CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - Fax: (+39) 06 5705 4593 - E-mail: codex@fao.org - www.codexalimentarius.net

Agenda Item 4 **CAC/33 CRD/02**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX ALIMENTARIUS COMMISSION

Thirty-third Session

Geneva, Switzerland, 5 - 9 July 2010

EXCERPT FROM FAO JECFA MONOGRAPHS 9 – PREPUBLICATION VERSION

ADDENDUM TO THE RESIDUE MONOGRAPH FOR RACTOPAMINE HYDROCHLORIDE

Background

The summary and appraisal with the conclusion and recommendations of the JECFA evaluation of the residue depletion studies on ractopamine in pig tissues performed by the People's Republic of China, are presented in the Annex to this document. The Annex is an excerpt from FAO JECFA Monographs 9 – prepublication version Addendum to the residue monograph for Ractopamine hydrochloride.

The evaluation is an addendum to the monographs prepared by the 40th, 62nd and 66th meetings of JECFA and published in FAO Food & Nutrition Paper 41/5, 41/16 and FAO JECFA Monographs 2, respectively. The prepublication of the FAP JECFA Monographs 9 is available on-line at the FAO JECFA website at: http://www.fao.org/ag/agn/agns/jecfa/JECFAMonographs9.pdf.

Annex

EXCERPT FROM FAO JECFA MONOGRAPHS 9 – PREPUBLICATION VERSION ADDENDUM TO THE RESIDUE MONOGRAPH FOR RACTOPAMINE HYDROCHLORIDE

Summary and appraisal

Background

The monograph addendum to the residue monograph on ractopamine hydrochloride in this volume of the FAO JECFA Monographs on the residues of, exposure to and statements on the studies on ractopamine residues in pig tissues submitted were prepared by the invited experts for this electronic meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during the period of January to May 2010. The tasks for the Committee was to evaluate three residue depletion studies on ractopamine in pig tissues performed by the People's Republic of China, consider any other relevant studies previously assessed in this context by the Committee, provide recommendations on whether the information contained in the three new studies would have an impact on the MRLs recommended for ractopamine and consider any other scientific issues arising from the evaluation of the studies. The additional study received in May 2010 was considered separately due to its late submission.

This request originated from the 32nd Session of the CAC, which had asked FAO and WHO to review the three residue studies in pigs using ractopamine hydrochloride medicated feed conducted by the Government of the People's Republic of China. These studies on ractopamine residue depletion used three different breeds in of pigs and were carried out at three different national laboratories, located in Wuhan, Guangzhou and Beijing. The Delegation of People's Republic of China had at the 32nd CAC expressed concern over the ractopamine residue levels in lung, stomach, heart, large and small intestine as well as in the tissues for which MRLs were proposed (muscle, liver, kidney and fat), particularly at early time points after withdrawal of the medicated feed.

Description of the three new residue studies in the pig

The Wuhan study used 40 Hubei White Swine. All pigs received a dose of approximately 20 mg ractopamine hydrochloride per kg medicated animal feed daily for 30 days. Average feed consumption was 2.0 kg/animal/day. Animals were slaughtered at 6, 12 hours, and 1, 2, 3, 5, 7 and 9 days following withdrawal of medicated feed. Muscle, liver, kidney, heart, lung, stomach, large intestine and small intestine were collected and analyzed. Ractopamine residue concentrations in liver, kidney, lung, and small intestine were greater than in the other tissues. Residue concentrations in the lung were greater than those in the liver and kidney, and were detected up to nine days following removal of medicated feed.

The Beijing study used Landrace x Large Yorkshire binary cross pigs. Animals received ractopamine hydrochloride in medicated feed at a dose rate of approximately 20 mg per kg daily for 30 days. Average feed consumption was 2.18 kg/animal/day. Samples of muscle, liver, kidney, heart, lung, stomach, large intestine, and small intestine were collected from all treated and control animals at 12 hours, 1, 2, 3, 5, 7 and 11 days. Ractopamine residue concentrations were above the limit of quantification (0.5 $\mu g.kg^{-1}$) in all the tissues collected 11 days after the treatment with the exception of muscle.

