PORCINE SOMATROPINS

First draft prepared by

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IDENTITY

Chemical name: Porcine Somatotropin

Synonyms: Porcine Growth Hormone, PGH, Porcine Somatotrophin, pST, Recombinant Porcine Somatotropin, rpST, CL, 326,061, AC 326,061, P-3895, Methionyl porcine somatotropin.

CAS Numbers:

9067-08-7	porcine growth hormone
96353-48-9	Somagrepor recombinant product

No CAS numbers were given for Grolene and Reporcin.

Products[1]:

Somagrepor ®	1-[N^2-(N-L-methionyl L-α-aspartyl)-L-glutamine] A6T:S11R:C183E:C191E Porcine Somatropin
Grolene ®	(8-191)-Porcine Somatotropin
Reporcin ®	Methionyl Porcine

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Active ingredient: recombinantly produced, containing:- not less than 90% monomer, not more than 10% dimer, not more than 10% aggregate, not more than 1% extraneous protein and not more than 1 pg DNA

Appearance: off-white, fluffy, odorless powder.

Melting point: Somagrepor not reported, Reporcin starts to decompose at 190°C.

Denaturation: 52°C (Somagrepor); 56°C (Reporcin)

Empirical Formulae:

$C_{979}H_{1527}N_{265}0_{287}S_8$	Reporcin
$C_{996}H_{1555}N_{271}O_{296}S_6$	Somagrepor
$C_{938}H_{1465}N_{257}O_{278}S_6$	Grolene

Molecular Weight:

21,837	(191 amino acids)	Reporcin
22,254	(193 amino acids)	Somagrepor
20,983	(183 amino acids)	Grolene

NOTE 1. The Committee was not able to identify common names for the three products reviewed. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO or FAO in preference to others of a similar nature that are not mentioned.

Structural Formula:

Porcine somatotropin is a protein whose primary structure is illustrated by the general 191 amino acid sequence below. Two disulfide bonds exist between cysteine residues at positions 53-164 and 181-189. In this sequence an amino acid is absent at position 10. Therefore, the natural form of porcine growth hormone has 190 amino acids. The reason for the structure as shown is that the basis for comparison of growth hormone among multiple species is based on a standard 191 amino acid protein sequence. The approach used is to compare regions where parts of the sequence are conserved and those where they are variable among species. The structure of porcine somatotropin differs from human somatotropin by 34% (Abrams, 1989).

Phe- Pro- Ala- Met- Pro- Leu- Ser- Ser- Leu-	10
Phe- Ala- Asn- Ala- Val- Leu- Arg- Ala- Gln- His-	20
Leu- His- Gln- Leu- Ala- Ala- Asp- Thr- Tyr- Lys-	30
Glu- Phe- Glu- Arg- Ala- Tyr- Isoleu- Pro- Glu- Gly-	40
Gln- Arg- Tyr- Ser- Isoleu- Gln- Asn- Ala- Gln- Ala-	50
Ala- Phe- Cys- Phe- Ser- Glu- Thr- Isoleu- Pro- Ala-	60
Pro- Thr- Gly- Lys- Asp- Glu- Ala- Gln- Gln- Arg-	70
Ser- Asp- Val- Glu- Leu- Leu- Arg- Phe- Ser- Leu-	80
Leu- Leu- Isoleu- Gln- Ser- Trp- Leu- Gly- Pro- Val-	90
Gln- Phe- Leu- Ser- Arg- Val- Phe- Thr- Asn- Ser-	100
Leu- Val- Phe- Gly- Thr- Ser- Asp- Arg- Val- Tyr-1	110
Glu- Lys- Leu- Lys- Asp- Leu- Glu- Glu- Gly- Isoleu-	120
Gln- Ala- Leu- Met- Arg- Glu- Leu- Glu- Asp- Gly-	130
Ser- Pro- Arg- Ala- Gly- Gln- Isoleu- Leu- Lys- Gln-	140
Thr- Tyr- Asp- Lys- Phe- Asp- Thr- Asn- Leu- Arg-	150
Ser- Asp- Asp- Ala- Leu- Leu- Lys- Asn- Tyr- Gly-	160
Leu- Leu- Ser- Cys- Phe- Lys- Lys- Asp- Leu- His-	170
Lys- Ala- Glu- Thr- Tyr- Leu- Arg- Val- Met- Lys-	180
Cys- Arg- Arg- Phe- Val- Glu- Ser- Ser- Cys- Ala-	190
Phe-	191

