ISSN 0254-4725

# Residues of some veterinary drugs in animals and foods

FAO
FOOD AND
NUTRITION
PAPER

41/12

*Deltamethrin*
*Dihydrostreptomycin*
*Doramectin*
*Estradiol-17ß*
*Neomycin*
*Phoxim*
*Porcine somatotropins*
*Progesterone*
*Streptomycin*
*Testosterone*
*Thiamphenicol*

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HEALTH
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Monographs prepared by the
fifty-second meeting of the
Joint FAO/WHO Expert Committee
on Food Additives
Rome, 2-11 February 1999

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Rome, 2000

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M-84
ISBN 92-5-104401-5

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**Rome, 2-11 February 1999**

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## ABBREVIATIONS USED IN THIS DOCUMENT

| | |
|---|---|
| ADI | -acceptable daily intake |
| AUC | -area under concentration-time curve |
| Av. | -average |
| b.i.d. | -twice a day |
| BP | -British Pharmacopoeia |
| Bq | -Becquerel (one disint/sec) |
| BST | -bovine somatotropin |
| bw, BW | -body weight |
| $^{o}C$ | -degrees Celcius |
| $^{14}C$ | -radioactive Carbon-14 |
| $C_{max}$ | -maximum concentration |
| µCi | -microcurie (radioactivity) |
| $cm^3$ | -cubic centimeter |
| conc | -concentration |
| CV | -coefficient of variation |
| D | -day |
| DPM, dpm | -disintegration per minute |
| ECD | -electron capture detector |
| e.g. | -for example |
| EP | -European Pharmacopoeia |
| Eq or EQ | -equivalents |
| F | -female |
| FDA | -Food and Drug Administration |
| G | -gram |
| µg | -microgram |
| GC | -gas chromatography |
| GI | -gastrointestinal |
| GLC | -gas-liquid chromatography |
| GLP | -Good Laboratory Practice |
| GVP | -Good Veterinary Practice |
| H | -hour |
| $^{3}H$ | -tritium |
| HPLC | -high performance liquid Chromatography |
| i.e. | -that is |
| i.m., IM | -intra muscular |
| i.m.i. | -intra muscular injection |
| i.p., IP | -intra peritoneal |
| i.v., IV | -intra venous |
| $k_{el}$ | -rate constant |
| kg | -kilogram |
| L or l | -litre |
| LC | -liquid chromatography |
| LOD | -limit of detection |
| LOQ | -limit of quantitation |
| LSC | -liquid scintillation counting |
| *M* | -molar or mole |
| M | -male |
| Max | -maximum |
| µm | -micrometer |
| mg | -milligram |
| min | -minute |
| ml | -millilitre |
| MR | -marker residue |
| MRL | -maximum residue limit |
| MRT | -mean residence time |
| MS | -mass spectrometry |
| n or No | -number |
| na | -not analysed, assayed or available |
| nd, ND | -not detected |
| NER | -non extractable residues |
| ng | -nanogram |
| Nm, NM | -not measured, if applicable |
| nm | -nanometer, if applicable |
| NMR | -nuclear magnetic resonance |
| NOEL | -no-observed-effect level |
| ppb | -parts per billion |
| ppm | -parts per million |
| R | -regression coefficient |
| RIA | -radioimmunoassay |
| RSD | -relative standard deviation |
| SA | -Specific Activity |
| s.c. | -subcutaneous |
| SD | -standard deviation |
| SEM | -standard error of mean |
| sic | -correctly spelled |
| s.i.d. | -once a day |
| $t_{1/2}$ | -half life |
| $t_{max}$ or $T_{max}$ | -time for maximum |
| TLC | -thin layer chromatography |
| TMS | -trimethyl silyl |
| TR | -total residues |
| TRA | -total radioactivity |
| TSD | -thermionic specific detection |
| UD | -unchanged drug |
| USDA | -US Department of Agriculture |
| USP | -United States Pharmacopeia |
| UV | -ultraviolet |
| $V_D$ | -volume of distribution |
| V/v | -volume/volume |
| wt | -weight |
| w/v | -weight/volume |
| WT | -withdrawal time |
| % | -per cent |
| > | -greater than |
| < | -less than |
| ≤ | -equal or less than |

## INTRODUCTION

The monographs on the residues of, or statements on, the 11 compounds contained in this volume were prepared by the fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, 2-11 February 1999. JECFA has evaluated veterinary drugs at previous meetings, including the 12th[1], 26th[2], 27th[3], 32nd[4], 34th[5], 36th[6], 38th[7], 40th[8], 42nd[9], 43rd[10], 45th[11], 47th[12], 48th[13] and 50th[14] meetings.

In response to a growing concern about mass-medication of food producing animals and the implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[15]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[15]. In response to these recommendations, the 32nd JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, 47th, 48th and 50th meetings of JECFA were also dedicated exclusively to evaluation of veterinary drugs.

The eleventh session of the CCRVDF, held in Washington, D.C., USA during September 1998, revised the priority list of veterinary drugs requiring evaluation. The drugs evaluated during the 52nd meeting of JECFA included these compounds, except cyhalothrin, metrifonate, permethrin, temephos and tilmicosin, the evaluation of which was postponed to a future meeting of the Expert Committee.

The present volume contains monographs of the residue data on 11 of the 13 compounds on the agenda. For two compounds, azaperone and carazolol, a monograph was not prepared For carazolol, a determination of an acute reference dose was considered at the request of CCRVDF because of the risk due to consumption of residues at the injection site. Data previously available to the Committee was used for this purpose. For azaperone, only an analytical method was reviewed for its ability to determine compliance with previously recommended MRLs. The performance of the method for azaperone and for azaperol did not differ significantly from previous methods and no data was provided using the proposed method with incurred residues in animal tissue. The Committee recommended that the method be further improved. Abamectin had been referred to the Committee by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) with a request that MRL residue concentrations be proposed for cattle meat The MRLs depend on the definition of the residues. The issue was resolved at a harmonisation meeting of JMPR and JECFA experts prior to the 52$^{nd}$ JECFA and, therefore, abamectin was removed from the agenda.

The anthelminthic agent, doramectin had been considered before by the Committee. Similarly, all four antimicrobial agents, dihydrostreptomycin, neomycin, streptomycin and thiamphenicol had been evaluated previously by the Committee.

The two insecticide compounds, deltamethrin and phoxim, had not been evaluated before by the Committee.

Of the production aids, the three naturally occurring hormones estradiol-17β, progesterone and testosterone had been evaluated previously by the Committee. The porcine somatotropins had not been evaluated previously by the Committee.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 52nd meeting is included in Annex 1.

The assistance of the experts and FAO consultant in preparing these monographs is gratefully acknowledged.

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15. Residues of Veterinary Drugs in Foods, Report of a Joint FAO/WHO Consultation, Rome, 29 October - 5 November 1984. FAO Food and Nutrition Paper No. 32, 1985.

16. Report of the First Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Washington D.C., 27-31 October 1986.

# DELTAMETHRIN

**First draft prepared by**
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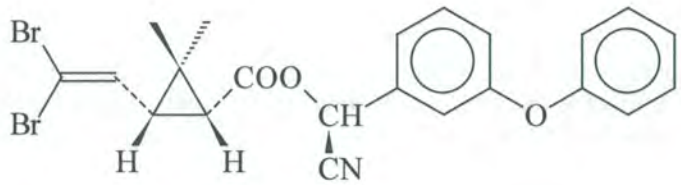
## IDENTITY

**Chemical name**: S-cyano-3-phenoxybenzyl-cis-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate

**Synonyms:** RU 22974, Decamethrin, BUTOX®

**Structural formula**:




**Molecular formula**: $C_{22}H_{19}Br_2NO_3$

**Molecular weight:** 505.2

## OTHER INFORMATION ON IDENTITY AND PROPERTIES

**Pure active ingredient:** deltamethrin

**Appearance:** white crystalline powder

**Melting point:** 98 - 101°C

**Solubility:** insoluble in water (<0.002 mg/kg)
soluble in acetone, DMSO, DMF, benzene, xylene, cyclohexanone, HMTP, ethyl acetate, THF, dioxan, acetonitrile (90 g/L); slightly soluble in ethanol, isopropanol,

**Optical rotation:** $[\alpha]_D$ +61° (c = 40 g/L, benzene)

**Stability**: Stable in acidic and neutral solutions. Unstable in alkaline solutions. Stable at 40°C in the dark and at room temperature in light.

## RESIDUES IN FOOD AND THEIR EVALUATION

## CONDITIONS OF USE

General

Deltamethrin is a synthetic insecticide belonging to the synthetic pyrethroid family and used particularly for dipteran flies and Mallophaga. It is the pure form (>99%) of one of eight possible diastereoisomers of α-cyano-3-phenoxybenzyl-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate. It is a neurotoxic agent that is widely

used in plant agriculture against insecticides. The ADI allocated by JMPR (1990) is 0 -10 µg/kg BW per day. It is authorised for veterinary use as an ecto-parasiticide in a number of countries. Withdrawal times vary between 0 and 10 days.

Dosage

Deltamethrin is prepared as a solution and used externally as a dip, spray or pour-on preparation for cattle, sheep, pigs, poultry and salmon.