The Guangzhou study used 30 Spotted Small-ear pigs. All pigs received a daily dose at a rate of approximately 20 mg ractopamine hydrochloride per kg medicated animal feed for 30 days. The average feed consumption was 1.63 kg/animal/day. Animals were slaughtered 6, 12 hours, and 1, 2, 3 and 5 days after treatment. Samples of muscle, liver, kidney, heart, lung, stomach, large intestine and small intestine, were collected from all treated and control animals. The distribution of ractopamine demonstrated tissue selectivity in the pig, with the highest residue concentrations at 12 h in kidney, with the lung containing the second highest concentrations, followed by stomach, liver, small intestine, large intestine and muscle. Ractopamine residues in the lung depleted slowly.

Additional residue study submitted in May 2010

The additional study used 25 Duroc × large White × Landrace pigs. All pigs received a daily dose at a rate of approximately 20 mg/kg ractopamine hydrochloride daily for thirty days. The average feed consumption was 2.61 kg/animal/day, higher than in the other three studies. Animals were slaughtered 6, 24,

48 and 72 hour after treatment. The tissues sampled were muscle, liver, kidney, fat, lung, heart, stomach, large and small intestine. Analysis was performed with and without enzymatic digestion of the tissue samples prior to extraction and purification The concentration of residues determined using the enzymatic step are all greater than those determined without the enzymatic step except for lung tissue where the ratio was approximately 1. i.e. the residues in lung tissue consisted of mostly free ractopamine. For stomach, large intestine and small intestine tissues, the ratios were 1.09 to 1.20. Large coefficients of variation (CV) for the intra-laboratory and inter-laboratory results shows concern with the enzymatic step regarding reproducibility for fat, kidney and liver tissues, in agreement observations made for the residue data for these tissues in the other three studies. The levels of residues in all tissue samples analyzed using the enzymatic hydrolysis step were of the same order of magnitude as in the other studies. No direct comparison to the dietary exposure assessments was performed using the three other studies, as no sampling was performed at a 12 hour withdrawal time in this new study.

Comparison of new data with previously evaluated data

A detailed analysis was undertaken of the three new studies in comparison with previously assessed studies and used to recommend MRLs. There were differences in study protocols and methodology for determination of ractopamine residues as well as in the determination of ractopamine residues in organ tissues other than liver and kidney. A comprehensive analysis was conducted to estimate the relationship of ractopamine residues as analyzed in the new studies using the enzymatic hydrolysis step with the ractopamine residues as determined in the studies in the previous evaluation by the Committee. The previous evaluation employed the relationship of marker residue to total residues determined without enzyme hydrolysis. Information on the ractopamine metabolites (A, B, C and D representing known ractopamine conjugates) from the previously reviewed ractopamine studies was used for this purpose.

In this analysis, the ratio between kidney and liver free ractopamine residues to total residues was 0.318 and 0.153, respectively, as estimated from data in the studies previously evaluated the by Committee. The most comparable values of marker to total residues for the data from the three new studies was estimated to 0.76 and 0.565, respectively, based on data from a 12 h withdrawal time point. These estimated values were derived with the aid of residue metabolism data from the previous evaluations. The 12 h time point was used as only the results of similarly designed studies can be compared with some confidence to obtain reliable results. This excludes *a priori* a comparison of the studies of the original dossier with the new studies at any withdrawal time other than at 12 hours. These ratios were applied in the estimation of dietary exposure.

A detailed analysis of kinetic data on residue depletion studies was carried out. The 66th meeting of the Committee concluded that it could pool the data from the studies submitted by the sponsor at the 62nd and 66th meeting of the Committee. Semi-logarithmic scale graphs of the depletion of the marker residue in liver and kidney are given in figures 7 and 8. From this analysis, a linear regression line and the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit") was derived. This limit is the value normally chosen for the MRLs recommended by the Committee. These data were compared with the results of the three new studies. The analysis shows that there were at times large differences in the results of the three studies, particularly with regard to maximum concentrations reached at short withdrawal times, slope of depletion, variability of the data obtained for the individual animals, and number of depletion phases. While comparable residue depletion graphs could be developed, the variability, measured as coefficients of variation (CV) on duplicate analysis, showed notable differences between the three new studies. The CVs on duplicate analysis were lowest for the Beijing study and highest for the Wuhan study.