Reporcin has an extra methionine added to phenylalanine at position 1, the N-terminus. Grolene may be described as a zinc complex of recombinantly produced porcine somatotropin which lacks Phe- Pro- Ala- Met- Pro- Leu- Ser-, the first seven amino acids at the N-terminal end of the protein. Somagrepor recombinant product also has several changes in the basic protein structure of porcine somatotropin. First, it has three additional amino acids at the N-terminus at position 1. They are methionine, aspartate, and glutamine. Second, there are four substitutions in the amino acid sequence. They are replacement of alanine at position 6 (position 3 above) with threonine, serine at position 11 (position 8 above) with arginine, and the cysteines at positions 183 and 191 (positions 181 and 189 in the above sequence) with glutamates. According to the declaration provided, the latter substitution that eliminates the second disulfide bond loop in the native structure does not lessen the potency of the recombinant product (Report FD 40-9.00.,1992 and Report FD 40-6.00.,1992).

Nomenclature

This monograph on porcine somatotropin (pST) describes experimental work conducted with both natural (native) and three different forms of recombinant porcine somatotropin. The natural porcine somatotropin is abbreviated as npST and recombinant porcine somatotropin is abbreviated as rpST throughout the remainder of the monograph.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Data for the three products, Somagrepor, Grolene and Reporcin, were considered by the Committee. The three products are genetically engineered protein hormones similar to natural porcine somatotropin. Their primary functions are to increase daily weight gain, improve feed efficiency, and increase carcass leanness.

Dosage

Porcine somatotropin is an injectable product administered to pigs in the last 30 days of their finishing phase, just prior to slaughter. The dosages vary with product, from single daily doses of 3-5 mg/day (60-70 μ/kg BW) to a sustained-release implant system containing 100 to 150 mg of active ingredient, equivalent to 3.3 to 5 mg/day over 30 days.

Other residues

Since many of the effects of pST are known to be mediated by insulin-like growth factors, especially insulin-like growth factor I (IFG I), IGF I concentrations following treatment with pST have been determined. Human, bovine and porcine IGF I are structurally identical (Francis *et al.*, 1989 and Tavakkol *et al.*, 1986).

METABOLISM AND PHARMACOKINETICS

Metabolism

No specific metabolism studies were available to the Committee.

Binding Studies

Porcine somatotropin does not bind to human liver preparations. However, monkey somatotropin partially competes with human somatotropin (^{125}I-hGH) in this *in vitro* test (Carr and Friesen, 1976). Also of importance is the demonstration by Evock *et al.* (1988) of the equivalent binding of rpST (Grolene) and npST in a pig liver microsome preparation containing somatotropin receptors. However, in the same study, these authors also noted a quantitative difference in binding of the protein rpST (2.7 μg/L versus 1.3 μg/L) to guinea pig antiserum raised against npST when measured in a half maximal binding assay using ^{125}I labeled natural pST. *In vivo* studies investigating the biological potency of npST and rpST, showed nearly identical results in many of the measured parameters, including growth rate, feed efficiency and induction of IGF-I production.

Throughout this monograph, concentration of pST should be understood as total porcine somatotropins because the RIA competitive binding methods used to measure plasma or serum pST were not able to distinguish between npST and rpST.

Pharmacokinetic Studies

Porcine Somatotropin

The pharmacokinetics of pST in pigs were investigated after intramuscular injection in all of the following studies except one study in which the intravenous (i.v.) route of administration was used. Quantitation in all cases was carried out using RIA methodology except in the i.v.-study where radiolabel counting techniques were employed.

Four groups of three pigs received single i.m. doses of 0, 0.01, 0.1, or 1 mg/kg BW of npST (Sillence and Etherton, 1987), whereby the highest dose was approximately 20 times greater than the recommended dose.. Serum pST

concentration in the control group ranged between 1.6 and 5.7 μg/L over the course of the experiment. Somatotropin levels peaked between 1 and 2 hours at 28, 112 and 286 μg/L in the treated groups, respectively, and returned to baseline values within 4, 12, and 24 hours for the 0.01, 0.1, and 1 mg/kg groups, respectively.

