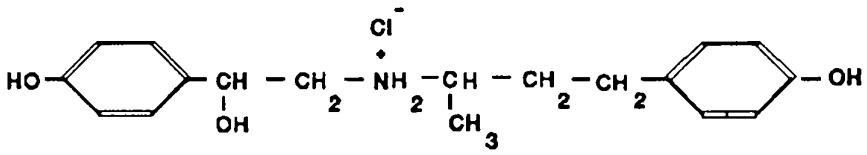
RACTOPAMINE

IDENTITY

Chemical name: dl-p-*α*-[[[Hydroxyphenyl-1-methylpropyl] amino]methyl]benzene methanol hydrochloride.

It exists in two diastereomeric forms, which are identified as RS,SR and RR,SS.

Structural formula:



Molecular formula: $C_{18}H_{24}O_3NCl$

Molecular weight: 337.5

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Ractopamine hydrochloride is a phenethanolamine salt which is an off-white to cream coloured solid. It is formulated as a premix containing ractopamine hydrochloride which is then sprayed onto animal feed, usually corn (maize) cob grits, dried and blended.

Melting Point: Not identified

Solubility: Soluble in polar solvents.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Ractopamine hydrochloride is recommended for continuous feeding to finishing pigs at concentrations of 5 -20 mg/kg for improved feed efficiency and increased rate of live weight gain (LWG). Concentrations of 10-20 mg/kg are recommended for increased carcass leanness and increased carcass dressing percent.

METABOLISM

Pharmacokinetics

Absorption and Bioavailability

The bioavailability of ractopamine hydrochloride was measured in rats and dogs. In both species ractopamine hydrochloride was

- rapidly absorbed into the blood following oral administration
- rapidly cleared from the blood circulation
- mostly associated with the plasma fraction of whole blood

There were large unexplained differences in male and female rats in the absorption and bioabailability of the drug. The results of studies are shown in Table 1.

Table 1. Absorption and bioavailability in rats

Species /Sex	Dose mg/kg	Peak Conc. μg-eq/L	Time to peak (hours)	AUC:Dose Ratio	Half-life (hours)
Rat MA	0.5	0.12	0.5	0.60	
	2.0	0.47	0.5	0.56	6.5
	20.0	3.85	0.5	1.08	14.4
Rat F	0.5	0.16	0.5	0.68	
	2.0	0.81	0.5	0.74	7.5
	20.0	9.02	2.0	3.21	7.5
Dog MA	0.05	0.02	1-2	2.40	
	0.5	0.38	2	3.56	4.0
	5	0.58	2	0.61	6.1
Dog F	0.05	0.02	0.5-2	2.60	
	0.5	0.26	0.5-1	2.72	7.7
	5	0.27	4-8	0.73	7.4

AUC is area under the curve. The values are based on measurements in whole blood. (Data from Elanco submission reports #RO3985, RO4085, RO4185 and DO2385).

Excretion of ractopamine

The excretion of ractopamine after oral dosing was measured in pigs, dogs and monkeys. In all species tested there is a rapid clearance of the drug from the animal with the major route of excretion via the urine. The results of studies are summarised in Table 2.

Table 2. Excretion of ractopamine

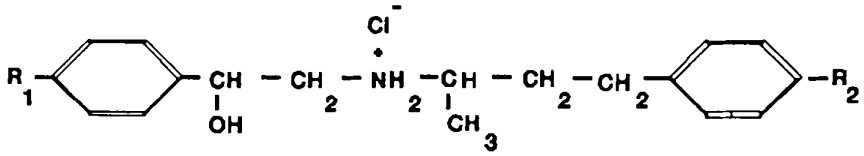
Species	Dose	Time (days)	Urine + faeces (%dose)	Urine (%dose)
Dog	0.125 mg/kg	1	>72	majority
		3	79.4	
Monkey	0.125 mg/kg	1	>63	majority
		3	69.8	
Pig	20 mg/kg + 40mg ^{14}C-Ractopamine HCl	1	84.7	82.2
		2	93.1	86.8
		3	95.4	87.6
		7	96.5	88.1

(Data from Elanco report #DO4686, PO3086 and ABC-0330).

