62</sup> by fitting a linear regression line (in general, other mathematical function – e.g. exponential or logarithmic or more complex ones – may be used to fit the data) and then using inverse prediction<sup>63</sup> to determine the BMD, i.e. the dose that leads to an a priori defined critical response (the benchmark response); the lower confidence limit (BMDL) of this BMD can be used as a ‘point of departure’, i.e. as an equivalent of a NOEL – then, the probability that a dose lower than this BMDL leads to the critical response is 5 %.

Since no critical response has been specified for any parameter, changes by 10 %, 20 %, 30 %, 40 % and 50 % of the mean of baseline values have been used as defaults in the FEEDAP Panel’s re-analysis – the corresponding BMDs and BMDLs are given in Table 1. Due to the small sample size, for the parameter QS2, no non-zero BMDL can be obtained for a 10 % change, and for the parameters HR and CO, even 20 % and 40 % changes, respectively, are not large enough to obtain a non-zero BMDL.

Table II.1. **BMDLs ( $\mu\text{g kg}^{-1}$ ) that lead to the specified percentage change with a probability of 5 %**

	10 %	20 %	30 %	40 %	50 %
<b>QS2</b>	0	33.3	217.1	394.8	567
<b>HR</b>	0	0	14.89	105.1	193.7
<b>CO</b>	0	0	0	0	105.3

<sup>62</sup> The Benchmark Dose Approach is widely applied method – as early as 1995, the EPA’s Risk Assessment Forum publishes the initial guidelines on the use of this approach in the assessment of non-cancer health risk. For more information on this approach, see <http://www.epa.gov/ncea/bmds/about.html>

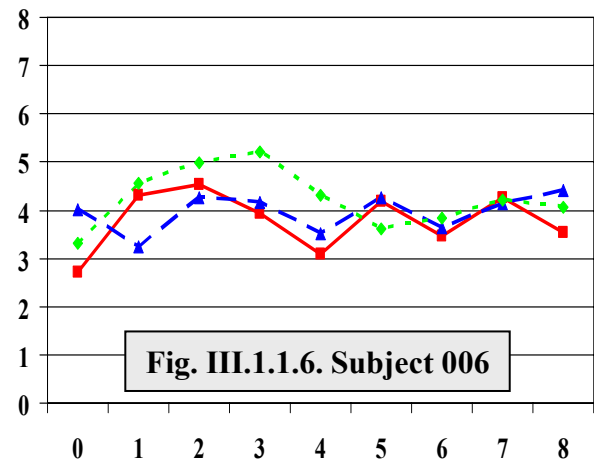
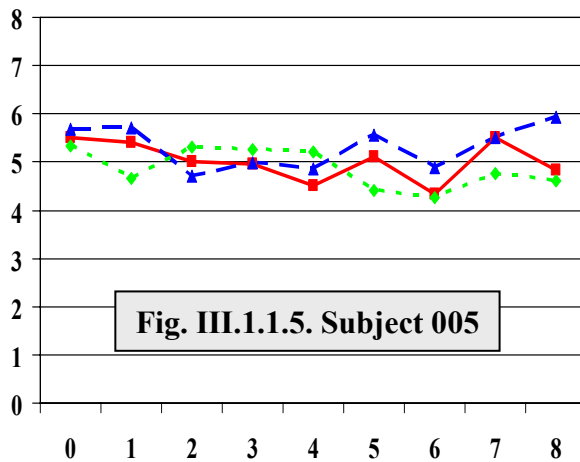
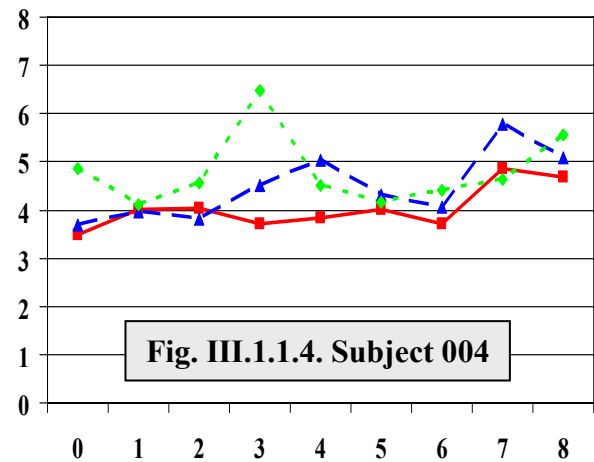
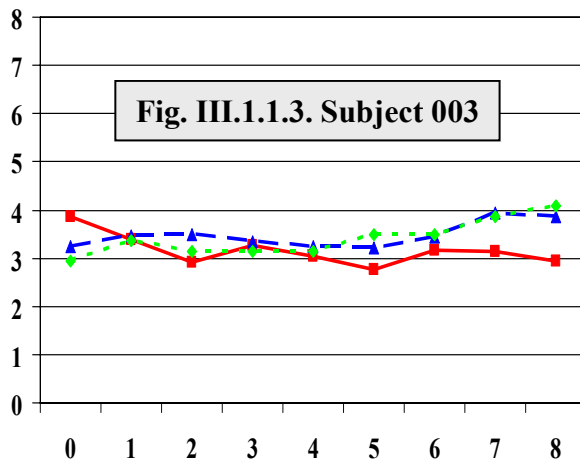
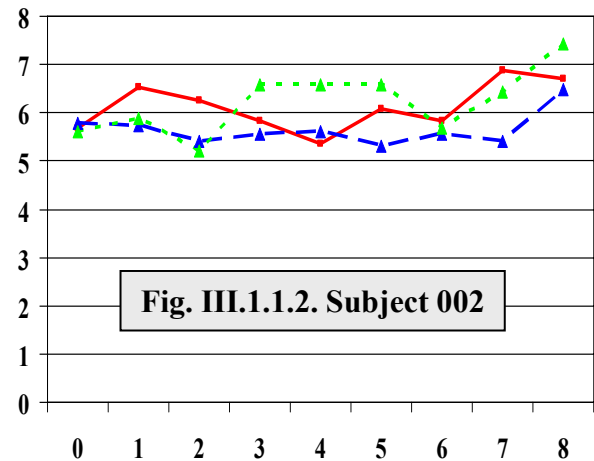
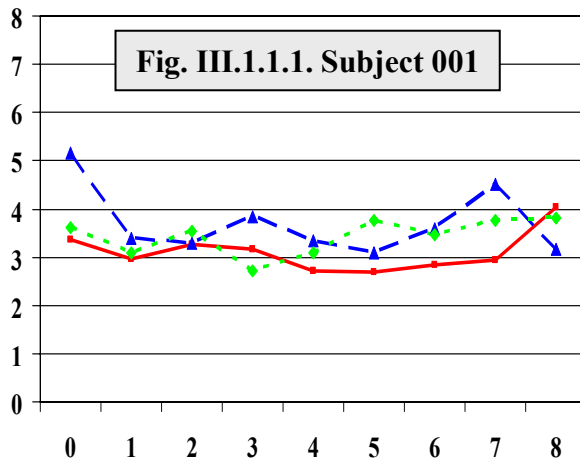
<sup>63</sup> For example, see J.L.Gill, Biases in Regression when Prediction is Inverse to Causation. J Anim Sci 1987, 64: 594-600.