Four groups of 12 pigs (40 kg barrows) received daily i.m. doses of npST of 0, 0.01, 0.03 or 0.07 mg/kg BW for 35 days (Etherton, *et al.*, 1987). Serum concentrations of pST were determined at day 17 with values found of 0.09 μg/L for controls and 9.5, 56.0, and 116.0 μg/L for the three treated groups, respectively.

Six groups of 12 pigs received daily i.m. doses 0, 0.035 or 0.070 mg/kg BW of npST and 0, 0.035, 0.070 or 0.140 mg/kg BW of rpST for 77 days (Evock *et al.,* 1988). Serum pST concentrations at day 49 were 6.0 μg/L for both control groups, and 24.6 μg/L (npST) or 19.6 μg/L (rpST) for treatment groups receiving 0.035 mg/kg BW, 36.5 μg/L (npST) or 43.6 μg/L (rpSTr) for treament groups receiving 0.070 mg/kg BW and 94.5 μg/L for the treatment group receiving 0.140 mg rpST/kg BW.

One group of 18 pigs received daily i.m. doses of 0.1 mg/kg BW of npST, starting at weights of 25 kg whilst a second group of 18 untreated pigs acted as controls (Campbell *et al.*, 1988). When the animals reached 50 kg body weight, serum pST values in controls ranged from 0.8 to 2.1 μg/L and in treated pigs from 19.7 to 21.4 μg/L.

Two groups of 12 (control) and 11 (treatment group) pigs received daily i.m. doses of 0 or 0.022 mg/kg BW npST for 30 days (Chung *et al.*, 1985). Blood samples were taken 3 hours after injection at day 0, 10, 20, and 30. Mean serum pST concentrations in control animals were 3.5, 3.5, 2.8, 2.5 μg/L, respectively, and in treated animals 3.4, 20.1, 22.8, 15.3 μg/L, respectively, at the four time points.

Two groups of 47 pigs were given i.m. doses of 0 or 14 mg per animal methionyl-rpST twice a week, for 6 weeks (from 60 to 100 kg BW) or for 13 weeks (from 60 to 140 kg BW) (Schams *et al.*, 1989). Blood samples were taken at slaughter 4.5 days after the last injection. Plasma pST concentrations of treated animals (1.6 μg/L) had declined to physiological concentrations of control animals (2.6 μg/L).

In a second trial reported by the same authors, groups of 12 pigs received i.m. doses of 0 or 14 mg per animal methionyl-rpST twice a week, for 9 weeks (from 60 to 120 kg BW) (Schams *et al.*, 1989). Blood samples were taken within 1 hour after the last injection and 26-27 hours later at slaughter. While plasma pST concentrations in treated animals were high (276 μg/L) within 1 hour after injection, they returned to physiological concentrations (2-3 μg/L) after 26-27 hours as compared to controls (2 μg/L).

Plasma base line levels of pST in swine have been reported in a number of studies, and values ranged from 1.7 to 6.8 μg/L with episodic peaks 2 to 3 times higher values (Klindt and Stone, 1984; Arbona *et al.*, 1988).

Plasma half-lives were determined in two groups of 3 pigs receiving one single i.v. dose of 50 ng ^{125}I labeled either npST or rpST (GhiasUddin 1988). The values reported were 4.12 (± 0.25) min (npST) and 4.03 (± 0.38) min (rpST) for the alpha-phase and 38.6 (± 2.6) min (npST) and 49.2 (± 10.0) min (rpST) for the beta-phase. In an older study, the half-life of pST in pigs was reported to be short, varying from 7–8 minutes (Althen and Gerrits, 1976)

Plasma concentrations of pST were also determined in a 28 day efficacy study in which three pigs were given a 123.1 mg sustained implant of the Somagrepor rpST product, representing a dose of 3-5 mg/animal/day (Tsalta *et al.*, 1994). Three control pigs were given a daily injection of buffer solution. On day 28 the animals were slaughtered and plasma and edible tissues were collected. Plasma values for the control pigs had a range of 1.3 to 2.5 μg/L, whereas the treated pigs exhibited values of 7.0 to 9.3 μg/L. The elevated levels of plasma pST demonstrated that the sustained release product was still releasing rpST.

Insulin-Like Growth Factor I (IGF-I)

Treatment of swine with pST increases the insulin-like growth factor I. IGF-I is a polypeptide containing 70 amino acids (Klapper *et al.*, 1983) and is similar to insulin (51 amino acids) in its 3-dimensional structure.