The logarithmically transformed concentration values from the three new studies were used for linear regression analysis. This model gave an acceptable fit of the linear model to most data sets except the data describing the kinetics in lung tissue. The parameters of the linear regression were used to estimate the "95/95-tolerance limits". The ratio between the tolerance limit and the median residue concentrations was used as an indicator of the variability of the results obtained for the groups of animals used in the studies.

The variability of the results of the Guangzhou study was greatest. Factors that may partly explain the variability include the initial body weights, which were significantly greater in this study compared to the other two studies (however, the end-of-treatment body weights of the Guangzhou study were not given and feed intake information for one treatment group was not provided); variability of body weight gain was also greatest in this study. The feed/body weight gain ratio was the lowest in the Guangzhou study. This result

would correspond to the fact that typically the residue concentrations found in the Guangzhou study were the lowest of the three studies.

An attempt was made to re-calculate selected results of the three new studies in equivalents of marker residue from the 62nd and 66th Committee evaluations (see table 4). The Committee recognized that there are unknown and possibly significant inherent uncertainties with this approach. However, such calculations assist in highlighting some of the major differences between the three new studies and the original studies evaluated by the Committee. The calculations were only possible for liver and kidney due to the absence of comparable data for the other tissues. The analysis suggested that the residue concentrations in liver, expressed as the marker residue, as defined by the Committee at its 66th meeting, were similar in all studies for median and the "95/95 tolerance limits". Contrary to this, residue concentrations in kidney were much higher in the three new studies. Considerable variability was also found in these data, as indicated by the distance between median and tolerance limits.

Dietary exposure estimates

For the purpose of conducting the dietary intake assessments, the Wuhan study was the only study providing kinetic residue data for all tissues of the model diet employed by the Committee. In addition, the residue concentrations found in muscle, liver and kidney were the highest of the three new studies. Therefore, using the data of the Wuhan study would result in the highest intake estimates. The predicted concentrations after 12 h withdrawal time were used, because only this time point provides comparable data for the ratio of (parent + conjugates)/total residue known from the studies of the original dossier. Sufficient information is available to interpolate any concentration data or marker/total ratios for this time point. Thus, 12 hours withdrawal time represents the only time point for which all data sets could be compared.

The comprehensive analysis of all the data suggests that the total residue concentrations in muscle and fat are of the same approximate magnitude as the concentrations of the parent compound and of the marker residue as measured in the Wuhan study. Therefore, at short withdrawal times the only relevant residue in muscle and fat is parent ractopamine. The proposed factor for the ratio of marker to total residue is therefore set to 1. For skin almost no data are available to estimate the ratio of marker residue to total residue. However, the contribution of residues in skin to the total intake is very low. Therefore, the choice of which factor to use is, within reason, not significant. For this estimate, the Committee used a factor of 1.

For liver and kidney, the relevant data base for ractopamine residue metabolite information is the study ABC-0369 evaluated at the 62nd and 66th meeting of the Committee. The Committee assumed that only metabolites A,B,C, and D (ractopamine conjugates) would be enzymatically hydrolysed to yield ractopamine and that all the other remaining endogenous residues are of equal toxicological concern. Using this conservative approach, the ratio of the equivalent marker residue to total residue from the new studies would be 0.565 for liver and 0.760 for kidney (see table 4), corresponding to conversion factors of 1.770 for liver and 1.316 for kidney.

Using the above factors, the estimated daily intake (using median residue concentrations) was calculated on the basis of the new study that showed the highest marker residue concentrations, i.e. the Wuhan study, and the most conservative conversion factors derived from the studies of the original dossier. Using these conversion factors, the estimated daily intake using muscle, liver, kidney and fat is 30.8 µg; using muscle, liver, kidney and skin, the value is 31.2 µg. Both values are well below the upper bound of the ADI (60 µg per day).

A modeling simulation was conducted to estimate the robustness of the calculations using the model diet and a diet with increased consumption of liver and kidney. Model intakes for 80 years lifespan (i.e. 29,220 days in 80 years), assuming daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat, were simulated. For each tissue log-normally distributed random values were generated for a 12 h withdrawal time and numerically ranging from the value predicted by the regression line of the tissue concentrations *plus or minus four times* the residual variance to the same predicted residual variance. Using the data of the Wuhan study and the normal model diet, typically 1.2 to 1.8% of the results would exceed the ADI with the highest results ranging around 1.5 times the upper bound of the ADI. If the 100 g of liver and 50 g of kidney of the model diet were replaced by 250 g liver, based on consumption data provided in the 2002 Chinese Survey on Nutrition, Diet and Health Status provided by the Chinese Centre for Disease Control, the distribution was slightly shifted to higher intake values with 8.3 to 8.8% above the upper limit of

the ADI. If the 100 g of liver and 50 g of kidney were replaced by 200 g of kidney, the distribution was further shifted to higher intake values. 50.6 to 51.7% would exceed the upper bound of the ADI.