APPENDIX III: FIGURES SHOWING INDIVIDUAL RESPONSE TO RACTOPAMINE

APPENDIX III.1: Cardiac output (L/min)

APPENDIX III.1.1: Comparisons of the absolute values of cardiac output ( $L \text{ min}^{-1}$ ) during observation period (the 2 hour values corresponds to 1 hour post-dose) for the individual subjects administered 0 (placebo), 5 and 10 mg ractopamine

—■— Control   
 —▲— 5 mg   
 -◆- 10 mg



**APPENDIX III.1.2: Effect on cardiac output calculated as  $E_{max}$ -(average of the two pre-dose values) for each of the six subjects for the 0 (placebo) and the 5 mg dose**

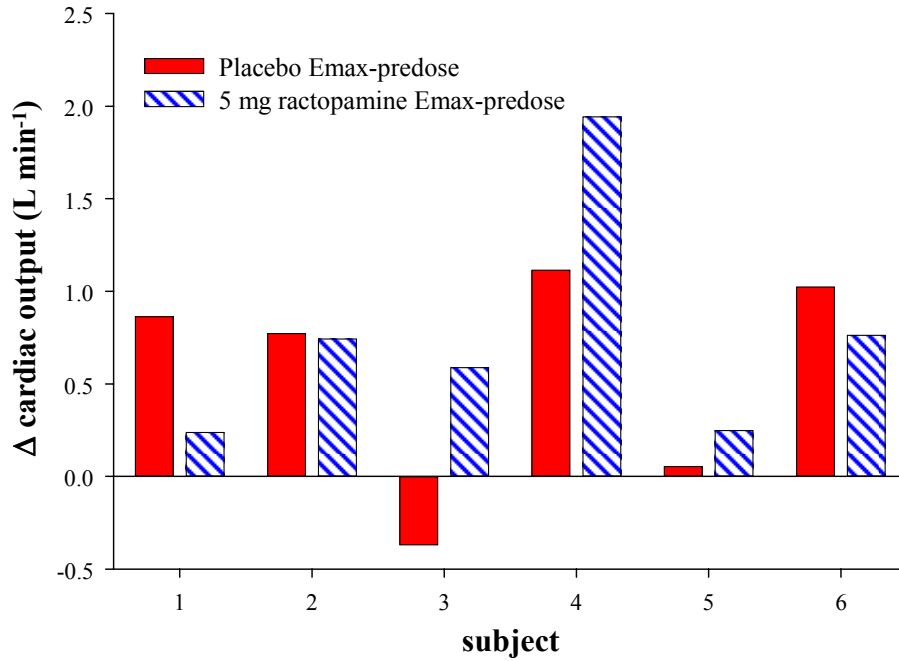
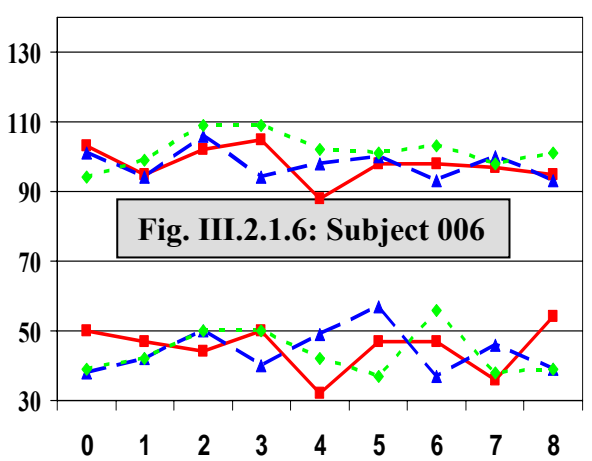
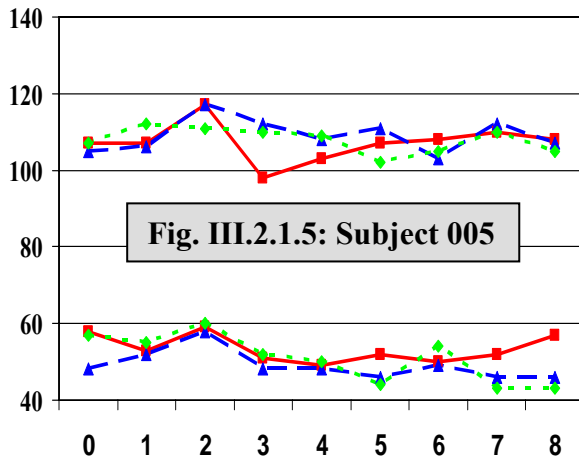
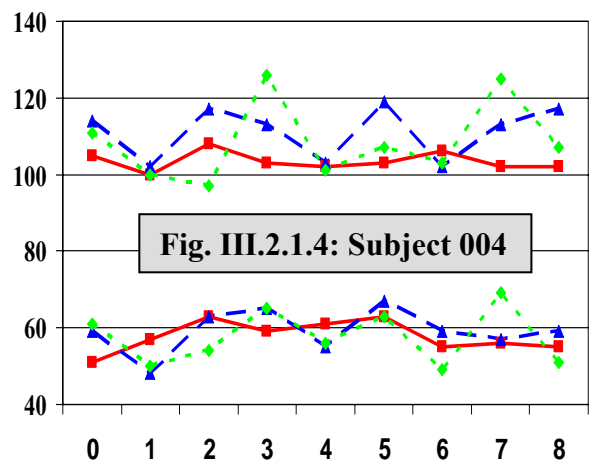
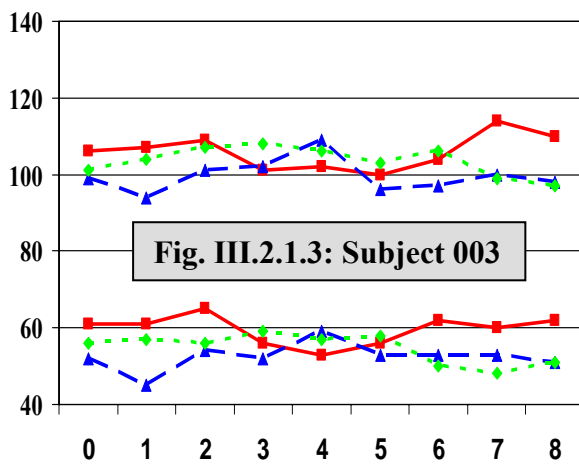
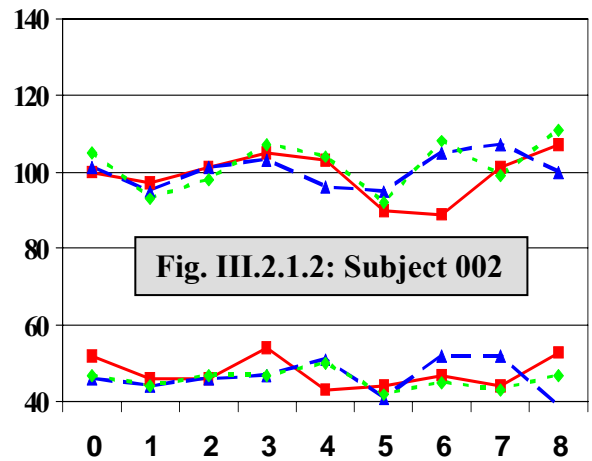
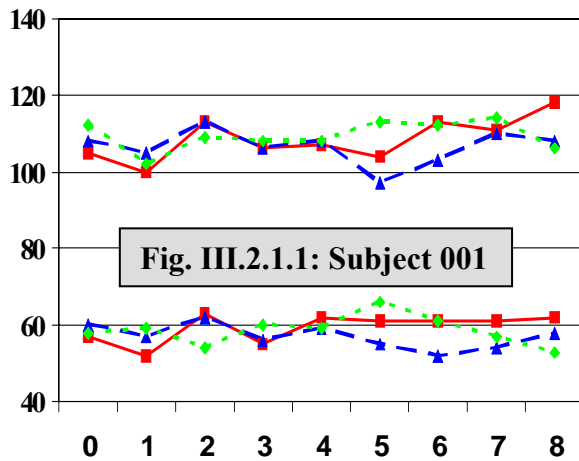