Two groups of 12 pigs (control) and 11 pigs (treatment group) received doses of 0 or 0.022 mg/kg BW npST for 30 days. This treatment caused a significant increase ($p<0.001$) to IGF-I of 305 IU/L[1] in treated pigs in comparison to 197 IU/L IGF I in controls to at 3 hours after the last injection (Chung *et al.*, 1985).

In a second study (Evock *et al.*, 1988), serum was sampled 3 hours after pigs (12 barrows per group) were treated for 49 days with either npST or Grolene rpST at doses of 0.035 mg/kg or 0.07 mg/kg BW. Control values of 145 IU/L were recorded. Pigs that were treated with the 0.035 mg/kg BW dose had values of 161 (npST) or 165 (rpST) IU/L, while those given 0.07 mg/kg BW had values of 227 (npST) or 215 (rpST) IU/L. Each dose level was significantly different ($p<0.05$) from control and from each other. However, there was no difference in IGF-I concentrations resulting from injection of either natural or recombinant sources of somatotropin.

Four groups of 3 pigs received single i.m. doses of of 0, 0.01, 0.1 or 1.0 mg/kg BW npST (Sillence and Etherton, 1987). Controls and pigs treated with 0.01 mg/kg BW had IGF-I values that ranged between 80 and 180 IU/L throughout the study. Pigs given 0.1 mg/kg BW had increased levels of IGF-I (200-250 IU/L) from 10 through 36 hours. However, pigs treated with 1.0 mg/kg BW had serum levels of IGF-I above 200 IU/L at 6 hours that increased to 380 IU/L at 24 hours, and were still at 340 IU/L at 36 hours after dosing.

In the first of two trials, two groups of 47 pigs were given i.m. doses of 0 or 14 mg BW methionyl-rpST twice a week, for 6 weeks (from 60 to 100 kg BW) or for 13 weeks (from 60 to 140 kg BW) (Schams *et al.*, 1989). Blood samples were taken at slaughter 4.5 days after the last injection. In trial 1 at 4.5 days after the last injection, IGF-I concentrations in control animals averaged 327 ng/mLμg/L and those in treated animals averaged 359 μg/L. With IGF-I concentration standard deviations well over 100, these values are not significantly different.

In a second trial reported by the same authors (Schams *et al.*, 1989), groups of 12 pigs received i.m. doses of 0 or 14 mg BW methionyl-rpST twice a week, for 9 weeks (from 60 to 120 kg bw). The results from the second trial show a comparison of values as follows: 1 hour, 340 μg/L control, 551 ng/mL μg/L treated; 26-27 hours, 271 ng/mL μg/L control, 941 μg/L treated. These results show the increase in IGF-I concentrations seen at 1 hour to continue to increase to higher values at 26-27 hours post administration. The plasma concentration of IGF-I in this study exceed somewhat the values seen in the residue study described below for the rpST sustained release implant study, which may be due to the higher dose administered in this study one day prior to analysis.

Plasma levels of IGF-I were also determined in a 28 day efficacy study in which three pigs were given a 123.1 mg sustained implant of the somagrepor rpST product (Tsalta *et al.*, 1994). Plasma values of IGF-I for the control pigs had a range of 175.6 to 401.6 μg/L, whereas the treated pigs exhibited values of 399.9 to 485.0 μg/L. Details of dosing are given the Section on pST pharmacokinetic above.

RESIDUE STUDIES

Little data exists on residue concentrations of pST and IGF-I in edible tissues of pST treated pigs. Two studies investigated tissue concentration of pST and one study tissue concentrations of IGF I.

Porcine Somatotropin

An argument was presented suggesting that there was no need to determine the concentrations of porcine growth hormone in edible tissues of pST treated pigs. This argument was based on work showing the species specificity of porcine growth hormone and, in particular, its lack of biological activity in humans (Knobil and Greep, 1959; Raben, 1959). Furthermore, the increase of IGF-I in response to pST administration is not considered to be of concern to human health, because IGF-I is not orally active (Hammond *et al.*, 1990).