METABOLISM

Pigs

The metabolism of ractopamine hydrochloride was studied in pigs fed 30 mg/kg ^{14}C-ractopamine hydrochloride and sacrificed 12 hours after the last dose of the drug. Ractopamine hydrochloride as parent drug is found as a major residue in liver and kidney tissues together with three other key metabolites. The metabolites are three distinct monoglucuronides.



	R1	R2	Isomers
Metabolite A	H	Glucuronide	RS,SR
Metabolite B	H	Glucuronide	RR,SS
Metabolite C	Glucuronide	H	Mixture

The metabolites A and B constitute 30.9% and metabolite C 4.6% of the extractable residues in liver. The extractable residues of the kidney contained 31% of metabolites A and B and 25.9% metabolite C. Similar metabolites are found in liver and kidneys of rats and dogs.

The metabolites were identified by a combination of extraction, enzyme hydrolysis, HPLC, TLC, GC-MS and NMR techniques. (Elanco report #ABC-0355 and later amendment of structures, June, 1992).

Rats and Dogs

One male dog and one female dog were administered by gavage ^{14}C-ractopamine hydrochloride dissolved in water. The dose was 0.5 mg/kg and given 3 times per day for 4 days and once on the fifth day. Samples of urine and faeces were collected . Six hours after the last dose the animals were sacrificed and liver, kidney and bile collected (Elanco report #ABC-0301).

Twelve male and twelve female rats were dosed once daily by gavage with 2 mg/kg ^{14}C-ractopamine hydrochloride for seven days. Each day urine and faeces were collected and liver and kidney tissues collected at slaughter (6 hours after last dose) (Elanco report #ABC-0285).

The samples were analyzed for parent drug and metabolites and the results for dogs, rats and pigs are summarised in Table 3.

Table 3. Metabolic profile in pigs, dogs and rats

	LIVER (mg/kg)			KIDNEY (mg/kg)		
	Pig	Dog	Rat	Pig	Dog	Rat
Parent	0.12	0.59	0.40	0.10	0.50	0.33
Metab A	0.03	0.46	0.17	0.05	0.18	0.52
Metab B	0.04	0.77	0.15	0.06	0.27	0.57
Metab C	0.02	1.76	0.10	0.09	0.63	0.08

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

All of the submitted studies describe radiometric studies in the pig, the species for which the drug is indicated. Several studies are reported and the radiolabel is always carbon-14 positioned either in ring A or ring B. In some studies the radiolabelled drug is a mixture of ractopamine hydrochloride labelled in either ring. The position of the label has no effect on the results since in almost all of the identified residues the parent drug molecule remains intact.

In five of the reported studies the dose is 30 mg/kg in the feed. This is 1.5 times the highest anticipated dose level. The sixth study used 20 mg/kg ractopamine in the feed for seven days.

Study 1. (Elanco Report #ABC-0231).

Three barrows and three gilts were fed 30 ppm ^{14}C-ractopamine hydrochloride for 7 days. The animals were sacrificed at 0.25, 3 and 5 day withdrawal period. The total residues are shown in Table 4.

Table 4. Total residues of ^{14}C-ractopamine hydrochloride (as mg/kg equivalent parent drug) in pigs

	0.25 day	3 days	5 days
Muscle	0.030	0.006	0.002
Kidney	0.738	0.024	0.021
Liver	0.176	0.093	0.045
Fat	0.016	0.006	0.006

Study 2. (Elanco Report #ABC-0368).

Three barrows and three gilts were fed 30 mg/kg ^{14}C-ractopamine hydrochloride for 4 days. The animals were sacrificed at 12 hours after the last dose. The total residues and the residue of parent drug are shown in Table 5.

Table 5. Total residues of ^{14}C-ractopamine hydrochloride (as mg/kg equivalents of parent drug) in pigs

	Concentration (mg/kg) Total	Parent drug	% Parent in Total
Kidney	0.405	0.094	23.4
Liver	0.410	0.111	27.2

Study 3. (Elanco Report #ABC-0273)

Pigs were fed 30 mg/kg ^{14}C-ractopamine hydrochloride for 4, 7 or 10 days. The residues in the tissues collected at the end of each treatment period showed that the total residues had reached a steady state by day 4 in both liver and kidney tissues. Residues in muscle and fat were below the level of reliable detection.