Figure III.1.2.

**APPENDIX III.2: Systolic and diastolic BP (mmHg)**

**APPENDIX III.2.1: Comparisons of the absolute values of systolic and diastolic BP (mm Hg) during observation period (the 2 hour values corresponds to 1 hour post-dose) for the individual subjects administered 0 (placebo), 5 and 10 mg ractopamine**

■ Control   
 ▲ 5 mg   
 ◆ 10 mg



**APPENDIX III.2.2: Effect on systolic and diastolic BP calculated as Emax-(average of the two pre-dose values) for each of the six subjects for the 0 (placebo) and the 5 mg dose**

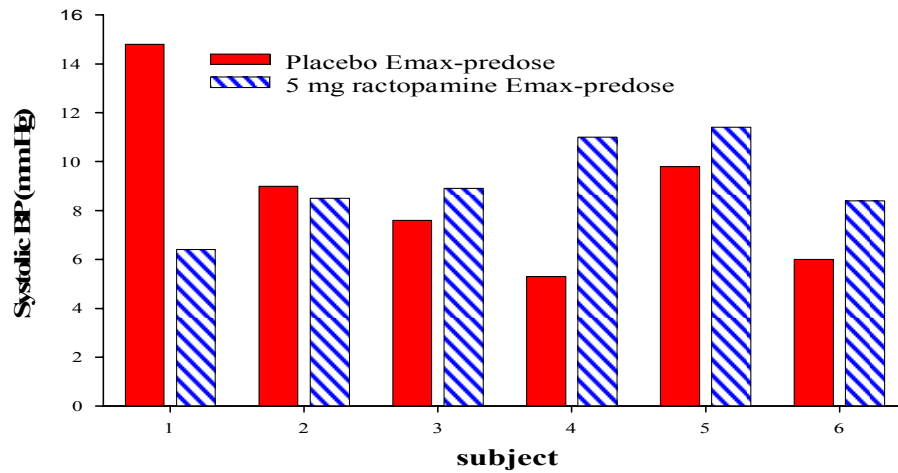


Figure III.2.2.1.