However, one group has determined tissue concentrations of pST and IGF-I in non-treated and treated pigs (Tsalta *et al.*, 1994). This study consisted of three control animals that were injected i.m. daily with a buffer solution, and three animals that were treated with a sustained release implant of Somagrepor containing 123.1 mg of rpST. The animals

[1] IU = International unit. A unit of enzyme activity equal to the amount of enzyme that catalyzes the conversion of one micromole of substrate or coenzyme per minute under specified conditions.

were slaughtered 28 days after implantation. Because the treated animals were implanted with a sustained release device, the residue data are obtained at zero withdrawal time. The concentrations of pST in the plasma of control pigs differed by about 2 fold or less, with concentrations ranging from 1.3 to 2.5 μg/L. In untreated pigs (control), the variability of pST concentrations in tissues were similar to those found in plasma : i.e., 11.7 to 18.1 μg/kg in muscle, 2.9 to 4.2 μg/kg in fat, 14.4 to 22.3 μg/kg in liver and 21.7 to 33.0 μg/kg in kidney. In rpSTtreated pigs, plasma pST values ranged from 7.0 to 9.3 μg/L. Liver and kidney samples also contained the highest pST concentration in the treated pigs, a situation similar to that found in the control pig tissues. The range of levels found in the treated pig tissues were 12.0 to 13.5 μg/kg in muscle, 2.3 to 3.2 μg/kg in fat, 17.0 to 24.5 μg/kg in liver, and 19.3 to 24.6 μg/kg in kidney.

The results showed that at 28 days the plasma pST concentration was elevated from a mean value of 1.7 μg/L in control pigs to 8.2 μg/L in treated pigs with the increased levels demonstrating the continuing release of the hormone from the implant. A test for significant differences in treatment versus control was applied to the pST data. The p value for plasma was $p<0.001$, confirming the presence of elevated levels of pST in plasma. However, pST concentrations in tissues of the treated group were essentially the same as the control group. The mean values (μg/kg) of pST in control versus treated groups were: 14.1 vs. 12.6 in muscle, 3.3 vs. 2.8 in fat, 17.4 vs. 20.0 in liver and 25.9 vs. 22.8 in kidney, respectively. The test for significance for the four tissues showed no significant differences between treated and control animals. The p values for muscle, fat, liver, and kidney were $p<0.42$, $p<0.38$, $p<0.49$, and $p<0.48$, respectively. The results are shown in Table 1.

Table 1. Mean plasma and tissue concentrations of pST in pigs treated with 123.1 mg of rpST in a sustained release implant formulation.

Sample	Treatment Group	PST Concentrations, (μg/L or μg/kg)	S.D.
Plasma	Control	1.7	± 0.6
	Treated	8.2	± 1.0
Muscle	Control	14.1	± 3.0
	Treated	12.6	± 0.8
Fat	Control	3.3	± 0.7
	Treated	2.8	± 0.7
Kidney	Control	25.9	± 5.7
	Treated	22.8	± 2.7
Liver	Control	17.4	± 3.7
	Treated	20.0	± 3.8

In the first of two trials, two groups of 47 pigs were given i.m. doses of 0 or 14 mg per animal methionyl-rpST twice a week, for 6 weeks (from 60 to 100 kg BW) or for 13 weeks (from 60 to 140 kg BW) (Schams *et al.*, 1989). In the second trial groups of 12 pigs received i.m. doses of 0, 14 mg per animal methionyl-rpST twice a week, for 9 weeks (from 60 to 120 kg BW) (Schams *et al.*, 1989). In trial 1, 2 controls and 4 treated animals were slaughtered at 4.5 days after the last of 12 treatments and in trial 2, 3 treated animals and 1 control were slaughtered at 26-27 hours after the last of 18 treatments.Residues of pST were measured in the shoulder muscle opposite to the shoulder receiving the injection in the slaughtered animals. Concentrations of pST in the shoulder muscle tissue were reported to be less than 5 μg/kg and were within the range of concentrations observed in plasma. No increased pST concentrations were observed in tissues of the treated animals.

Insulin-Like Growth Factor-I

A control group of 3 pigs received daily buffer injections, a treatment group of 3 animals received an implant with 123.1 mg rpST 28 days before slaughter (Tsalta *et al.*, 1994). The highest IGF-I concentration in the non-treated pigs was

found in the plasma. The basal levels varied from each other by 2 to 3 times, with concentrations ranging from 175 to 401 μg/L. The variability in IGF-I levels in the control pig tissues was similar to that found in the plasma. However, they were quantitatively much lower, and values of 1.7 to 3.5 μg/kg in muscle, 3.8 to 8.8 μg/kg in fat, 9.5 to 28.6 μg/kg in liver, and 25.8 to 64.4 μg/kg in kidney were reported.