## PHARMACOKINETICS AND METABOLISM

### General Comments on the Pharmacokinetics of Deltamethrin

The important aspect for laboratory animals is the pharmacokinetics following oral administration, because this route is to be compared with human consumption. The pharmacokinetics in the target animals also reviews the fate of the drug when applied externally to the animals, since this is the route of administration for the ecto-antiparasitic action of the drug. However, the target animals are also exposed to this compound as a contaminant present on or in plant foods. This aspect was covered by JMPR (1990).

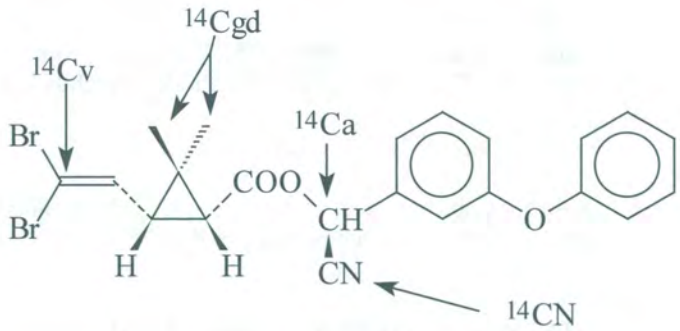
### Pharmacokinetics in Laboratory Animals

Rats

The pharmacokinetics of deltamethrin following oral administration is reported in the open literature for both rats (Ruzo *et al.*, 1978) and mice (Ruzo *et al.*, 1979).

Male rats, 140 - 160 g, were dosed orally at 0.64 - 1.6 mg/kg BW with three differently labelled preparations of [$^{14}$C]-deltamethrin. The $^{14}$C labels were placed at positions indicated in Figure 1 and each compound identified hereafter as $^{14}$Cv, $^{14}$Ca or $^{14}$CN. Rats were also dosed with the 1RS-trans-$^{14}$Cv- isomer of deltamethrin. The excretion pattern was similar to that for the $^{14}$Cv-deltamethrin.

**Figure 1. Positions of the radiolabel in deltamethrin metabolite studies**



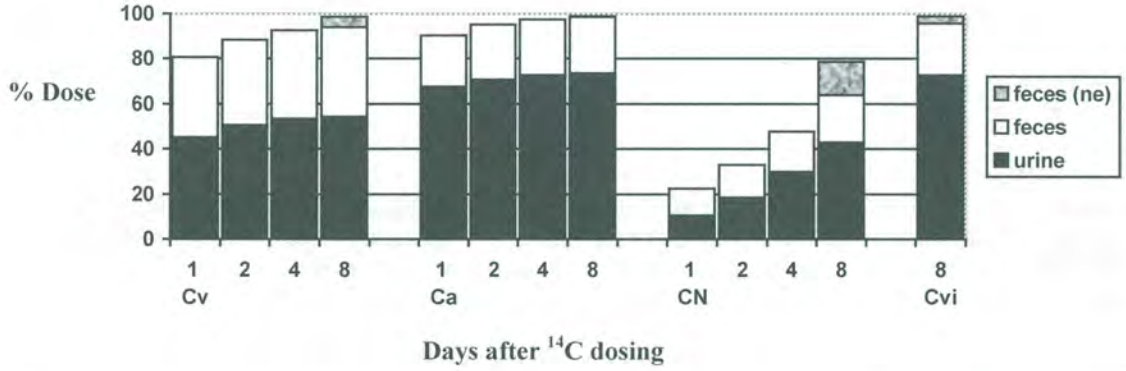

Urine, faeces and expired $CO_2$ were collected for 2 - 8 days. No radiolabelled $CO_2$ was expired and the cumulative totals for each labelled compound are shown in Figure 2. The excretion of radioactivity into the urine was greater than in the faeces and indicates that the drug was readily absorbed. The radiolabels at $^{14}$Cv and $^{14}$Ca were very rapidly excreted in the first day and almost completely excreted after 8 days. This contrasts with the excretion pattern for the $^{14}$CN label where there was a more gradual excretion and <80% was excreted after 8 days. This difference is caused by the metabolism of deltamethrin and suggests that the metabolites containing the $^{14}$CN label are incorporated into less readily excreted components. This was also supported by the distribution of the three different radiolabelled

deltamethrins in tissues collected at 8 days after dosing. The concentration of $^{14}C$ as deltamethrin equivalents was higher for the $^{14}CN$ label in most of the tissues and especially in bone, intestine, muscle, lung, heart, skin, spleen, stomach and testes. The differences were less marked in fat, blood, brain, kidney and liver.

Mice

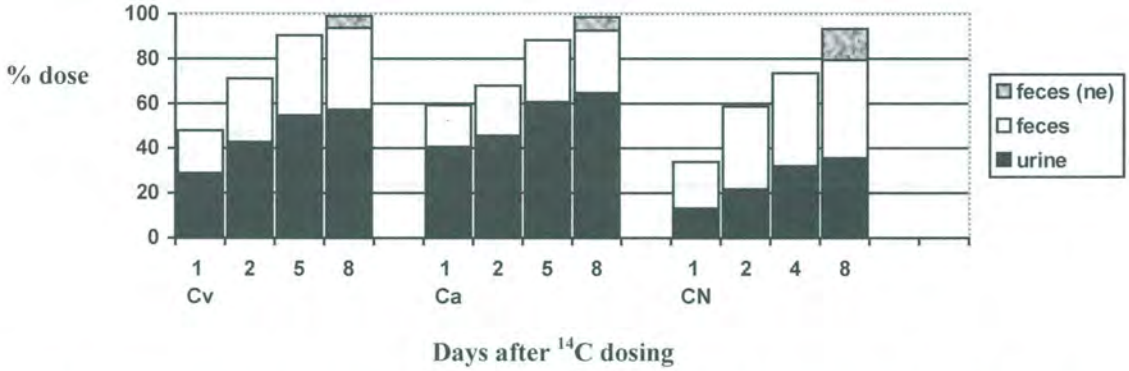
Male mice (18 - 20g) were orally dosed with the same three $^{14}C$-radiolabeled deltamethrin compounds in a similar pharmacokinetics study to that employed for the rat studies discussed above (Ruzo *et al.*, 1979). The excretion of the same three specifically labelled compounds is shown in Figure 3.

**Figure 2. Percent of radiolabelled dose excreted in rats.**




Cv = $^{14}Cv$ –deltamethrin at 0.9 mg/kg BW; Ca = $^{14}Ca$-deltamethrin at 1.6 mg/kg BW; CN = $^{14}CN$-deltamethrin at 0.64 mg/kg BW; Cvi = 1RS-$^{14}Cv$-deltamethrin at 0.94 mg/kg BW; faeces (ne) is the total of non-extractable $^{14}C$ in the faeces over the 8-day period.

**Figure 3. Percent of radiolabelled dose excreted in mice.**




Cv = $^{14}Cv$ –deltamethrin at 4.4 mg/kg BW; Ca = $^{14}Ca$-deltamethrin at 1.7 mg/kg BW; CN = $^{14}CN$-deltamethrin at 2.2 mg/kg BW; faeces (ne) is the total of non-extractable $^{14}C$ in the faeces over the 8 day period.

The distribution of residues in the tissues was similar in both rats and mice for each of the three separately labelled deltamethrins.

No measurements were made of the bioavailability, absorption and blood pharmacokinetics in either rats or mice.

**Pharmacokinetics in Food Animals**

Cattle

Two studies, using the same cow for each study, was carried out to measure the pharmacokinetics parameters for both oral and dermal application of $^{14}$Ca-deltamethrin [$^{14}$C-benzyl-deltamethrin, see Figure 2], (Dowling *et al.*, 1979). The radiochemical purity was ≤ 98.9%. A lactating cow weighing 350 kg was dosed intrarumenally with 0.27 g of $^{14}$Ca - deltamethrin. Blood, milk, faeces and urine were collected over a 6 or 10 day period after dosing. Over a 6-day period 51% and 28% of the dose were excreted into the faeces and urine respectively. The concentration of radiolabel in the blood and whole milk were similar, reaching peak values within 24 hours and declining to < 1 μg/kg in 5 - 8 days with half lives of <1 day. The majority (95%) of the radioactivity in milk was in the fat phase, of which 89% was radiolabelled parent drug.

Forty-nine days after the intrarumenal administration, 0.21 g of $^{14}$Ca-deltamethrin was applied topically to the cow. The dose was in a 1L solution and applied using a brush to paint the hair of the cow but not to the udder. Blood and milk were collected over a 10-day period and hair samples were taken for 45 days post-dosing. No measurements were made on excreta. The concentrations of radiolabel in the blood and whole milk were again similar, reaching peak values within 2.5 days and declining to < 1 μg/kg in about 9 weeks, with half lives in milk and butterfat of 4.3 and 4.4 days, respectively. The majority (95%) of the radiolabel in milk was contained in the fat phase. The radiolabel, probably as intact $^{14}$Ca-deltamethrin, remained above 1mg/kg in the hair for approximately 75 days around the head and for about 100 days on the body hair. The mechanism of absorption of the drug from the coat was not clear but was most likely by ingestion through the cow licking herself.