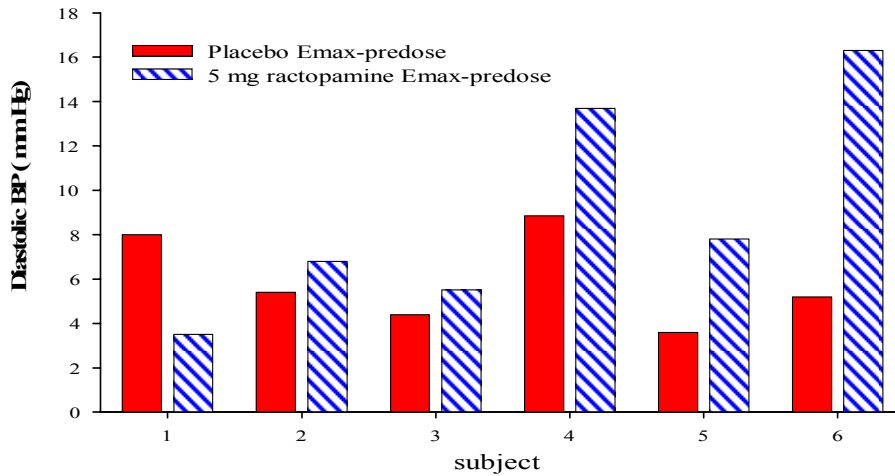
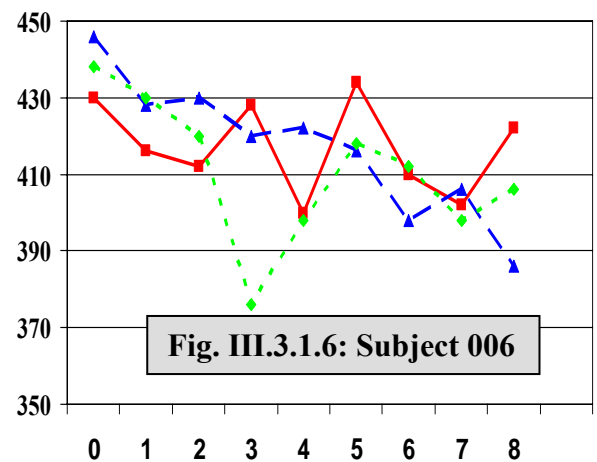
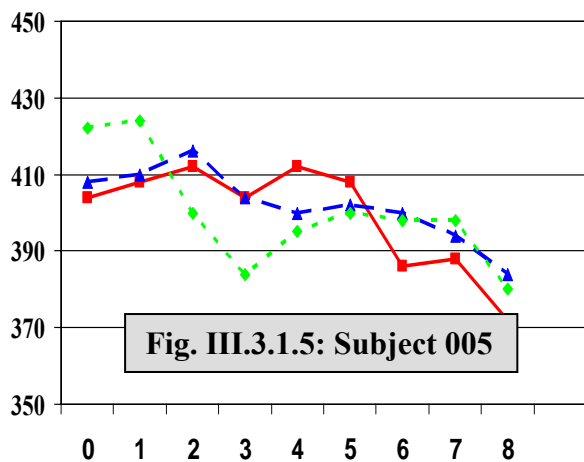
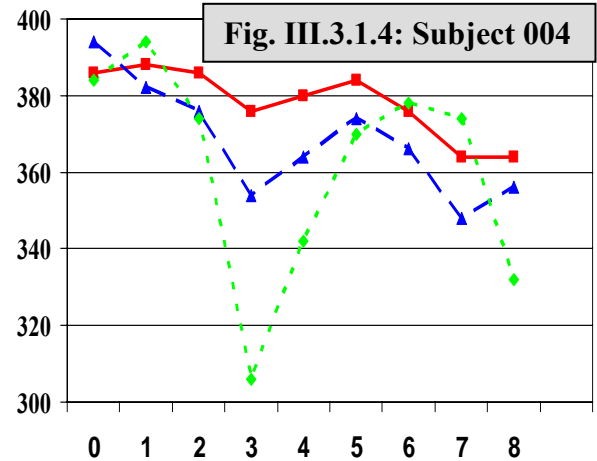
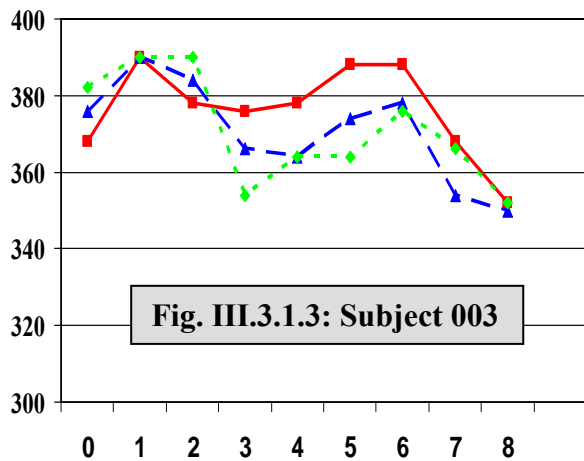
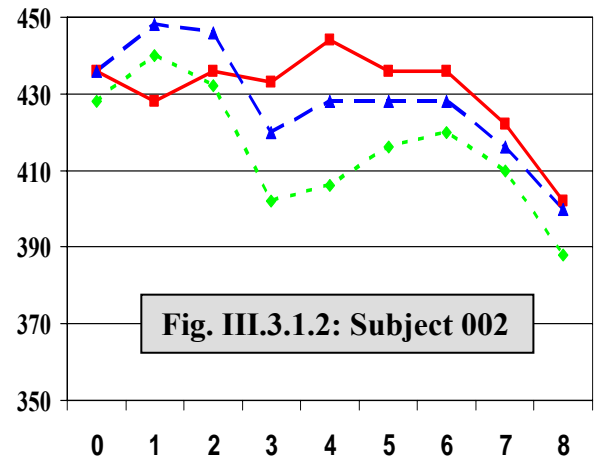
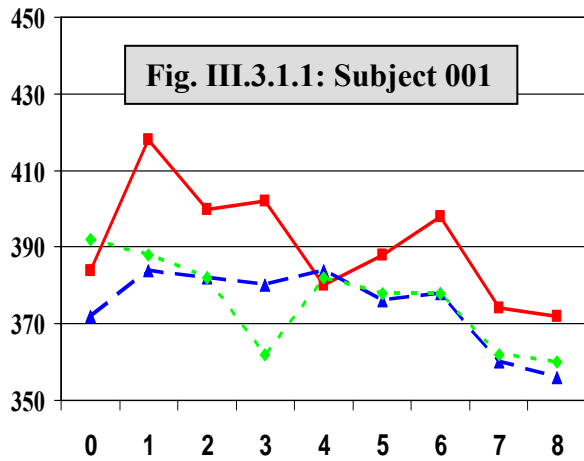


Figure III.2.2.2.

**APPENDIX III.3: Systolic time interval (STI, QS2 in msec)**

**APPENDIX III.3.1: Comparisons of the absolute values of systolic time interval during observation period (the 2 hour values corresponds to 1 hour post-dose) for the individual subjects administered 0 (placebo), 5 and 10 mg ractopamine**



**APPENDIX III.3.2: Effect on systolic time interval (QS2) calculated as Emax-(average of the two pre-dose values) for each of the six subjects for the 0 (placebo) and the 5 mg dose**

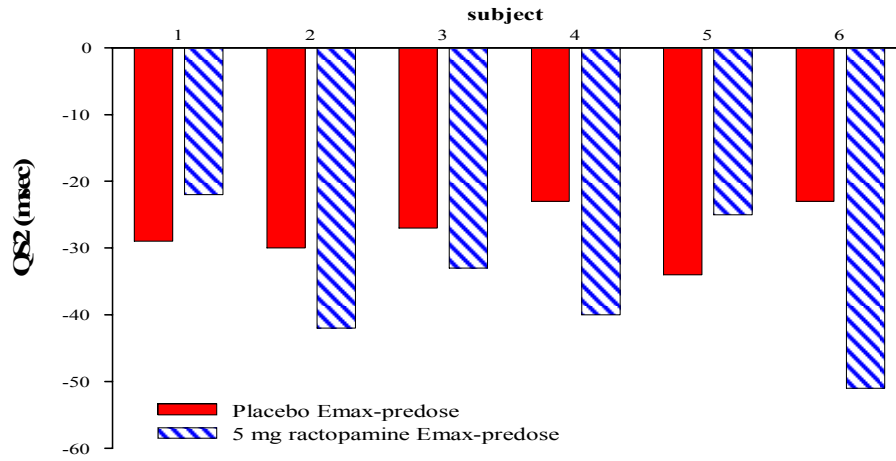


Figure III.3.2.



APPENDIX IV: RESIDUE DATA IN PIGS AND CATTLE

Table IV.1. Data on total residue in pigs

Study	Dosage mg kg <sup>-1</sup> feed	Withdrawal (hours)	Total residues (expressed as mg equivalent ractopamine kg <sup>-1</sup> )			
			Liver	Kidney	Muscle	Fat
Ref. 21B ABC-0283	30	12	0.314	0.435	0.014	0.015
			0.305	0.481	0.014	0.013
Ref. 22B ABC-0291	30	12	0.258	0.382	0.013	0.014
			0.627	0.858	0.030	0.025
			0.375	0.553	0.024	0.024
Ref. 23B ABC-0368	30	12	0.363	0.411		
			0.333	0.443		
			0.420	0.427		
			0.472	0.317		
			0.408	0.423		
			0.463	0.408		
Average ± SD			<b>0.394 ± 0.102</b>	<b>0.467 ± 0.142</b>	<b>0.019 ± 0.008</b>	<b>0.018 ± 0.006</b>
Ref. 20B ABC-021	30	6	0.166	0.829	0.022	0.015
			0.192	0.676	0.039	0.017
Average ± SD (all studies)			<b>0.361 ± 0.123</b>	<b>0.511 ± 0.171</b>	<b>0.022 ± 0.010</b>	<b>0.018 ± 0.005</b>

Ref. 24B T4V739003 T4V739004	20	24	0.106 ± 0.030	0.116 ± 0.14	<0.030	<0.030
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			Ractopamine + conjugates (mg kg <sup>-1</sup> )			
Quiang <i>et al.</i> (2007)	18	0 (12)	0.046	0.169	0.003	0.007