In the rpST-treated pigs, plasma IGF-I concentrations ranged from 399.9 to 485.0 μg/L and remained the highest among all samples analysed. However, the variability among the treated pigs was not as pronounced as the variability in the non-treated control group. This was also true for the tissues, which were reported to contain 5.7 to 8.5 μg/kg in muscle, 9.5 to 15.9 μg/kg in fat, 36.9 to 56.7 μg/kg in liver, and 46.9 to 74.9 μg/kg in kidney.

The results, detailed in Table 2, show that there was an increase in IGF-I concentrations in the rpST-treated pigs. A maximum of a two to three fold increase in IGF-I levels was noted when mean values (μg/L or μg/kg) of the control group were compared with the treated group. Comparative values were: 299.4 vs. 428.8 in plasma, 6.7 vs. 12.4 in fat, 2.7 vs. 7.1 in muscle, 20.2 vs. 44.8 in liver and 44.7 vs. 65.4 in kidney. However, the variation in individual pig values was sufficiently high such that the high end of the IGF-I concentration range in the control group approached or exceeded the low end of IGF-I concentration range observed in the treated pigs. A test for significance indicated that treated muscle and liver were significantly different from the controls with p values of $p<0.01$ and $p<0.04$, respectively. The p values for fat and kidney were $p<0.08$ and $p<0.22$.

Table 2. Mean plasma and tissue concentrations of IGF-I in pigs treated with 123.1 mg of rpST in a sustained release implant formulation.

Sample	Treatment Group	IGF-I Concentrations (μg/L or μg/kg)	S.D.
Plasma	Control	299.4	± 101.0
	Treated	428.2	± 53.7
Muscle	Control	2.6	± 0.8
	Treated	7.1	± 1.3
Fat	Control	6.4	± 2.5
	Treated	12.2	± 2.9
Kidney	Control	44.7	± 17.5
	Treated	65.5	± 14.9
Liver	Control	20.2	± 8.6
	Treated	44.8	± 10.1

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METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A radioimmunoassay (RIA) method of analysis was used to determine pST and IGF-I concentrations in plasma and tissues. As the method was primarily used for research purposes, only a limited description and validation of the method were available to the Committee. The IGF-I assay achieved reported recoveries of 83% in plasma and 73% to 110% in tissues with liver at 48%. The pST assay achieved similarly reported recoveries of 89% in plasma and 75% to 128% in tissues. In this assay protocol, recovery determinations were run concurrently in each assay matrix as a quality control of analysis to determine pST and IGF-I in plasma and tissues.

The method does not distinguish between natural and recombinant pST.

As IGF-I is known to be highly associated with a binding protein that can interfere with the analysis, IGF-I was first dissociated with cold acetic acid. After centrifugation and filtration, a sample of filtrate was placed on each of two C_{18} solid phase extraction cartridges on a vacuum manifold. Elution with acetic acid and methanol was used to remove the free and then bound IGF-I, respectively. After reconstitution in buffer, a competitive binding double antibody procedure employing ^{125}I-IGF-I was used. Calculations used a Ria/Fia Calc program by LKB/Wallac, which provides Rodbard's 4 parameter logistic (4-PL) curve fitting program with a dose response curve that ranged from 0 to 240 pg/tube.

The pST assay was also an RIA based assay in which plasma or tissue was initially dissolved in 3% sodium dodecylsulfate followed by a brief incubation at 90-98^0 C and then cooled to room temperature with subsequent centrifugation. Aliquots of supernatant were then employed in a standard competitive binding double antibody assay. ^{125}I-rpST was used in the assay and the results were calculated using the same program described above with a dose response curve that ranged from 0 to 320 pg/tube.

In conclusion, no validated methods for the analysis of pST or IGF-I have been provided for any of the three recombinant products. However, this deficit may not be relevant if analytical methods are not deemed necessary for residue control purposes.

APPRAISAL

pST

Three recombinant porcine somatotropin products have been evaluated by the Committee. All three somatotropins differ from the natural porcine protein by one or more amino acids.