A more recent study in two lactating cows provides more information on the absorption of the residue following a topical application in which the cows were not allowed to lick the application sites (Whalen, 1995). A 2.0% deltamethrin cattle pour-on formulation containing either $^{14}$Ca-deltamethrin or $^{14}$Cgd-deltamethrin (see Figure 1)) was administered topically to a dairy cow for three consecutive days. One cow received 1.47 mg/kg BW/d and 44.5 μCi/kg/d of $^{14}$Ca-deltamethrin and the other cow received 1.50 mg/kg BW/d and 46.4 μCi/kg/d of $^{14}$Cgd-deltamethrin. The radiochemical purity for both compounds was > 96%. The high dose level, about four times the recommended dose rate, was necessary to allow accurate evaluation of the extent of absorption and to facilitate metabolite identification. The doses were administered to the skin within an enclosure encircling the lumbar region of the back. The dose site was covered with a non-occlusive cover. Blood samples were collected pre-dose, at 1, 6, and 12 hours after the first dose, immediately before the 2nd and 3rd dose applications, and at sacrifice. Milk, urine, and faeces were collected twice daily (a.m. and p.m.). The animals were sacrificed approximately 24 hours after the last dose application and tissue samples were collected. Samples were analysed for radioactivity.

The total recovery of radioactivity from the cow treated with $^{14}$Ca-deltamethrin was 80% that of the administered dose. A majority of the dose was recovered in the skin wash and wipe (48%) and in the dose enclosure (18.9%). The skin at the dose site contained 10.3% of the administered dose. Excretion of radioactivity in the faeces accounted for 0.6% of the administered dose and urine accounted for 0.3%. Less than 0.01% of the administered radioactive dose was recovered in the milk, bile, or tissues collected. The radioactivity was well contained within the dose site because only 0.51% was recovered in a wash of the area surrounding the dose application site, 1.95% in the non-occlusive cover and 0.06% in the stall wash and wipe.

For the cow treated with $^{14}$Cgd-deltamethrin, the total recovery of radioactivity was 85% that of the administered dose. A majority of the dose was recovered in the skin wash and wipe (36%) and in the dose enclosure (37%). The skin at the dose site contained 10.1% of the administered dose. The excretion of radioactivity in faeces and urine accounted for 0.2% and 0.5% of the administered dose, respectively. Less than 0.01% of the administered dose was recovered in the milk or in the bile and tissues collected at sacrifice. The radioactivity was well contained within the dose site because only 0.5% was recovered in a wash of the skin surrounding the dose application site, 1.1% in the non-occlusive cover, and 0.1% in the stall wash and wipe.

The results were similar for both $^{14}$Ca-deltamethrin and $^{14}$Cgd-deltamethrin and indicate that at least 11% of the radioactive dose was absorbed and that about 70% of the administered dose remained at the dose application site. No metabolism of deltamethrin was observed in the skin at the dose site because essentially all radioactivity (greater than 95%) found in skin was unchanged $^{14}$C-deltamethrin. Data for blood levels of radioactivity indicate that the radioactivity is rapidly absorbed and transported systemically. However, the blood concentrations remain low

throughout the study, with values of 1 and 4 μg/L for $^{14}$Ca-deltamethrin and $^{14}$Cgd-deltamethrin, respectively, at 1 hour post dose and less than 1 μg/L for either radiolabelled deltamethrin at 12 hours post dose.

Radioactive residues were low for all tissues analysed, ranging from 1 μg/kg in muscle to 13 μg/kg in liver. No radioactivity was detected in the blood collected at sacrifice. Whole milk contained up to 2 μg/L deltamethrin equivalents and the radioactivity was located in the cream and not in the skim milk.

Swine and Sheep

No pharmacokinetics studies were carried out.

Chickens

The fate of deltamethrin in laying hens, after both topical and oral dosing, has been determined in a study performed to GLP (Whittle, 1997). $^{14}$Ca-deltamethrin and $^{14}$Cgd-deltamethrin were administered, either topically or orally, to four individual groups of hens, six hens in each group, once daily for three consecutive days at a nominal dose level of 0.15 mg/kg BW. Birds were sacrificed approximately 23 hours after the final dose administration.

After topical application of $^{14}$Cgd-deltamethrin, between 32% and 62% of the dose was recovered from the application site feathers and between 3.0% and 12.6% was found on the application site dressings. Radioactivity in the excreta accounted for between 1.2% and 3.7% that of the total applied dose. Concentrations of radiolabelled residues in eggs were below the LOD (1.0 μg/kg). Concentrations of radiolabelled residues in tissues remote from the application site were highest in the liver (5.0 - 17.5 μg/kg), followed by whole blood (1.1 - 3.5 μg/kg) and skin/fat (2.0 - 6.4 μg/kg), respectively. Residues in muscle samples were at or below the LOD while plasma concentrations were between 1.1 μg/kg and 3.5 μg/kg.

Similarly, for hens dosed topically with $^{14}$Ca-deltamethrin, between 41% and 53% of the administered dose was found on the application site feathers and 1.9 - 8.3% found on the dressings. Radiolabelled residues in excreta accounted for between 1.0% and 2.5% of the total dose whereas levels of residues in eggs were below the LOD (1.0 μg/kg). Concentrations of radiolabelled residues in the tissues were highest in the liver (1.4 - 5.6 μg/kg), followed by whole blood (0.5 - 1.2 μg/kg) and skin/fat (1.0 - 19.7 μg/kg). Residues in muscle samples were at or below the LOD. Levels of radiolabelled residues in plasma were 0.5 - 1.4 μg/kg.

After oral administration of the labelled compounds, 73 - 99% of the total radioactivity was recovered, of which radioactivity in the excreta accounted for a mean of 95% of the administered $^{14}$Cgd-deltamethrin and 84% of the administered $^{14}$Ca- dm, respectively. Concentrations of radioactivity in eggs were below the LOD (3.8 μg/kg). Radiolabelled residues were present in the liver, whilst in the remaining tissues they were below the LOD. The concentration of radiolabelled residues in plasma were 0.6 - 15.3 μg/kg for $^{14}$Cgd-deltamethrin but were <0.42 μg/kg (LOD) for the $^{14}$Ca- deltamethrin.

Salmon

55 Atlantic salmon (140 ± 26 g), maintained at 12°C in sea water, were dipped for 30 min. in sea water containing 5 μg/L of a 1:1 mixture of $^{14}$Cgd-deltamethrin and $^{14}$Ca- deltamethrin. The fish were sampled between 1 hour and 10 days. In another study 13 salmon, similarly maintained, were administered the radiolabelled mixture as an intravascular dose of 0.25 mg/kg BW. The drug was absorbed after the dip treatment and residues were present in the tissues. The distribution of residues was followed after the parental administration and a tentative half-life of in blood was calculated as 54 hours (Horsberg and Ingebrigtsen, 1998).

**Metabolism in Toxicological Test Species**

Rats

The metabolic pathway of deltamethrin in rats was based on identified metabolites with support, by analogy, with permethrin metabolism in rats (Gaughan et al., 1977a). The pathways involved in rat metabolism of the deltamethrin isomers are similar to those utilised for other pyrethroids in many segments of the ecosystem (Elliott, 1977; Gaughan *et al.,* 1977a, b; Miyamoto, 1976).

Male rats, 140 - 160g, were dosed orally at 0.64 - 1.6 mg/kg BW with three labelled $^{14}$C-deltamethrins. Figure 1 shows the positions of the radiolabel in $^{14}$Cv-, $^{14}$Ca - or $^{14}$CN-deltamethrins used in this study (Ruzo *et al.*, 1978). The metabolites were identified by a series of TLC separations, combined with autoradiography using authentic standards. A portion of an oral dose was excreted in the faeces without metabolism. Since there is no significant production of expired $^{14}CO_2$, the pathways did not include extensive fragmentation of the acid and alcohol moieties. The principal mechanisms of metabolism were ester cleavage and oxidation at the 4'-position of the *m*-phenoxyphenyl- moiety. Additional minor oxidation sites were at the 5 and 2' positions of the *m*-phenoxyphenyl moiety and on the methyl group *trans* to the carboxyl group of the acid moiety. The ester metabolites did not form conjugates but the corresponding acids underwent extensive conjugation at both the phenolic hydroxyl and carboxylic acid groups.

The acid moiety was rapidly excreted, principally as the glucuronide with smaller amounts free acid and the glycine conjugate. The *trans*-hydroxymethyl derivative was also excreted as free acid and as the glucuronide. All major metabolites of the aromatic portion of the alcohol moiety were rapidly excreted and probably arose from ester cleavage of deltamethrin or its ester metabolites, conversion of the released cyanohydrins to the aldehydes which rapidly gave the corresponding acids, and conjugates of these acids. 3-Phenoxybenzoic acid was excreted either unconjugated or as their glucuronide and glycine conjugates. The major metabolite, the sulfate of 4'-hydroxy-3-phenoxybenzoic acid, was probably formed by hydroxylation of the phenoxy group and ester cleavage, in an undetermined sequence, followed by oxidation to the benzoic acid and sulfate conjugation. Cleavage of the deltamethrin ester group led to release of cyanide which was converted mainly to thiocyanate and a small amount of 2-iminothiazolidine-4-carboxlylic acid, the latter via reaction with cystine and related substances (See Casida, et. al)

The slow release of thiocyanate from the body was due in part to selective tissue retention. The localisation pattern of $^{14}$C from $^{14}$CN-deltamethrin was probably a characteristic of thiocyanate localisation rather than an indication of the site of deltamethrin metabolism.