Table IV.2. Marker residues in pigs

Study	Dosage mg kg <sup>-1</sup> feed	Withdrawal (hours)	Free ractopamine (mg kg <sup>-1</sup> )			
			Liver	Kidney	Muscle	Fat
Ref. T4V759003	20	12	0.0198	0.0653	0.0069	0.0038
			0.0053	0.0073	0.0036	0.0005
			0.0026	0.0051	0.0057	0.0017
			0.0145	0.0425	0.0053	0.0005
			0.0212	0.0600		
			0.0030	0.0067		
			0.0038	0.0105		
			0.0188	0.0571		
Ref. 33B T4V629501	20	12	0.0087	0.0134		
			0.0180	0.0397		
			0.0027	0.0042		
			0.0246	0.0270		
			0.0179	0.0345		
			0.0165	0.0139		
Average ± SD			<b>0.013 ± 0.008</b>	<b>0.028 ± 0.022</b>	<b>0.005 ± 0.001</b>	<b>0.002 ± 0.002</b>

Table IV.3. Data on total residues in cattle

Study	Dosage* mg kg <sup>-1</sup> feed DM	Withdrawal (hours)	Total residues (mg ractopamine equivalent kg <sup>-1</sup> )			
			Liver	Kidney	Muscle	Fat
Ref. 06B ABC-0398	45	12	0.682	0.537	<0.030	<0.008
			0.827	0.418	<0.030	<0.008
			0.752	0.538	<0.030	<0.008
Ref. 26B ABC-0375	45	12	0.780	0.508	0.018	0.016
			0.541	0.494	0.023	0.013
			0.555	0.379	0.025	0.009
<b>Average ± SD</b>			<b>0.690 ± 0.119</b>	<b>0.479 ± 0.066</b>	<b>0.026 ± 0.005</b>	<b>0.021 ± 0.010</b>
Ref. 28B T4V739301	40	48	0.264	0.337	<0.025	<0.025
			0.066	0.150	<0.025	<0.025
			0.146	0.223	<0.025	<0.025
			0.149	0.245	<0.025	<0.025
<b>Average ± SD (all studies)</b>			<b>0.156 ± 0.081</b>	<b>0.239 ± 0.077</b>	-	-
Ref. 27B ABC-0408	30	12	0.338	0.212	-	-
			0.141	0.177	-	-
			0.272	0.177	-	-
<b>Average</b>			<b>0.250</b>	<b>0.189</b>	-	-

\* Ractopamine was administered intra-uminally by capsule. Feed concentration was derived by calculation using NRC data.

Table IV.4. Marker residue in cattle

Study	Dosage mg kg <sup>-1</sup> feed	Withdrawal (hours)	Free ractopamine (mg kg <sup>-1</sup> )			
			Liver	Kidney	Muscle	Fat
Ref. 27B ABC-0408	30	12	0.066	0.057	-	-
			0.013	0.033	-	-
			0.030	0.039	-	-
<b>Average</b>			<b>0.037</b>	<b>0.043</b>	-	-
Ref. 28B T4V739301	40	48	0.007	0.014	-	-
			0.002	0.006	-	-
			0.004	0.012	-	-
			0.005	0.008	-	-

## References

Quiang, Z., Shentu, F., Wang, B., Wang, J., Chang, J., Shen, J. 2007. Residue depletion of ractopamine and its metabolites in swine tissues, urine, and serum. *J. Agric. Food Chem.* 55, 4319-4326.

## APPENDIX V: SAFETY OF RACTOPAMINE FOR TARGET ANIMALS

Although not requested in the Terms of Reference, the safety of the target animal(s) has been evaluated on the basis of published literature.

### APPENDIX V.I: PIGS

Marchant-Forde et al. (2003) studied the effects of ractopamine on the behaviour and physiology of pigs during handling and transport. A total of 72 gilts (85.5 kg body weight; six replicates of six pigs each per treatment) were fed for four weeks diets mainly consisting of corn (64.4%) and soybean meal (29.2 %) and containing 0 or 10 mg ractopamine kg<sup>-1</sup>. All pigs were weighed individually on a weekly basis; feed intake was recorded daily. The behaviour of gilts was recorded over a 22-hour period once a week using ceiling mounted cameras. During week 4 of the trial, heart rate responses to unfamiliar human presence were measured in all pigs, and on different days blood samples were taken from a single pig in each pen. Catecholamines and cortisol in plasma were measured. At the end of week 4, all pigs were monitored during transport to processing (2 minutes loading, 18 minutes transportation, 2 minutes unloading). Behaviour and heart rate data were analysed using the repeated option of Proc GLM of SAS. Student's *t*-tests were used to compare hormone plasma concentrations.

During week 1 and 2, ractopamine-fed pigs spent more time active ( $P < 0.05$ ), more time alert ( $P < 0.05$ ) and less time lying in lateral recumbency ( $P < 0.05$ ). They also spent more time at the feeder in week 1 ( $P < 0.05$ ). These differences disappeared in week three and four.

At start of the trial, there were no differences in behavioural responses to handling. However, over the next four weeks, fewer ractopamine-fed pigs exited the home pen voluntarily, they took longer to remove from the home pen, longer to handle into the weighing and needed more pats, slaps and pushes from the handler to enter the scale. There appeared to be no habituation to the handling and weighing routine for all pigs (including those of the control); therefore, it may be that the weighing routine was not carried out frequently enough for habituation to occur.

The authors mentioned that pigs that are more difficult to move and more likely to be subjected to rough handling and increased stress during transportation.

Ractopamine seemed to chronically elevate heart rate compared to control pigs. At the end of week 4, ractopamine-fed pigs had higher heart rates in presence of an unfamiliar human ( $P < 0.05$ ) and during transport ( $P < 0.05$ ), but not during loading and unloading.

At the end of week 4, ractopamine-fed pigs had higher circulating catecholamine concentrations (epinephrine: 253 vs. 102 pg mL<sup>-1</sup>; norepinephrine: 991 vs. 480 pg mL<sup>-1</sup>) than control pigs ( $P < 0.05$ ). Cortisol concentrations were not affected. It is assumed that a down regulation of  $\beta$ -adrenergic receptors occurred, which would in turn result in an increase of the catecholamine production of the sympathetic nervous to compensate for the fewer available receptors.

### Conclusions

Ractopamine at the lower recommended dose (10 mg kg<sup>-1</sup> feed) affects the endocrine homeostasis in pigs as concluded from the higher plasma catecholamine concentrations. The FEEDAP Panel expresses concerns with regard to the safety of the compound to pigs.