Study 4. (Elanco Report #ABC-0283)

This feeding study is superseded by study 5 which provides more time points under the same experimental conditions.

Study 5. (Elanco Report #ABC-0291)

Six barrows and six gilts were fed 30 mg/kg ^{14}C-ractopamine hydrochloride for 4 days. Groups of three animals of mixed sex were sacrificed at 0.5, 2, 4 and 7 days after the last dose. The total residues are shown in Table 6.

Table 6. Total residues of ^{14}C-ractopamine hydrochloride (mg/kg equivalents parent drug) in pigs

	0.5 day	2 days	4 days	7 days
Muscle	0.02	0.00	0.00	0.00
Kidney	0.60	0.06	0.03	0.02
Liver	0.42	0.10	0.05	0.06
Fat	0.02	0.00	0.00	0.00

Study 6. (Elanco reports T4V739003 and T4V739004)

In this radiolabeled residue depletion study, eight barrows and eight gilts received ^{14}C-ractopamine hydrochloride at 20 mg/kg in the feed for seven days. Three barrows and three gilts were sacrificed after 24 and 48 hour withdrawal periods and two barrows and two gilts were sacriced after a 72 hour withdrawal period. After each sacrifice the ^{14}C-residue concentration was determined in liver, kidney, muscle and fat. The ractopamine (as hydrochloride equivalents) concentration was determined in liver and kidneys. The mean total radioactivity and the mean concentration of ractopamine (as hydrochloride equivalents) calculated as μg/kg of ractopamine in liver and kidney is summarized in Table 7. Muscle and fat did not contain detectable residues at any of the withdrawal periods in this study.

Table 7. Residues of ^{14}C-ractopamine (μg/kg) in pigs fed 20 mg/kg medicated feed

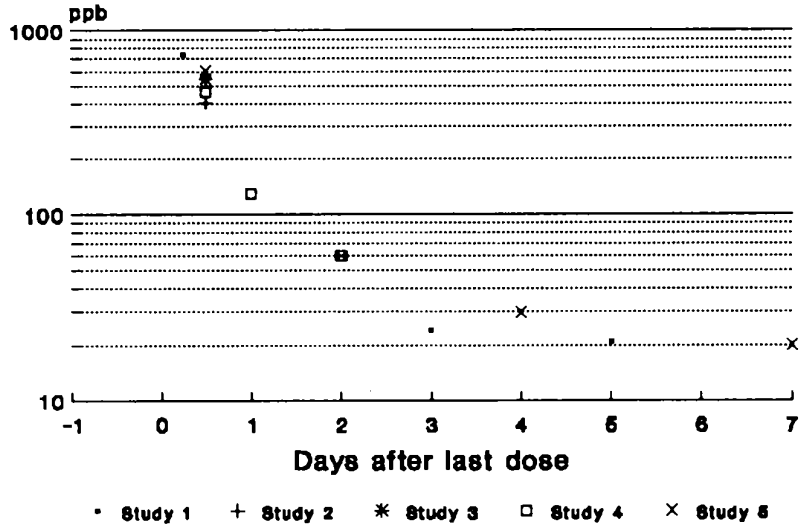
Withdrawal Time (hours)	Liver Total residues	Liver Ractopamine	Kidney Total residues	Kidney Ractopamine
24	106	14.8	116	32.1
48	73	3.7	48	8.3
72	56	1.7	36	3.4

(Ref. Dalidowicz, J.E., Macy, T.D., Cochrane, R.L., Elanco Reports T4V739003 and T4V739004)