Mice

Male mice (18 - 20 g) were orally dosed (Ruzo *et al.*, 1978) with the same three $^{14}$C-radiolabeled deltamethrin compounds in a similar pharmacokinetics study to that for rats (Ruzo *et al.*, 1979, *vide supra*). The identification of the metabolites was by methods similar to those used in the rat study. Two factors contributed to the rapid detoxification of deltamethrin in mice: the relevant esterases were present in many tissues and the oxidases in at least liver microsomes. Deltamethrin metabolism and the resulting detoxification in mice involved 4 sites of oxidative attack (trans methyl group of the acid moiety and 2', 4', and 5 positions of the *m*-phenoxyphenyl moiety), hydrolysis, and a variety of conjugation processes. Mice excreted less unmetabolised deltamethrin than rats (Ruzo *et al.*. 1978), suggesting a more efficient absorption and/or metabolism. Whereas rats hydroxylated deltamethrin predominantly at the 4' position, mice produced considerable amounts of the *trans*-2'- and 5-hydroxy derivatives. Portions of the deltamethrin and 4'-hydroxydeltamethrin were detected as the $\alpha$-R-epimers probably due to an artefact resulting from isomerisation of the proton $\alpha$- to the cyano-group (Ruzo *et al.*, 1977. 1978) during sample handling. The acid moiety was rapidly excreted as the glucuronide with smaller amounts of free and the glycine conjugate. The *trans* hydroxymethyl derivative was also excreted free, as well as the glucuronide and the sulfate conjugate. This sulfate conjugate was not detected in rats.

Deltamethrin hydrolysis yielded phenoxybenzylcyanohydrin (Shono *et al.*, 1979), which readily degraded to 3-phenoxybenzaldehyde and HCN (Ruzo *et al.*. 1977). The metabolites of the benzyl alcohol moiety formed from 3-phenoxybenzaldehyde generally follow the same pathways in mice as in rats (Ruzo *et al.*, 1978) with some important exceptions. Extensive conjugation of 3-phenoxybenzoic acid with taurine was found only in mice (Hutson and Casida, 1978) and only mice excreted glucuronides of 4'-hydroxyphenoxybenzyl alcohol and 5-hydroxy-3-phenoxybenzoic acid. Mouse excreta contained 3-phenoxybenzaldehyde and 3-phenoxybenzyl alcohol and its glucuronide, not present in the excreta of rats. Also, the aldehyde metabolite appeared to be less easily oxidized in mice than in rats, so that

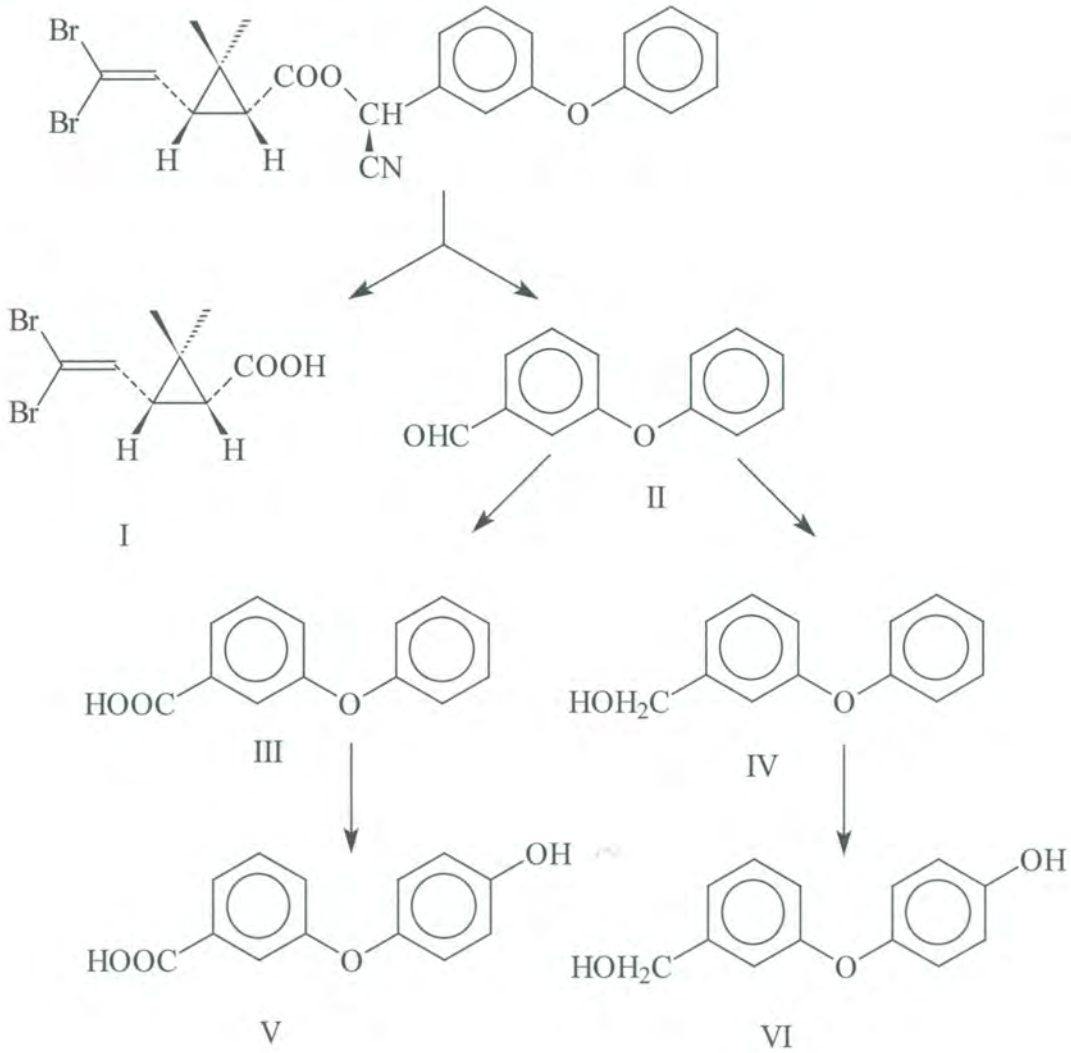
some was excreted *per se* and a portion was also reduced to the benzyl alcohol. Whereas rats converted HCN into thiocyanate and iminothiazolidine carboxylic acid, only thiocyanate was obtained by metabolism of HCN in mice.

**Metabolism in Food Animals**

Cattle

The metabolism of deltamethrin in farm animals is extensive and this, associated with the rapid degradation and clearance of the drug, presents a difficult challenge to identifying the biotransformations that do take place. Akhtar (1984) investigated the metabolism *in vitro* using bovine and chicken liver enzyme preparations incubated with either $^{14}$Cgd-deltamethrin or $^{14}$Ca-deltamethrin. Using chromatography and mass-spectrometry he was able to identify 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxlyic acid ($Br_2CA$)(I), 3-phenoxybenzaldehyde (II), 3-phenoxybenzoic acid (III), 3-phenoxybenzyl alcohol (IV), 3-(4-hydroxyphenoxy)benzoic acid (V) and 3-(4'-hydroxyphenoxy)benzyl alcohol (VI). The metabolic pathway proposed for bovine and chicken liver metabolism is shown in Figure 4.

**Figure 4. The biotransformation of Deltamethrin in *in vitro* liver preparations cattle and poultry.**



A 2.0% deltamethrin cattle pour-on formulation, containing either $^{14}$Ca or $^{14}$Cgd-deltamethrin,, was administered topically to dairy cows for three consecutive days (Whalen, 1995) (see Pharmacokinetics section for detail). The liver, kidneys, renal fat and cream were analysed to determine the nature of the radioactive residues. No individual radioactive metabolites, extracts, or residual radioactivity accounted for more than 10 µg/kg deltamethrin equivalents. $^{14}$C-Deltamethrin was the primary radioactive component in the renal fat and cream accounting for 48.4% (4 µg/kg) and 42.1 (4 µg/kg) % of total residues, respectively, for $^{14}$Ca-deltamethrin and for 59.4% (7 µg/kg) and 54.7% (5 µg/kg) of total residues, respectively, for $^{14}$Cgd-deltamethrin. Little, if any, $^{14}$C-deltamethrin was found in the liver and kidney, indicating that considerable metabolism occurred in these tissues. Polar metabolites, including N-(3-phenoxybenzoyl)-L-glutamate, were found in the liver (31% of total residues) and kidney (33% of total residues) of the $^{14}$Ca-deltamethrin treated cow, and up to seven metabolites (including trace amounts of $Br_2CA$) were found in the and kidney tissue from the $^{14}$Cgd-deltamethrin treated cow. Residual radioactivity that remained in liver and kidney tissues after extraction with organic solvents, was released (solubilised) by hydrolysis of the residue with 3*N*-HCl. The percentage of deltamethrin of the total residues in the edible tissues is shown later in the discussion of Table 4.

Chickens

The metabolism of deltamethrin was studied in liver preparations by Akhtar (1984) and is similar to that for cattle (see above).

Excreta from birds dosed topically (with both $^{14}$Ca-deltamethrin and $^{14}$Cgd-deltamethrin), were extracted and analysed by HPLC and TLC (Whittle, 1997). The major metabolites identified were polar materials and deltamethrin accounting for ca 0.l% of the dose. Analysis of pooled excreta extracts from orally dosed birds showed deltamethrin to be the major component accounting for approximately 30% of the sample radioactivity in birds dosed with both labelled forms of deltamethrin. Other minor components were resolved but these accounted for 1% or less of the sample radioactivity and did not correspond to available reference compounds except for a metabolite (from $^{14}$Cgd-deltamethrin-dosed birds) which was tentatively identified as $Br_2CA$ (I in Figure 4).