### Reference

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## APPENDIX V.II: CATTLE

Baszczak et al. (2006) and Gruber et al. (2007) examined the effects of ractopamine supplementation and biological type on behaviour during routine handling and on growth performance and carcass characteristics, respectively. Equal numbers of British, Continental crossbred and Brahman crossbred calf-fed steers ( $n = 420$ , average bw: 520 kg) were blocked by BW within type and allocated to pens, resulting in two pens (ten cattle per pen) representing each block  $\times$  type subclass. Pens within each block  $\times$  type subclass were then randomly assigned to ractopamine supplementation treatments (0 or 200 mg steer<sup>-1</sup> d<sup>-1</sup>), which were administered during the final 28 days of the finishing period. At the time final BW were obtained (28 days after treatment initiation, approximately 565 kg), a single, trained observer, blinded with respect to treatment designations, recorded subjective scores to characterise the behaviour of each animal. Scores included entry force score (degree of force required to load the animal into the chute; 1 = entered chute voluntarily or after encouragement without physical contact, 2 = entered chute with limited physical encouragement, 3 = required a single impulse from an electric prod to move into chute, 4 = required more than 1 electrical impulse to move into chute.); entry speed score (walk, trot, run; 1 = walk, 2 = trot, 3 = run or gallop.); chute behaviour score (calm, restless shifting, moderate struggling; 1 = calm behaviour, 2 = restless shifting, 3 = moderate struggling); and exit speed score (walk, trot, run; 1 = walk, 2 = trot, 3 = run or gallop). Ractopamine supplementation had no effect on entry force score (2.4 for both groups), chute behaviour score (1.2 vs. 1.1 for control) or exit speed score (1.8 vs. 1.9 for control); however, cattle supplemented with ractopamine entered the chute more rapidly than did control cattle (score 1.5 vs. 1.4 for control,  $P < 0.05$ ). Biological cattle type was a significant source of variation in entry force score and exit speed score, but did not affect scores for entry speed or behaviour during restraint in the chute. No adverse effects of ractopamine supplementation on cattle behaviour were observed in this study.

## Conclusions

Ractopamine (200 mg head<sup>-1</sup>, corresponding to approximately 20 mg kg<sup>-1</sup> DM) did not affect the behaviour of feedlot cattle. In contrast to the pig study, heart rate or circulating catecholamines were not measured.

## References

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#### APPENDIX VI: MICROBIOLOGICAL PROPERTIES OF RACTOPAMINE

The antimicrobial activity of ractopamine hydrochloride was assessed and the minimum inhibitory activity (MIC) against a list of bacterial strains was calculated. The test was performed by using twofold dilution procedures (from 0.008 to 256 mg L<sup>-1</sup> of ractopamine hydrochloride) in agar media. The full details of the experiment, based on in-house method (Lewis, 1985), were not accessible.

The MIC of ractopamine was determined for 55 strains belonging to 37 different species of commensal and human pathogenic bacteria. Information on the origin and the culture collection deposition number of bacterial strains used at this purpose was not provided in the study (Lewis, 1985). The calculated MICs were higher than 128 mg L<sup>-1</sup> for the tested aerobes and 256 mg L<sup>-1</sup> for all anaerobes, with the exception of *Bacteroides vulgatus* for which the MIC was 128 mg L<sup>-1</sup>.

These data indicate that ractopamine hydrochloride has no detectable antimicrobial activity against the tested bacteria at maximum used feed level.<sup>64</sup>

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<sup>64</sup> Original reports/Reference 82A

**APPENDIX VII: MEAT QUALITY ASPECTS**

According to the European feed legislation (Regulation (EC) No. 1831/2003, Article 5.2.c), a feed additive shall not harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products. An increase in lean carcass (muscle and protein accretion) in livestock animals is one of the effects claimed for ractopamine. The FEEDAP Panel examined therefore the potential influence of ractopamine on pork and beef quality based on published literature.

**Meat quality: Definitions**

Meat quality has commonly three different aspects: organoleptic, nutritional and technological.

The organoleptic qualities are defined by colour, flavour, tenderness and juiciness of meat:

- Tenderness is often considered as one of the major attributes of importance. It is influenced by the collagen content and its cross-linking state, and by the level of post-mortem proteolysis (also called meat maturation).
- Colour determines the purchase decision by the consumer. It is essentially influenced by the content and the chemical state of the myoglobin, the pigment of meat, but it is also affected, to a lesser extent, by the lipid content and the chemical evolution of the post-mortem muscle.
- Flavour essentially appears during the meat cooking process due to Maillard reactions and thermally induced lipid oxidation. Intramuscular fat (IMF) improves meat flavour.
- Juiciness is linked to the water holding capacity of meat before and after cooking, and brings the flavour components in contact with the taste buds.

The technological qualities are considered as the ability of meat to be processed. It includes the ability of meat to be stored and cooked, limiting the drip and cooking losses (water holding capacity).

The nutritional quality of meat is often associated to lipid and amino acid content and composition, and to the ability of the muscle to keep micronutrients and preserve a good protein digestibility during processing. Then, here again, the water holding capacity (WHC) is of importance because it affects the loss of hydrosoluble compounds and micronutrients during drip and cooking losses.

Those criteria are quantified by trained sensory panels of consumers and/or by physical measurements. In this last case, colour is generally measured by the CIELAB L\* a\* b\* system using a Minolta chromameter, tenderness by Warner Bratzler Shear Force (WBSF) measurements using an Instron Testing machine, flavour can be measured by electronic nose and water holding capacity by measurement of processing yields or physical measurement based on compression of meat and estimation of juice.

**Ractopamine and meat quality**

Ractopamine improves average daily gain, feed efficiency, carcass leanness and increases carcass weight in pigs (Uttaro et al., 1993, Dunshea et al., 1993, Smith et al., 1995, Rinker et al., 2005, See et al., 2005, Carr et al., 2005a, Weber et al. 2006, Carr et al. 2009) and cattle (Schroeder et al. 2003a, Avendano-reyes et al., 2006, Walker et al. 2006, Winterholler et al. 2007). Carcass meat lean yield is improved by increasing the percentage and diameter of white (Type IIB) fibres in pigs (Aalhus et al. 1992, Depreux et al., 2002) and in cattle by increasing the percentage of IIA fibres (Gonzalez et al. 2008) and the IIA and IIB fibre areas (Strydom et al. 2009).

Dunshea et al. (2005) reviewed data from 19 studies with  $\beta$ -agonists. The authors performed a meta-analysis for the effects of the  $\beta$ -agonist on meat quality of pork taking into account dose, muscle, sex and breed of the animals. Ten out of nineteen studies were conducted with ractopamine. The overall data indicates that ractopamine had no effect on intramuscular fat content and drip loss but increased shear force by approximately 0.5 kg (4.72 vs. 4.23 kg) and decreased redness by 12.5 % ( $a^*$  7.4 vs 8.5).

More recent data on pork quality as influenced by ractopamine are shown in Table VII.1.