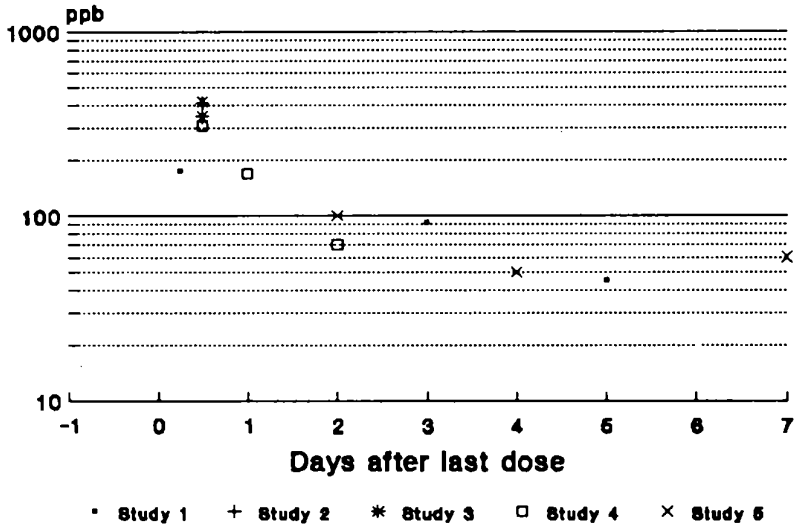
The percent ractopamine compared to total residues in liver declined from 14% at 24 hours to 5% at 48 hours and to 3% at 72 hours. Similarly, the percent ractopamine residues in kidney declined from 30% at 24 hours to 17% at 48 hours and to 9% at 72 hours.

All the results for the total residues in liver and kidney tissue are combined in the following two figures. They show a steady log-based decline with a flattening out after 3-4 days. This latter effect is due to the persistence of nonextractable bound residues.

Total residues in kidneys



Total residues in liver.



OTHER RESIDUE DEPLETION STUDIES

Residue Depletion Studies with Unlabeled Drug

Two studies were carried out by the sponsors using HPLC to measure the residues of parent drug in pigs administered ractopamine-HCl. The HPLC method with electrochemical end-point detection is a validated method which measures the concentration of parent drug in biological samples. The assay does not measure residues of the glucuronide metabolites which are a major fraction of the total residue (see Table 3) since no hydrolysis step is incorporated into the assay. The information would be more meaningful if the parent drug and the ractopamine-glucuronides had been measured. The total of parent drug and it's glucuronide metabolites could have provided information regarding a possible marker residue. There is no firm indication of what percentage of the total residues the parent drug represents because the ratio of parent drug to glucuronides appears to be highly variable.

In another residue depletion study, six pigs were fed 31 mg/kg ractopamine-HCl for seven days. The pigs were sacrificed 12 hours after withdrawal of the drug and the residues of ractopamine-HCl were measured in kidney and liver samples by HPLC. Residues of ractopamine-HCl were 0.058 ± 0.027 mg/kg in liver and 0.118 ± 0.054 mg/kg in kidney.

In a similar study forty eight crossbred pigs were fed ractopamine-HCl at 20 mg/kg for 14 days. Groups of eight pigs (4MA, 4F) were slaughtered at 12 h, 1, 2, 3, 4 and 5 days after drug withdrawal. The residues were measured in the edible tissues by HPLC and the results are shown in Table 8.

Table 8. Residues of ractopamine-HCl in pigs (μg/kg) measured by HPLC

WT (days)	Liver	Kidney	Muscle	Fat	Skin
0.5	11.1	31.8	5.4	<2.0	7.5
1	5.8	12.7	1.9	<1.0	NA
2	3.4	6.7	NA	NA	NA
3	1.7	3.0	NA	NA	NA
4	1.2	2.2	NA	NA	NA
5	NDR	<1.0	NA	NA	NA

NDR is no detectable residue; NA is not analyzed.

The residues measured in pigs given a 31 mg/kg oral dose are 5.2 times higher in liver and 3.7 times higher in kidney than pigs fed 20 mg/kg.

In the most recent study on ractopamine tissue residue depletion in pigs, 30 crossbred pigs were fed 20 mg/kg ractopamine treated ration for nine days. Six pigs, three barrows and three gilts, were sacrificed at one, two, three, four and five days withdrawal. Tissues were collected and subjected to quantitative analysis using HPLC with electrochemical detection. Results are summarized in Table 9.