Liver samples from each group of hens were pooled, extracted with organic solvent and the extracts analysed by both HPLC and TLC. For birds dosed with $^{14}$Cgd-deltamethrin, the major components resolved were polar metabolites (18.5% total radioactivity (TR) in orally dosed hens and 1.8% TR in the topically dosed hens) and deltamethrin (1.6% TR in oral hens and 5.3% TR in topically dosed hens). A further metabolite was also detected in orally dosed hens but this did not correspond to available reference compounds. Similarly, for hens dosed with $^{14}$Ca-deltamethrin, the major components resolved were polar material (5.7% TR for orally dosed hens and 5.4% TR for topically dosed hens) and deltamethrin (3.1% TR for orally dosed hens and 10.9% for topically dosed l hens). The remaining metabolites did not correspond with the available reference compounds. Extracts of the application site skin, feathers, occlusive cover and swabs from the topically dosed hens were analysed by HPLC and TLC. In all the samples the major component was unchanged deltamethrin and no other significant component was separated. The percentage of total deltamethrin radioactivity in the edible tissues and excreta is shown in Table 1.

**Table 1. Percentage of total residues (TR) found as parent drug measured by HPLC and non-extractable residues in cows and hens following dermal application of $^{14}$C-deltamethrin.**

| Tissue | % TR as deltamethrin ($^{14}$C-gem-dimethyl) | | % TR as deltamethrin ($^{14}$C-benzyl) | | % Non-extractable | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $^{14}$C-gem-dimethyl | | $^{14}$C-benzyl | |
| | Cow | Hen | Cow | Hen | Cow | Hen | Cow | Hen |
| Skin* | 95 | 94.4 | 97.3 | 88.9 | 1.5 | | 0.8 | |
| Liver | 3.9 | 5.3 | ND | 10.9 | 63.7 | 42.9 | 63.6 | 68.5 |
| Kidney | 3.1 | nm | ND | nm | 33.9 | nm | | nm |
| Fat | 59.4 | nm | 48.4 | nm | NA | nm | 30.5 | nm |
| Excreta | nm | 32 | nm | 32 | nm | 6-10 | 0 | 3-6 |
| Cream | 54.7 | NA | 42.1 | NA | 0 | NA | nm | NA |

* skin at the application site nm = not measured; NA = not applicable.

Pigs and Sheep

No metabolism studies reported.

## TISSUE RESIDUE DEPLETION STUDIES

### Radiolabelled Residue Depletion Studies

The depletion of radiolabelled $^{14}$C-deltamethrin following topical application as a veterinary drug was studied in cattle and chickens. Studies using orally administered drug have been reported and assessed by JMPR (1990) as part of the assessment as an agricultural pesticide. Successful long acting topical application necessitates a parasiticidal dose on the exterior of the treated animal for the duration of the claimed antiparasitic activity. Thus, typical of the characteristic residue pattern is the high and persistent residues of deltamethrin at the site of application. The relatively small percentage of the parent that is absorbed through the skin or by ingestion, enters the fat compartments or is rapidly metabolised.

Cattle

The depletion of $^{14}$C-deltamethrin following topical application of a pour-on formulation of 2 mg/kg BW to three heifers (200 – 240 kg BW) has been reported (Dowling et al, 1981). Heifers were sacrificed at 3, 7 and 14 days after dosing and tissue samples collected for analysis of total residues and parent drug. The results are shown in Table 2. The residues in fat persisted over 14 days and were almost entirely deltamethrin. A significant proportion of the very low residues detected in muscle were also parent drug. Less than of the total residues in kidneys was deltamethrin, except in one heifer at 14 days, which is probably an erroneous result,. High residue levels persisted in liver. However, only less than <20% of these residues were extractable and residues of parent deltamethrin were too small to be measured.

**Table 2. Total residues and percentage as parent drug after a pour-on dose of 2 mg/kg BW of $^{14}$C-deltamethrin to heifers**

| Tissue | | 3 days(µg/kg) | (%) | 7 days(µg/kg) | (%) | 14 days(µg/kg) | (%) |
|---|---|---|---|---|---|---|---|
| Muscle | Gluteous | 6 | 33 | 8 | 75 | 6 | 33 |
| | Psoas | 12 | 58 | 7 | 57 | 7 | 67 |
| Liver | | 214 | 16* | 32 | 12* | 309 | 6.7* |
| Kidney | | 81 | 7 | 79 | 8 | 48 | 60? |
| Fat | Renal | 119 | 82 | 221 | 86 | 185 | 81 |
| | Omental | 69 | 96 | 121 | 92 | 129 | 99 |

- * = % of radioactivity which was extractable with organic solvents. ? = possibly an erroneous result

A 2.0% deltamethrin cattle pour-on formulation containing either $^{14}$Ca-deltamethrin or $^{14}$Cgd-deltamethrin was administered topically to dairy cows for three consecutive days (Whalen, 1995). One cow received $^{14}$Ca- deltamethrin (1.47 mg/kg BW/day and 44.5 µCi/kg/day) and one cow received $^{14}$Cgd-deltamethrin (1.50 mg/kg BW/day and 46.4 µCi/kg/day). The radiochemical purity for both compounds was > 96%. The dose level, was about 3 - 4 times the label dose rate. The animals were sacrificed 24 hours after the last dose. The liver, kidneys, renal fat, and cream (from the Day 4 a.m. milk collection) were analysed to determine the nature of the radioactive residues. The total residues in edible tissues and application site are shown in Table 3.

The liver, kidneys, renal fat and cream were analysed to determine the nature of the radioactive residues. No individual radioactive metabolites, extracts, or residual radioactivity accounted for more than 10 µg/kg deltamethrin equivalents. $^{14}$C-Deltamethrin was the primary radioactive component in the renal fat (4 -7 µg/kg) and cream (4 - 5 µg/kg). Little, if any, $^{14}$C-deltamethrin was found in the liver and kidney indicating that considerable metabolism occurred in these tissues. 3-Phenoxybenzoyl-L-glutamate was found in the liver and kidney of the $^{14}$Ca-deltamethrin-treated cow and up

to six metabolites (including trace amounts of $Br_2CA$) were found in the liver and kidney tissues of the cow treated with $^{14}$Cgd-deltamethrin-. The skin at the application site contained mostly unchanged deltamethrin.

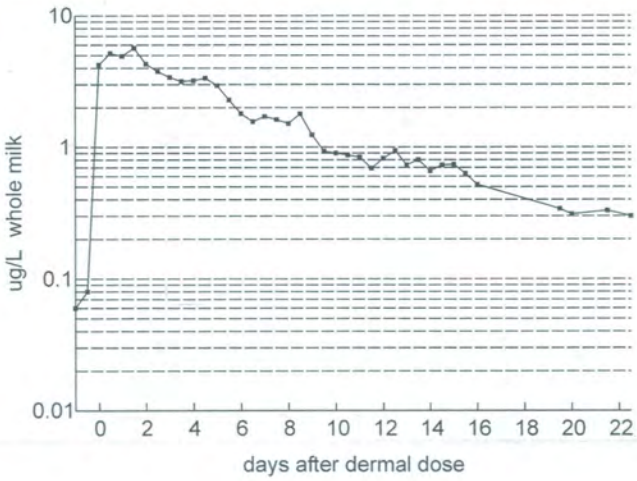
**Table 3. Total residues and percentage as parent drug in cows 1 day after 3 daily topical doses of 1.47 mg/kg BW of $^{14}$Ca-deltamethrin or 1.5 mg/kg BW of $^{14}$Cgd-deltamethrin.**

| Tissue | $^{14}$Ca-deltamethrin | | $^{14}$Cgd –deltamethrin | |
|---|---|---|---|---|
| | (µg/kg) | (%) | (µg/kg) | (%) |
| Muscle | 2 | - | 1 | - |
| Liver | 13 | - | 9 | 3.9 |
| Kidney | 10 | - | 10 | 3.1 |
| Fat omental | 8 | - | 4 | - |
| Renal | 9 | 62.2 | 11 | 59.4 |
| Skin (test site) | 226 | 97.3 | 214 | 95.8 |
| Cream (at sacrifice) | 9 | 42.1 | 10 | 54.7 |
| Milk (at sacrifice) | 1 | | 2 | |

Residue Depletion in Milk

A dairy cow was treated externally with 0.21 g (ca. 0.55 mg/kg BW) of $^{14}$Cgd-deltamethrin ((Dowling *et al.*, 1979). Milk samples were collected twice daily and the radioactivity in the whole milk determined. The results are shown in Figure 5. The butter fat was rendered and the radioactivity measured. There was a consistent ratio of 20 between the concentration in the butterfat and the whole milk indicating that most of the residue was in the fat. The maximum level in whole milk was 5.7 µg/L, two and a half days after dosing. The terminal half-lives in milk and butter fat were about 4 days.

**Figure 5. The depletion of radioactivity (as equivalents of $^{14}$C-deltamethrin) in bovine milk after a topical application of 0.21g (ca. 0.55 mg/kg BW) $^{14}$Cgd- deltamethrin.**




Chickens

$^{14}$Ca-deltamethrin and $^{14}$Cgd-deltamethrin were administered topically to individual groups of hens once daily for three consecutive days at a nominal dose level of 0.15 mg/kg BW. Birds were sacrificed approximately 23 hours after the

final dose administration. Radioactivity of the residues was measured in muscle, liver, skin/fat and eggs. The results are shown below in Table 4.