Table VII.1. **Recent studies on the effect of ractopamine on meat quality in pigs. Data are expressed as percentage change from the respective control values**

Reference	RAC (mg kg <sup>-1</sup> feed)	IMF <sup>8)</sup>	Consumer panel score			Shear force (kg)	pH <sub>24</sub>	Drip loss (%)	Colour (CIE-scale)		
			Flav.	Juic.	Tend.				L*	a*	b*
Armstrong et al. (2004) <sup>1)</sup> (27 days treatment)	5								-1.9	-10.8	-5.1
	10								-2.3	-1.2	-1.3
	20								2.5	-14.5	-5.1
Carr et al. (2005 a) <sup>2)</sup>	10		-0.7	-5.0	-12.7	14.1	-1.3	-15.6	-1.3	-13.8	-24.5
	20		-2.0	-2.3	-12.1	17.5	-1.3	-20.2	0.4	-18.2	-27.6
Bridi et al. (2006) <sup>3)</sup>	10					9.8	1.4	-11.9	0.8	-13.7	-0.5
Xiong et al. (2006) <sup>4)</sup>	20					20.0	0.0				
Weber et al. (2006) <sup>5)</sup>	10	-21.3					0.0	-2.8			
Stahl et al. (2006) <sup>6)</sup>	5	-10.7				10.9	0.5		2.3	-11.3	2.6
Fernandez-Duenas et al. (2008) <sup>7)</sup>	5	-5.3	0.2	-4.9	-3.3	10.6	-0.2		-0.9	-4.2	-14.8
	7.4	-4.8	-0.7	1.4	-2.3	1.8	0.5		-1.4	-6.0	-19.1

<sup>1)</sup> Sex: mixed, breed: Dekalb EB, Tissue: Musculus longissimus dorsi

<sup>2)</sup> Sex: barrow, breed: Dekalb EB, Tissue: Musculus longissimus dorsi

<sup>3)</sup> Sex: mixed, breed: PIC NN and Nn, Tissue: Musculus longissimus dorsi

<sup>4)</sup> Sex: mixed, breed: crossbreed, Tissue: Musculus longissimus dorsi

<sup>5)</sup> Breed: crossbreed

<sup>6)</sup> Sex: barrow, breed: crossbreed, Tissue: Musculus longissimus dorsi

<sup>7)</sup> Sex: mixed, breed: crossbreed, Tissue: Musculus longissimus dorsi

<sup>8)</sup> IMF: Intramuscular fat; Flav.: flavour; Juic.: juiciness; Tend.: tenderness

The data confirmed the above conclusions that ractopamine increases shear force and reduces redness. The data may also indicate a tendency for yellowness to decrease. The results on IMF and drip loss are contradictory to the results of the meta-analysis.

The database for ractopamine effects on beef is rather limited (see Table VII.2: on five studies, none provides a full dataset of meat quality). The only consistent effect of ractopamine can be described as an increased shear force. In contrast to pork, colour appears not to be affected by ractopamine.

Table VII.2. Studies on the effect of ractopamine on meat quality in beef. Data are expressed as percentage change from the respective control values

Reference	RAC mg/head /day	IMF <sup>6)</sup>	Consumer panel score			Shear force (kg)	pH <sub>24</sub>	Drip loss (%)	Colour		
			Flav.	Juic.	Tend.				L*	a*	b*
Schroeder et al. (2003) <sup>1)</sup>	100	-0.6	-1.4	-0.1	-1.3	-1.3	-0.4				
	200	0.4	0.2	1.3	-1.5	2.6	0.0				
	300	-0.8	-1.8	-1.2	-6.5	11.5	0.0				
Avendano-Reyes et al. (2006) <sup>2)</sup>	300					9.9	0	43.2	3.2	-1.5	4.6
Quinn et al. (2008) <sup>3)</sup>	200					2.2		3.1	-2.1	-3.2	-1.6
Gruber et al. (2008) <sup>4)</sup>	200		-3.7	-8.2	-9.6	9.0					
Strydom et al. (2009) <sup>5)</sup>	400					2.5	0.3	8.7			
						8.7	0.3				

<sup>1)</sup> Sex: Steer, breed: mixed, Tissue: Musculus longissimus dorsi

<sup>2)</sup> Sex: Steer, breed: crossbreed, Tissue: Musculus longissimus dorsi

<sup>3)</sup> Sex: heifer, breed: crossbreed, Tissue: Musculus longissimus dorsi

<sup>4)</sup> Sex: Steer, breed: British continental crossbreed and Brahm crossbreed, Tissue: Musculus longissimus dorsi

<sup>5)</sup> Sex: Steer, breed: Bonsmara, Tissue: Musculus longissimus dorsi (first raw of data), Musculus semitendinosus (second raw of data).

<sup>6)</sup> IMF: Intramuscular fat; Flav.: flavour; Juic.: juiciness; Tend.: tenderness

## Ractopamine and meat tenderness

### Pork

Feeding pigs with ractopamine resulted in tougher meat independently of the ractopamine dose. Results in WBSF of 13 studies are reported in Table VII.3. Eight experiments showed significant increase in WBSF from pigs fed ractopamine compared to controls. A 5 mg ractopamine kg<sup>-1</sup> feed is sufficient to increase shear force by about 10 % as shown by the results of Stahl et al. (2006) and Fernandez-Duenas et al. (2008).

Table VII.3. Effect of ractopamine on shear force value in pork meat

Reference	Muscle	sex	Ractopamine (mg kg <sup>-1</sup> feed)						
			0	5	7.4	10	12.5	15	20
Aalhus et al. (1990)	LD	Mixed	5.56 <sup>a</sup>			6.32 <sup>b</sup>		6.46 <sup>b</sup>	6.41 <sup>b</sup>
Aalhus et al. (1992)	LD	Mixed	3.14 <sup>a</sup>			3.38 <sup>b</sup>		3.28 <sup>ab</sup>	3.35 <sup>b</sup>
Dunshen et al. (1993)	LD	Boar	4.18						4.91
		Barrow	3.39						4.0
		Gilt	4.81						4.17
Uttaro et al (1993)	LD	Mixed	4.23 <sup>a</sup>						4.72 <sup>b</sup>
	SM		3.88						3.79
Stites et al.(1994)	LD	Mixed	2.84			3.15		3.76	2.78
	SM		1.20			1.3		1.29	1.32
Smith et al. (1995)	LD	Boar	4.89	5.39			4.85		5.41
		Gilt	5.53	5.67			5.21		5.39
Stoller et al. (2003)	LD	Mixed	5.56			5.79			
Carr et al. (2005 a)	LD	Barrow	3.76 <sup>a</sup>			4.29 <sup>b</sup>			4.42 <sup>b</sup>
Carr et al. (2005 b)	LD	Mixed	3.51 <sup>a</sup>			3.92 <sup>b</sup>			
Bridi et al. (2006)	LD	Mixed	8.41			9.45			
Xiong et al. (2006)	LD	Mixed	3.06 <sup>a</sup>			3.68 <sup>b</sup>			
Stahl et al. (2006)	LD	Barrow	3.66 <sup>a</sup>	4.06 <sup>b</sup>					
Fernandez-Duenas et al.(2008)	LD	Mixed	2.74 <sup>a</sup>	3.03 <sup>b</sup>	2.79 <sup>b</sup>				

<sup>ab</sup> Different superscript within a raw indicate significant difference (p < 0.05)

## Beef



Feeding steers with 300 and 200 mg ractopamine head<sup>-1</sup> day<sup>-1</sup> increased shear force compared to controls significantly (Schroeder et al. 2003b, 300 mg: 3.95 versus 3.54 kg,  $p < 0.05$ ; Avendano-Reyes et al. 2006, 300 mg: 4.83 versus 4.39 kg,  $p < 0.05$ ; Gruber et al., 2008, 200 mg: 4.60 versus 4.22 kg,  $p < 0.01$ ). The findings of Schroeder et al., 2003b and Gruber et al., 2008 on WBSF are confirmed by the sensory panel, tenderness score decreased ( $p < 0.05$  and  $p < 0.01$ , respectively).