Although a limited amount of information has been presented on pharmacokinetics and residues concentrations of pST the data presented is sufficient to demonstrate that:

- there are similar rapid depletion characteristics of natural and recombinant pST in plasma after use of an injectable product (half-life 4-8 minutes for the initial and 38-49 minutes for the terminal phase)
- there is an absence of significantly elevated levels of pST in edible tissues after use of a sustained release or injectable forms of rpST
- there is species specificity of npST and its resulting biological inactivity in humans
- there is destruction of pST by gastric and intestinal proteases when orally ingested.

IGF-I

There is a well-known link between the action of somatotropins and other body constituents including growth factors. One of the most significant of these growth factors is IGF-I because it has the same growth promoting action in many species and its structure is identical in pigs and humans. For this reason, concentrations of IGF-I were determined in tissues of pST treated pigs. Although the concentrations of the IGF I were low, a 2 to 2.5 fold increase in tissue concentrations resulted from the use of the sustained release recombinant product. While the highest levels were seen in kidney, this tissue is consumed in very low amounts when compared to muscle where the smallest amounts of IGF-I were observed. Table 3 below is based on the information previously described above for the sustained release formulation of rpST.

Table 3. Theoretical Mean concentrations and dietary intake of IGF-I in tissues of rpST untreated and treated pigs.

Tissue	Food Factors*	IGF-I (µg/kg) untreated pigs	Total IGF-I untreated pigs (µg)	IGF-I (µg/kg) treated pigs	Total IGF-I treated pigs (SYMBOLµg)
Muscle	300	2.6	0.78	7.1	2.1
Liver	100	20.2	2.02	44.8	4.5

Kidney	50	44.7	2.24	65.5	3.3
Fat	50	6.4	0.32	12.2	0.6
Total	500		**5.4**		**10.5**

- WHO TRS 788, 1989

Treatment of pigs with rpST may lead to an increase of IGF-I tissue concentrations. This would result in a 5.1 μg higher daily intake of IGF-I when compared to the consumption of meat from non-treated animals. This difference is exceedingly small in comparison to the estimated daily production rate of 10,000 μg in humans for IGF-I (Guler *et al.*, 1989). This together with the evidence that IGF-I is not absorbed to any significant extent in several neonatal animal models (Burrin, 1997, Donovan *et al.*, 1997, Hammon and Blum, 1997, Phillips *et al.*, 1995)), as well as the comparison of the consumption value (10.5 μg or an increase of only 5.1 SYMBOLμg over control) with a calculated value of 383 SYMBOLμg of IGF-I secreted daily into the GI tract (WHO FAS 41, 1998) shows that the exposure to IGF-I in the diet is insignificant compared with endogenous production.

As IGF-I concentrations may be increased in tissues due to the use of rpST, the Committee used these tissue concentrations in an exposure assessment (see Table 3). The Committee concluded that the use of rpST products will not lead to a significant increase of human exposure to IGF I. There is also a lack of oral activity of IGF-I.

Conclusions

It has been demonstrated that recombinant and natural pST are indistinguishable in biological activity and binding. The use of sustained release or injectable forms of rpST does not lead to significantly elevated residues pf pST. Both rpST and npST are species specific and orally inactive.

However i.m. and i.v. dosing of pigs with pST results in the increase of IGF-I in pig plasma and tissues.

In a comprehensive review of bovine somatotropin (bST), the 50th Meeting of JECFA concluded that IGF-I, resulting from use of bST, was orally inactive (WHO TRS 888, 1999). The 50th Meeting also discussed results that suggested that casein could protect IGF-I, present in cow's milk, from degradation during oral ingestion (Kimura *et al.*, 1997). However, the 50th meeting was not persuaded that such results established that IGF-I was orally absorbed in an unchanged form as a result of such a protection. Additionally, when considering increased IGF-I tissue concentrations resulting from pST use in swine, the previously suggested protecting effect of milk casein on IGF- I degradation is not relevant in this context.

Moreover, in view of the extremely low increases of IGF-I concentrations in swine tissues after rpST treatment in comparison to the endogenous human daily IGF-I production, it must be concluded that these concentrations have no impact on human health.

As the Committee has recommended that an ADI " not specified" be adopted and along with the Committee's finding on residues, it follows that an MRL of "not specified" be recommended for r-pST and IGF-I for the use of recombinant pST in pigs.

These results led the Committee to conclude that it is not necessary to recommend MRLs for the three rpST products reviewed.

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