All residues in eggs and breast muscle and in some of the leg muscles were below the limits of detection of the analytical method. The dose applied to the hens is 10 times lower than that used in the cattle studies and thus it is not surprising that the residues are lower in hens than cattle

**Table 4. Total residues in hens 23 hours after dermal dosing daily for three days with $^{14}$C-deltamethrin at 0.15 mg/kg BW.**

| Tissue | $^{14}$Ca- deltamethrin | | $^{14}$Cgd-deltamethrin | |
|---|---|---|---|---|
| | Range (µg/kg) | Mean ± SD (µg/kg) | Range (µg/kg) | Mean + SD (µg/kg) |
| Muscle Leg | 0.8 – 1.8 | NA | <0.8 – 4.9 | NA |
| Muscle Breast | <0.8 | NA | <1.0 | NA |
| Liver | 1.4 – 5.6 | 3.85 ± 1.77 (21%)* | 5.0 – 17.5 | 9.2 ± 4.9 (13%)* |
| Skin/Fat | 1.0 – 19.7 | 5.53 ± 7.12 | 2.0 – 6.4 | 4.47 ± 1.91 |
| Eggs | <1 | | <1 | |

The results are expressed as equivalents of $^{14}$C-deltamethrin. There were six hens per group. * = percentage of total residues found as parent drug.

Pigs and Sheep

No radio-depletion studies were reported.

Salmon

55 Atlantic salmon (140 ± 26 g) were dipped for 30 minutes in sea water at 12°C containing 5 µg/L of a 1:1 mixture of $^{14}$Cgd- deltamethrin and $^{14}$Ca-deltamethrin. The fish were sampled between 1 hour and 10 days (Horsberg and Ingebrigtsen, 1998). The depletion of total residues in edible tissues is shown in Table 5.

**Table 5. Total residues (µg/kg) of $^{14}$C-deltamethrin in tissues of salmon maintained at 12°C.**

| Hours after treatment | Muscle | Liver | Kidney | Skin |
|---|---|---|---|---|
| 1 | 1.57 | 95.4 | 40.7 | 11.4 |
| 4 | 3.83 | 82.3 | 18.9 | 12.9 |
| 8 | 2.88 | 25.3 | 9.61 | 10.6 |
| 12 | 2.59 | 18.0 | 8.00 | 8.37 |
| 24 | 3.6 | 9.80 | 7.02 | 5.93 |
| 48 | 5.29 | 6.53 | 4.52 | 4.82 |
| 72 | 8.52 | 4.80 | 2.15 | 4.64 |
| 96 | 2.46 | 2.70 | 2.49 | 4.55 |
| 168 | 1.02 | 2.50 | 1.95 | 3.92 |
| 240 | 1.00 | 2.96 | 2.28 | 3.31 |

**Residue Depletion Studies with Unlabelled Drug**

The residues of deltamethrin following topical and parental administration were fully reviewed by JMPR (1990). Since then only one new study is reported (McKinney and Crotts, 1995) in which two doses of a pour-on preparation were applied to lactating cattle. A summary of the highest residues found in the several species is shown in Table 6.

**Table 6. Maximum residues (µg/kg) of Deltamethrin following topical administration.**

| Species | No | Treatment | No | Dose | M | L | K | F | S | E | Mk | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cow | 12 | Bath | 1 | 62 mg/L | <2 | <2 | <2 | 70 | | | | HIDH 80-1 |
| Cow | 9 | Bath | 27 | 50 mg/L | | | | 148 | | | | HIBH 82-5 |
| Cow | 17 | Bath | 57 | 18-52 mg/L | | | | 130 | | | | HIDH 81-1 |
| Calf | 9 | Pour-on | 1 | 0.5 mg/kg BW | | | | 180 | | | | HCAH 83-5 |
| Cow | 6 | Pour-on | 1 | 0.25 mg/kg BW | | | | | | | 9 | HIBH 83-5 |
| Cow | 3 | Pour-on | 4<br>+ 1 | 0.66 mg/kg BW<br>1 mg/kg BW | | | | | | | 11 | CIBH 85-C1 |
| Cow | 12 | Pour-on | 4<br>+ 1 | 0.66 mg/kg BW<br>1 mg/kg BW | | | | 155 | | | | CIBH 85-C2 |
| Cow | 2<br>2 | Pour-on<br>Pour-on | 1<br>1 | 0.2 mg/kg BW<br>2 mg/kg BW | | | | | | | 16<br>53 | VENANT et al, 19?? |
| Cow | 6 | Pour-on | 6 | 0.75 mg/kg BW | | | | | | | 11 | E 236 |
| Calf | 24 | Pour-on | 1 | 0.75 mg/kg BW | 30 | 5 | 34 | 220 | | | | E 249 |
| Cow | 24 | Pour-on | 1 | 0.75 mg/kg BW | 14 | <2.5 | 20 | 370 | | | | E 316 |
| Cow | 6 | Spray | 1 | 1 mg/kg BW | | | | | | | 7 | E 224 |
| Cow | 6 | Spray | 1 | 1 mg/kg BW | | | | | | | 11 | E 240 |
| Calf | 24 | Spray | 1 | 1 mg/kg BW | 14 | <2.5 | 13 | 361 | | | | E 248 |
| Sheep | 10 | Bath | 1 | 15 mg/L | | | | 430 | | | | HIBH 81-1 |
| Sheep | 12 | Bath | 1 | 15 mg/L | | | | 500 | | | | AITH 81-3 |
| Sheep | 12 | Bath | 1 | 11 mg/L | 32 | <5 | <5 | 19 | | | | HIBH 83-2 |
| Sheep | 9 | Pour-on | 1 | 2.5 mg/kg BW | | | | 42 | | | | CIBH 85-C2 |
| Sheep | 9 | Pour-on | 1 | 4 mg/kg BW | | | | 60 | | | | HITH 80-1 |
| Sheep | 11 | Pour-on | 1 | 4 mg/kg BW | <30 | <30 | <30 | 80 | | | | HITH 80-2 |
| Pig | 9 | Pour-on | 1 | 1 mg/kg BW | <10 | 11 | <10 | 201 | 3243[a] | | | HIBH 84-1 |
| Pig | 9 | Pour-on | 1 | 0.75 mg/kg BW | <7 | <10 | <7 | <7 | | | | Scheid 1986 |
| Hen | 75 | Spray | 1 | 50 g/L | <12 | <7 | <8 | <13 | | <15 | | Ansoborlo |

[a] skin from site of administration 3 days after dosing, highest residue in skin at non-application site was 40
M = muscle, L = liver, K = kidney, F = fat, S = skin, E = eggs, Mk = milk

Cattle

Six lactating cows were dosed with a pour-on formulation of deltamethrin at 0.4 mg/kg BW, followed by a repeat dose 7 days later. A further group of twelve lactating cows were similarly treated but dosed with 1.6 mg/kg BW. Cows were milked twice daily until slaughter on days, 3, 7 or 14 after the second treatment. Samples of edible tissues were taken for residue analysis for total deltamethrin (the sum of α–R, *cis*- and *trans*- deltamethrin) using gas chromatography with electron capture (GC-ECD) (McKinney and Clayton, 1995). The results for the residues are shown in Table 7.

**Table.7. Concentration range of residues of deltamethrin after treatment with pour-on preparations to lactating cattle (McKinney and Clayton, 1995).**

| Tissue | Dose | LOQ (µg/kg) | 1 day (µg/kg) | 3 days (µg/kg) | 7 days (µg/kg) | 14 days (µg/kg) |
|---|---|---|---|---|---|---|
| Muscle | Low | 15 | <LOQ | NA | <LOQ | NA |
| | High | | <LOQ | <LOQ | <LOQ | <LOQ |
| Liver | Low | 15 | Nm | NA | Nm | NA |
| | High | | <LOQ | <LOQ | <LOQ | <LOQ |
| Kidney | Low | 15 | Nm | NA | Nm | NA |
| | High | | <LOQ | <LOQ | <LOQ | <LOQ |
| SC. fat | Low | 45 | <LOQ – 106 | <LOQ | <LOQ | <LOQ |
| | High | | <LOQ - 48 | <LOQ | <LOQ | <LOQ |
| Renal fat | Low | 45 | <LOQ | NA | Nm | NA |
| | High | | 64 – 67 | 46 – 90 | 46 – 74 | 58 – 70 |
| Milk | Low | 15 | Nm | Nm | Nm | Nm |
| | High | | <LOQ | <LOQ | <LOQ | <LOQ |
| Milk fat | Low | 75 | <LOQ – 95 | <LOQ – 80 | Nm | Nm |
| | High | | 111 - 531 | 119 – 282 | 100 – 113 | <LOQ |

Nm = not measured because values predicted <LOQ. NA = not applicable because no low dose animals were slaughtered on days 3 and 14. The values for the tissues are the ranges for 3 animals. All animals not sacrificed were used for the milk measurements. The low dose = 0.4 mg/kg BW and the high dose = 1.6 mg/kg BW.

In an Australian study, cattle were dipped, in a bath containing 62 mg/kg deltamethrin and 320 mg/kg ethion. The animals were slaughtered 1, 3, 6 or 10 days after treatment and edible tissues sampled for residue analysis. Residue levels peaked at 6 days, the maximum residue being 70 µg/kg in perirenal fat. Most residues were 30 µg/kg or lower and residues in samples of liver, kidney and neck muscle did not exceed 2 µg/kg (Dowling *et al.*, 1980a).