Table 9. Mean concentration of ractopamine (as HCl equivalents) residue depletion in pig tissue in μg/kg

Withdrawal time (days)	Liver	Kidney
1	11.1	24.6
2	3.2	7.9
3	2.2	2.0
4	1.2	2.3
5	1.4	1.9

(Ref. Tuberg, M.P., Macy, T.D., Cochrane, R.L., Elanco study T4V739001)

Bound Residues and Bioavailability

Pigs were fed 30 mg/kg ^{14}C-ractopamine-HCl for four days. The mean concentration of nonextractable residues, calculated as mg/kg ractopamine-HCl, remaining in the liver and kidney after a one time extraction with 1.0*N* perchloric acid - ethanol (1:1) is summarised in Table 10. The values in parentheses are the non extractable residues as a percentage of the total residues.

Table 10. Nonextractable residues (mg/kg) in pig tissues

Tissue	0.5 d	2 d	4 d	7 d
Liver	0.12 (29%)	0.06 (60%)	0.04 (80%)	0.04 (67%)
Kidney	0.08 (13%)	NA	NA	NA

NA is not assayed

The nature of the nonextractable residues was not fully investigated. The residue pattern is typical of many drugs in that as the total residues in the tissues decrease, the nonextractable portion of the total residues increases.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A method based on HPLC with electrochemical detection is available for the measurement of residues of ractopamine in the edible tissues of pigs. The method measures the concentration of ractopamine as the sum of the four possible stereoisomers. The method is described below.

Ractopamine is extracted from tissues with methanol. Water is added and the extract evaporated down under nitrogen to yield an aqueous extract. The extract is buffered to pH 10.5 with sodium carbonate and the ractopamine extracted with ethyl acetate. The ethyl acetate fraction is further purified on an acid-washed silica cartridge. An aliquot of the purified extract is assayed by HPLC with electrochemical detection.

The method has been carefully validated for

a) linearity of response; standard curve 2 to 300 ng/ml , r = 0.9998

b) precision, reproducibility (interassay CV) in liver and kidney for concentrations 25 to 100 μg/kg was between 2.2 to 3.8%

c) recovery; the recoveries of spikes of ractopamine-HCl at 25, 50 and 100 μg/kg were 77 to 79%

d) specificity; no significant interferences were present in the chromatograms

e) the limit of detection; based on the response from the control tissue (muscle, liver, kidney or fat) at 3 times the signal to noise ratio (SNR) the limit of detection was calculated as 1.5 μg/kg. The sponsors indicate that the sensitivity of the assay has been improved to 0.5 μg/kg (Personal communication to JECFA, June 1992)

f) the limit of quantification based on 10 times the SNR was 5 μg/kg

The method does not measure the ractopamine glucuronides. The sponsors reported some success with kidney tissue but with liver the process required so many clean-up steps that they concluded the method repeatability would be too low (Personal communication to JECFA, June 1992).

APPRAISAL

Ractopamine hydrochloride is specifically indicated for use in the feed of pigs. The highest recommended dose in feed is 20 mg/kg.

The metabolism is similar for the rat, dog and pig. The drug is rapidly absorbed from the gut and is rapidly excreted, mostly via the urine. The major metabolites are the ractopamine glucuronides which together with parent drug account for almost all of the extractable residues.

There is adequate information from six radiometric studies about the total residues in the edible tissues. The highest concentrations are found in the liver and kidney. The total residues decline rapidly after drug withdrawal. This decline over seven days is mostly associated with the extractable residues. The nonextractable residues appear to have a longer half-life.

The parent drug is a significant fraction of the extractable residues, but less than half of the extractable residues are found in liver or kidney at 12 hours after drug withdrawal. The lack of more information on the ratios of parent drug to metabolites in the edible tissues makes it inappropriate to use the parent drug as a marker residue for total residues especially at withdrawal times longer than 24 hours.

The establishment of an MRL will have to consider the total residues because there is no information on the toxicity of the bound residues.

The HPLC assay for parent drug is well validated but it does not measure the extractable glucuronides and therefore may not be appropriate for measuring a marker residue. Determining the acceptability of an analytical method for compliance with the MRL requires consideration of how the HPLC method can be applied or even modified to take into account the metabolites.