Strydom et al. (2009) fed steers with 400 mg ractopamine head<sup>-1</sup> day<sup>-1</sup> and observed a significant increase in WBSF only for the musculus semitendinosus (5.0 versus 4.6 kg,  $p < 0.05$ ) but not for the musculus longissimus dorsi.

Quinn et al. (2008) found no significant difference in WBSF (heifers fed 200 mg ractopamine head<sup>-1</sup> day<sup>-1</sup>).

### Comments

Aalhus et al. (1992) explained part of the increase in meat toughness by the increase in white fibres percentage which exhibit larger diameters and is associated with decreased tenderness, independent of connective tissue strength or age (Swatland, 1984).

Moreover, the calpain/calpastatin proteolytic system which is mostly involved in meat maturation (Koochmaraie and Geesink, 2006) is inhibited by ractopamine (Xiong *et al.*, 2006, Strydom et al. 2009). This phenomenon contributes to a decrease of the post-mortem proteolysis rate and hence to meat tenderness (Xiong et al. 2006). This effect disappeared in pig meat after ten days post-mortem in the study of Xiong et al. (2006) while the increase in shear force of about 0.5 kg in steers remained constant even after 21 days post-mortem (Gruber et al. 2008).

The consumer thresholds for meat tenderness reported by Miller et al. (2001) indicate that meat is considered as tender when WBSF is ranging from 1.62 to 2.29 kg; WBSF of 3.92 to 4.50 characterises intermediate tenderness and 5.42 to 7.42 kg toughness. The authors concluded that the transition consumer perception from tender to tough beef occurred between 4.3 and 4.9 kg (acceptability for tenderness decreased from 86 % at 4.3 kg for a 'slightly tender' rating to 59 % at 4.9 kg for a 'slightly tough' rating).

### Ractopamine and meat colour

The decrease in redness of meat from ractopamine-fed pigs (see Dunshea et al., 2005 and Table 1) could be due to the increased percentage and size of fibres IIB which contain less oxymyoglobin, the red pigment of muscle (Aalhus et al. 1990, Uttaro et al. 1993, Carr et al. 2005a). Although the effects of ractopamine were mostly statistically significant, the absolute differences of about 1 point in the  $a^*$  value may be considered as of questionable commercial significance. However, not yet published data indicates that in red meat a difference of less than 1 point in  $a^*$  (from 15.33 to 15.95) is realised by the consumer (P. Gatellier, personal communication), suggesting a possible commercial depreciation of meat coming from ractopamine fed animals. The absence of a comparable effect in beef could be linked to the increase in proportion and size of IIA fibres, which show a more intensive red colour than IIB fibres (Gonzalez et al. 2008, Strydom et al. 2009).

### Ractopamine and Water Holding Capacity (WHC)

#### Pork

There is no clear evidence of the effect of ractopamine on WHC. The meta-analysis of Dunshea et al. (2005) did not indicate any effect. Other findings (Table 4) are controversial.

Apple (2007) used the data in drip loss of ten experiments to perform a meta-analysis (Table VII.4). He concluded that feeding ractopamine, regardless of dietary inclusion level does not impact ( $p < 0.603$ ) measures of pork water holding capacity.

Table VII.4. Effect of ractopamine hydrochloride on drip loss percentages of fresh pork (from Apple, 2007)

References	Ractopamine hydrochloride (mg kg <sup>-1</sup> feed)		
	0	10	20
Aalhus et al. (1990)	3.76	3.84	3.87
Dunshea et al. (1993a)	6.59	-	7.43
Dunshea et al. (1993b)	5.83	-	5.92
Uttaro et al. (1993)	6.45	-	4.31
Stoller et al. (2003)	2.47	2.31	-
Apple et al. (2004)	2.89	2.44	-
Carr et al. (2005a)	4.79 <sup>a</sup>	4.04 <sup>ab</sup>	3.82 <sup>b</sup>
Carr et al. (2005b)	4.11	4.53	-
Rinker et al. (2005)	2.6	2.4	-
Weber et al. (2006)	2.84	2.76	-
<b>Meta-analysis (p=0.603)</b>	<b>4.39</b>	<b>4.12</b>	<b>4.11</b>
Standard Error	± 0.554	± 0.581	± 0.596

<sup>ab</sup> Different superscript within a row indicate significant difference ( $p < 0.05$ )

A small decrease in cooking loss was observed in pork from pigs fed 20 mg ractopamine kg<sup>-1</sup> (Uttaro et al. 1993 : 25.7 versus 24.3,  $p < 0.05$ ; Smith et al. 1995 : 28.3 versus 24.4, linear effect in females only,  $p < 0.05$ ) while Carr et al. (2005b) and Stahl et al. (2007) did not find significant differences in pork fed 10 and 5 mg ractopamine kg<sup>-1</sup>, respectively.

## Beef

Avendano-Reyes et al. (2006) found an increase of 43 % of drip loss in steers fed 300 mg ractopamine head<sup>-1</sup> day<sup>-1</sup> compared to controls (5.93 % versus 4.14 %,  $p < 0.001$ ). However, a physical measurement of WHC did not confirm those differences. The slight increases in drip loss found by Quinn et al. (2008) and Strydom et al. (2009) were not statistically significant.

Whatever the ractopamine dose tested (100–300 mg ractopamine head<sup>-1</sup> day<sup>-1</sup>), cooking loss was not affected (Schroeder et al. 2003, Quinn et al. 2008).

## Conclusions

The use of ractopamine in feeding affects pork and beef tenderness and pork redness.

Pork and beef from ractopamine-treated animals are less tender, as shown by increased WBSF values. This may be due to an increase in fibre size or more likely to inhibition of proteolytic enzymes involved in meat maturation.

The redness of pork is reduced by ractopamine probably because of an increase of the less oxymyoglobin containing type IIB fibres.

In the majority of the findings, other parameters of meat quality (flavour, juiciness, intramuscular fat and water holding capacity, lightness) are not influenced. However, the database is limited, particularly for beef, and some results are contradictory.

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