In a South African study, cattle were dipped approximately weekly in a bath containing 0.0018 to 0.0057% deltamethrin, slaughtered, and omental and renal fat analysed for residues. For a group of cattle dipped 57 times and slaughtered 14 days after the last dipping, mean residue levels were 30 and 40 µg/kg deltamethrin in omental and renal fat, respectively. For cattle dipped only 3 times and slaughtered 4 days after the last dipping, the corresponding levels were 100 and 110 µg/kg in omental and renal fat, respectively. This latter group were smaller animals and in poorer general condition (Dowling *et al.*, 1980b).

Cattle were treated, in another South African study, by dipping on 27 occasions, at weekly intervals, in a bath that contained a nominal concentration of 50 mg/kg deltamethrin. Selected samples of body fat were removed from animals slaughtered at 1, 4 and 7 days after the last dipping. The highest mean levels of deltamethrin residues detected were 120 µg/kg and 11 µg/kg in omental and perirenal fat, respectively (HIBH 82-5, 1982).

Nine calves were treated with 10 mL of 1% deltamethrin in Miclycol 812 and slaughtered in groups of 3 at intervals of 3, 7 and 14 days after treatment. A further group of 3 animals served as controls. Samples of omental and perirenal fat were analysed for residues. One animal, slaughtered after 3 days, had residues of 175 µg/kg and 99 µg/kg in perirenal and omental fat, respectively. All other samples contained less than 60 µg/kg deltamethrin (HCAH 83-5, 1984).

Six lactating cows were each treated topically with 10 mL of a 1% deltamethrin in Miglycol 812 formulation. The cows were milked twice daily and milk from each cow was retained once or twice daily for 10 days and processed into rendered butterfat, which was analysed for residues of deltamethrin. The highest residue found was 150 µg/kg in butterfat from the milk of one cow collected 2 days (fourth milking) after treatment. It was calculated that this corresponded to a residue of 9 µg/kg in whole milk (HIBH 83-5, 1984).

Three cows were treated with a 1% pour on preparation weekly for 4 weeks with 0.66 mg deltamethrin per kg BW and milk samples were collected. Seven days after the last treatment, the cows were given a single pour on treatment of 1 mg/kg BW and milk samples again collected. The milk samples were processed to rendered butterfat. The highest level of residues was equivalent to 10 µg/kg in whole milk, after treatment with 1 mg/kg deltamethrin (CIBH 85-Cl, 1985).

Four groups, each comprising 3 animals, were dosed weekly at 0.66 mg/kg BW with a 1% w/v deltamethrin pour-on for 6 months. Three groups were killed at 1, 7 or 14 days after the last treatment. The fourth group was treated with a further dose of 1 mg/kg BW and killed 7 days later. There were less than 200 µg/kg deltamethrin residues in all analytical samples of perirenal and omental fat obtained from slaughtered animals (CIBH 85-C5, 1987).

In a study to determine the metabolic fate of deltamethrin in lactating cows, two cows were treated with 0.1 g and two others with 1 g of deltamethrin by pour-on application. Samples of urine, faeces, milk and blood, collected during 8 days, were analysed. About 95% of the total eliminated deltamethrin was excreted in the faeces, with less than 1% excreted in both urine and milk. The maximum residue levels of 16 µg/kg (low dose) and 53 µg/kg (high dose) in milk were observed after 2 days. Residues in milk were l µg/L after 8 days (Venant *et al.*, undated).

Cattle were dipped, in a bath containing 62 mg/kg deltamethrin and 320 mg/kg ethion in an Australian study. The animals were slaughtered 1, 3, 6 or 10 days after treatment and edible tissues sampled for residue analysis. Residue levels peaked at 6 days, the maximum being 70 µg/kg in perirenal fat. Most residues were 30 µg/kg or lower. Residues in samples of liver, kidney and neck muscle did not exceed 2 µg/kg. (HIDH 80-1, 1980)

Cattle dipped approximately weekly, in a South African study, in a bath containing 0.0018 to 0.0057% deltamethrin were slaughtered, and omental and renal fat analysed for residues. For a group of cattle dipped 57 times and slaughtered 14 days after the last dipping, mean residue levels were 30 and 40 µg/kg deltamethrin in omental and renal fat, respectively. For cattle dipped only 3 times and slaughtered 4 days after the last dipping, the corresponding levels were 100 and 110 µg/kg. This latter group were smaller animals and in poorer general condition (HIDH 81-1, 1981).

In a South African study, cattle were treated by dipping at weekly intervals for 27 weeks, in a bath containing a nominal concentration of 50 mg/kg. Selected samples of body fat were removed from animals slaughtered at 1, 4 and 7 days after the last dipping. The highest mean level of deltamethrin residues was 120 µg/kg and 11 µg/kg in omental and perirenal fat respectively (HIBH 82-5, 1982).

Nine calves were treated with 10 ml of 1% deltamethrin in Miclycol 812 and slaughtered in groups of 3 at intervals of 3, 7 and 14 days after treatment. A further group of 3 animals served as controls. Samples of omental and perirenal fat were analyzed for residues. One animal, slaughtered after 3 days, had residues of 18 µg/kg and 10 µg/kg in perirenal and omental fat respectively. All other samples contained less than 60 µg/kg deltamethrin (HCAH 83-5, 1984).

Six lactating cows each were treated topically with 10 ml of a 1% deltamethrin in Miglycol 812 formulation. The cows were milked twice daily and milk from each cow was retained once or twice daily for 10 days and processed into rendered butterfat, which was analysed for residues of deltamethrin. The highest residue found was 15 µg/kg in butterfat from the milk of one cow collected 2 days (fourth milking) after treatment. It was calculated that this corresponded to a residue of 9 µg/kg in whole milk (HIBH 83-5, 1984).

Six lactating cows were treated with deltamethrin pour-on at 0.75 mg/kg BW weight and the residues of deltamethrin determined in the butterfat from the milk of each cow, up to the 10th milking after application. Some variations between animals were observed but the results generally confirm other studies. The highest residue in the milk of an individual cow was 10 µg/kg after 2 days, and the highest mean residue was 4 µg/kg after 3 days (E 236, 1985).

Twenty-four calves, 12 male and 12 female, were divided into four groups of 3 males and 3 females each and treated with deltamethrin pour-on at 0.75 mg/kg BW. The groups of animals were slaughtered after 12 hours, 24 hours, 3 days and 5 days, and samples of muscle, liver, kidney and fat taken for analysis. In muscle and liver, traces did not reach the LOQ, 3 µg/kg for muscle and 5 µg/kg for liver. In kidney, the maximum concentration was 34 µg/kg. The maximum concentration in fat was 220 µg/kg reached on day 3 after treatment (E249. l986).

Six cows were treated with a deltamethrin 50g/L formulation, sprayed on after dilution to a 50 mg/L solution. The dose per animal was ca 1 mg/kg BW. Samples of milk were taken from the 10 milkings after treatment, and butterfat was extracted and analysed. The maximum level of 0.007 mg/kg deltamethrin was observed in whole milk at the 7th milking

on day 3. (Report E224, 1986). A second study using a formulation of 12.5 g/L but the same dose rate showed a maximum level of 10 μg/kg in whole milk (E 240, 1986).

Twenty-four calves were treated with a 12.5 g/L formulation of deltamethrin at 1 mg/kg BW by spraying. Four groups, each of 3 males and 3 females were used and the groups were slaughtered 12 h, 24 h, 3 days and 5 days after treatment and samples of muscle, liver, kidney and fat taken for analysis. Levels were below the limit of determination in liver. Maximum levels in kidney were 13 μg/kg, in muscle 14 μg/kg and in fat 360 μg/kg (at 3 days after application) (Report E 248. 1986).

Sheep

In an Australian study, ten sheep were treated by dipping in a bath with a nominal concentration of 15 mg/L deltamethrin. Omental and perirenal fat, gluteus muscle, kidney and liver collected from animals slaughtered after 1, 3 and 7 days were analysed. The maximum residue of deltamethrin in muscle was 2 μg/kg, 1 day after treatment. One sample of perirenal fat taken at 1 day contained 430 μg/kg deltamethrin; all other fat samples contained less than 200 μg/kg deltamethrin (HIBH 81-1, 1981).

Twelve sheep, nine dipped in a solution of 15 mg/L deltamethrin and three used as untreated controls, were slaughtered in groups of three (controls slaughtered at day 1) at 1, 3 and 7 days after treatment. At slaughter, samples of perirenal and omental fat, liver, kidney and neck muscle were taken for analysis. The maximum individual residue in omental fat was 35 μg/kg at 1 day; the maximum individual residue in perirenal fat was 470 mg/kg, again at 1 day. However, the highest mean residues of 14 μg/kg and 80 μg/kg occurred on day 3 for omental fat and on day 1 for perirenal fat, respectively (AITH 81-3, 1981).

Twelve sheep were dipped in a bath containing 100 mg/L deltamethrin and slaughtered in groups of three at 3, 7, 14 and 21 days after treatment. A further group of three sheep served as controls. Samples of fat, muscle, kidney and liver were taken for analysis. Residues of deltamethrin in muscle, kidney and liver were all below 5 μg/kg with the exception of one muscle sample with a residue level of 32 μg/kg at 7 days. Residues at 3 days were 36 μg/kg in one sample of omental fat and l5 μg/kg in perirenal fat; residues in fat samples at 7, 14 and 21 days were less than 5 μg/kg with one exception, which had a deltamethrin residue of 6 μg/kg (HIBH 83-2, 1983).