The Committee recognizes consumption of lung tissue to be a specific issue that has not been addressed in other residue evaluations. There is no international consensus value to estimate an appropriate consumption of lung tissue. In addition, there are no data to derive conversion factors for marker to total residue concentrations for ractopamine in lung tissue. It was noted that in the overall assessment of the three new studies, there is significant variability for residues in lung tissue (the Guangzhou study exhibits the greatest variability of all 27 data sets provided for individual tissues in the three studies). Therefore, in modeling experiments like the ones described above, replacing liver and kidney by 300 g of lung and using a conversion factors of 1 for marker to total residues, the estimated daily intake (EDI) exceeds the upper bound of the ADI for the Wuhan and Beijing studies. The EDI estimated for the Guangzhou study would remain significantly below the upper bound of the ADI.

Analytical Methods

The analytical method used in the three new studies included a step where the tissue samples were hydrolyzed with β-glucuronidase/aryl sulfatase. The hydrolysed sample was then extracted with ethyl acetate-25% ammonium hydroxide (95-5), purified with solid phase extraction and analyzed by LC/MS/MS using deuterium labelled (D6)-ractopamine hydrochloride or tri-deuterium labeled ractopamine (D3) as an internal standard. The limit of quantification (LOQ) reported for this method was 0.5 $\mu g.kg^{-1}$; the limit of detection (LOD) was 0.2 $\mu g.kg^{-1}$. This analytical procedure for quantifying ractopamine residues is different from the methods considered in previous evaluations by the Committee where enzymatic hydrolysis was not used in the analytical determination of ractopamine residues.

The analytical method applied in the Chinese studies is considered as fit for the purpose. It is based on the use of isotopic dilution (deuterated internal standards) and LC-MS/MS (reverse phase separation, electrospray ionization, and acquisition of the signals in the selected reaction monitoring mode on a triple quadrupole instrument) for characterization of the analytes. However, uncertainties arise through the first steps of the method, especially the phase II metabolite deconjugation process with different enzymatic sources and the first extraction step used to recover hydrolyzed metabolites. No data regarding validation of the enzymatic hydrolysis was provided and it was concluded that this step in the analysis was not validated. Furthermore, the conditions used for the deconjugation differed appreciably. The deconjugation step is probably the most critical stage in the methods that can lead to differences in the results. Moreover, it is common practice to conduct the hydrolysis step after a first extraction of the solid matrix. In addition, the original tissue sample is usually either digested (e.g. using a protease), or lyophilised and ground. In these studies, the deconjugation was performed directly on homogenized tissue. The accessibility of the enzyme to the substrate may be compromised and it is possible that less quantities of the ractopamine conjugates were hydrolysed, however, there was insufficient data to verify this hypothesis.

In summary, even if some shortcomings were identified for the different analytical methods used to detect residues in the three new studies, it was noted that the analytical data provided are of acceptable quality, and even if the strategies used by the three different laboratories are slightly different, the performance between the three studies is somewhat different, in the final analysis, it is concluded that all data are valid for use in the analysis presented in this monograph.

Conclusion and recommendations

The Committee concluded that, based on the data provided, including those from the three breeds of pigs in the studies undertaken by the People's Republic of China, and corresponding dietary information, the recommended MRLs are compliant with the ADI as regards consumption of pig tissues of muscle, liver, kidney and fat. The estimated daily intake is approximately 50% of the upper bound of the ADI for a 60 kg person. Substituting specific organ tissue data in the model diet employed by the Committee for liver and kidney would result in dietary intakes that are still below the upper bound of the ADI, with the exception of lung tissue, where specific risk management measures may need to be considered. International food consumption data on offal and other organ tissues such as lung are lacking and further work should be undertaken to address this issue.