Nine sheep were treated topically with 10 mL of a 1% deltamethrin pour-on formulation and subsequently slaughtered at 3, 7 and 14 days. A further group of three sheep served as controls. At slaughter, omental and renal fat were excised, rendered and the rendered fat was analysed for deltamethrin. All samples contained < 50 μg/ kg (CIBH 85-C2, 1985).

Nine sheep were treated topically with a 4 mg/kg BW dose of a deltamethrin pour-on formulation and subsequently slaughtered at 3 and 7 days. At slaughter, muscle, liver, kidney, omental and renal fat were excised and analysed for deltamethrin. All fat samples contained between 0 and 80 μg/ kg (HITH 80/1, HITH 80/2), and all residues were <LOQ (<30 μg/kg) in muscle, liver and kidney (HITH 80/2, 1980).

Hens

Laying hens and their enclosures were sprayed with either a 25 mg/kg or a 50 mg/kg solution of deltamethrin in water. Eggs were collected daily and the hens slaughtered at 1, 2, 3, 4 and 8 days after treatment. Tissue samples were collected and analysed for residues of deltamethrin. All residues were < LOQ and the results are shown in Table 6. (Ansoborlo, 1988).

Animal feeding studies.

Three trials, one with pigs, one with chickens and one with laying hens were carried out to determine the residues generated by feeding a diet containing deltamethrin equivalent to the maximum residue in cereals. The maximum residues in the pig study were in fat (40 μg/kg) and in muscle (3 μg/kg). In liver and kidney, residues were below the limit of determination of 1 μg/kg (E.3O7, 1989). The maximum residues in the chicken studies were 3 μg/kg in fat and 10 μg/kg in skin. Muscle, liver and eggs, residues were below the limit of quantification of l μg/kg for muscle and liver and 2 μg/kg for eggs (E.3O8, 1988; Mougon and Protais, 1989).

**Bound Residues/Bioavailability**

There were <10% bound residues in fat, milk and muscle. The extensive metabolism in the liver of all species resulted in low molecular weight substances, which are either bound or incorporated into the cellular components. The non-extractable residues in bovine liver and kidney were ca. 64% and 32% respectively. In hens the bound residues were 43-68% in liver. The nature of the bound residues is not known but the radioactivity could be released (extracted) after hydrolysis with 3*N*-HCl.

## METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The JMPR considered the analytical methods for deltamethrin in 1990. Some of the analytical methods used to determine the concentrations of deltamethrin in tissues claimed limits of quantification (LOQs) lower than those in the latest method (see below). For example, in cattle, LOQs were claimed to be as low as 2 µg/kg for muscle, liver and kidney and 5 µg/kg for fat (HIDH 80-1).

Since then, the sponsor has provided full details of separate methods, in ISO78/2 format, developed under GLP and dated 1997, for both bovine and chicken, muscle, liver, kidney and fat; for bovine milk and chicken's eggs (Sponsor volume 18, reports 1 - 10). The methods are modifications of those of EN-CAS (1994). The methods are suitable for cattle and chickens but have not been validated for sheep. A method for residues in salmon tissues is also provided (Alpharma, 1997).

The method provided by the sponsor consists of the following steps: Alpha-R-deltamethrin, cis-deltamethrin, trans-deltamethrin and tralomethrin are extracted from tissues, milk and eggs with hexane/acetone. Partition and filtration steps are used where needed to remove particulate matter. The extract is taken through gel-permeation chromatography followed by clean up with alumina column chromatography using hexane - dichloromethane as eluents. The eluate is dried and reconstituted in hexane prior to analysis by gas chromatography with electron-capture detection. The principal characteristics of the methods are summarised in Table 8.

**Table 8. Performance characteristics of analytical methods (Sponsor vol 18 & 19)**

| Species | Tissue | LOQ (µg/kg) | Sample Size (g) | Recovery (%) Mean ± SD (n) | Repeatability CV% | Reproducibilty CV% |
|---|---|---|---|---|---|---|
| **Cattle** | Muscle | 15 | 10 | 91 ± 7.5 (8)[P] | | |
| | Liver | 15 | 10 | 104 ± 8 (12)[P] | | |
| | Kidney | 15 | 10 | 92 ± 13 (13)[P] | | |
| | Fat | 45 | 2 | 90 ± 12 (20)[P] | | |
| | Whole Milk | 15 | 10 | 101 ± 13 (70)[P] | | |
| | Milk Cream/fat | 15 | 10 | 95 ± 11 (49)[P] | | |
| **Chicken** | Muscle | 15 | 10 | 89 ± 10 (6)[M] | | |
| | Liver | 15 | 10 | 93 ± 4.3 (6)[M] | | |
| | Fat | 45 | 2 | 93 ± 8.6 (7)[M] | | |
| | Eggs | 15 | 10 | 92 ± 13 9 (8)[M] | | |
| **Salmon** | Muscle | 2 | 15 | 82[A] 79[B] | <17[A] <5[B] | <23[A] <9[B] |
| | Liver | 2 | 8 | 73[C] 46[D] 65[E] | <16[C] <2[E] | <23[C] <17[E] |
| | Skin | 2 | 3 | 80[C] 57[D] 71[E] | <8[C] <5[E] | <10[C] <15[E] |

The GC - ECD range for the calibration curve was 0.015 –15 ng; $r^2 = 0.997$

[A] is at 25 µg/kg [B] is at 250 µg/kg [C] is at 20 µg/kg [D] is at 50 µg/kg [E] is at 500 µg/kg

P = the procedural recovery samples across all batch analyses. M = method validation recovery results.

## CONCLUSION

Deltamethrin is widely used as an agricultural pesticide and is also licensed in many countries as an ectotopic parasiticide. In 1990 JMPR established an ADI and recommended MRLs to also cover the veterinary usage. These MRLs would appear to be for all species and, in addition, there are values specific for poultry. The sponsors have submitted the same data as to JMPR and also provided some new information; in particular, the analytical method for use with cattle and poultry food products is well documented. The method was applied to a new study to determine the cold residues in cattle given a pour-on preparation.

There are some deficiencies in the data package; namely:-

1. The expert reports are old and too brief to be of much value.
2. There are no pharmacokinetics, metabolism or radio depletion studies for pigs and sheep.
3. The marker compound, parent drug, may only be suitable for fat and milk and maybe muscle.
4. It is not possible to estimate the total unlabelled residues using the residue data in liver, kidney or eggs. This will depend on the Committee allotting a toxic activity to the other residues. The drug is extensively metabolised in the liver and much of the total residues in cattle and poultry liver is in the bound form.
5. The residues at the site of application may not be a problem in animals where the skin is removed but residues are very high on the skin of pigs.

## APPRAISAL

The JMPR established an ADI of 0 – 10 µg/kg BW (0 - 600 µg / 60 kg person) for both veterinary and pesticide uses. The JMPR recommended MRL for Deltamethrin as a pesticide taking into account its use as a veterinary drug: namely; Meat (fat), 500 µg/kg; Poultry meat, 10 µg/kg: Offal, 50 µg/kg; Poultry offal, 10 µg/kg; Eggs, 10 µg/kg; Milk 20 µg/kg. Note that the definition of species for meat and offal is not clarified.

In evaluating MRLs using the JECFA procedures the following factors are considered;

1. The ADI and MRL set by JMPR.
2. The radiodepletion studies, metabolism and analytical methods are only provided for cattle and poultry.
3. There are unlabelled drug residue depletion studies for pigs and sheep.
4. The route of administration is ectodermal.
5. The parent drug is absorbed and residues as parent drug are predominantly distributed in the body fat and milk fat.
6. There is extensive metabolism of the parent drug by the liver (and kidney?) with rapid excretion of the products.
7. The parent drug is the proposed marker residue and is a good indicator of residues in body fat, milk fat and at the site of application. Because of the extensive metabolism it is extremely difficult to monitor the total residues in liver and kidney. Residues of marker compound in muscle are very low and the LOQ of the analytical method will be a determining factor.
8. The LOQ of the methods for cattle and poultry tissues are 15µg/kg for muscle, liver, kidney, eggs, milk and milk fat and 45 µg/kg for body fat.
9. The maximum total residues in cattle and poultry using $^{14}$C-Deltamethrin are shown in Table 9. They are <7% of the ADI.

**Table 9. Theoretical total residues of $^{14}$C-Deltamethrin in "food basket" for cattle and poultry.**

| Tissue | Cattle (µg) | | Poultry (µg) | Maximum for both (µg) |
|---|---|---|---|---|
| | (µg) | MR as % TR | | |
| Muscle (300g) | 3.6 | (58%) | 1.5 | 3.6 |
| Liver (100g) | 21.4 * | (16%) | 1.8 | 21.4 |
| Kidney (50g) | 4.1 | (7%) | Nm | 4.1 |
| Fat (50g) | 6.0 | (82%) | 0.5 | 6.0 |
| Milk (1500g) | 3 | (55 - 89%) | | 3.0 |
| Eggs | | | <0.2 | 0.2 |
| Total | 38.1 | | 4.0 | 38.3 |

10. The toxicological activity of the residues other than parent drug may be assumed not to have the neuronal and toxicological activities of a pesticide/parasiticide. Much of the residues in liver and kidney are the products of extensive metabolism and a large percentage of those in liver are non-extractable, i.e. bound residues.

The Committee took account of the MRLs recommended by JMPR and recommended the same MRLs for liver, kidney and fat. The Committee noted that the concentrations of residues in muscle, milk and eggs are less than twice the limit of quantification of the analytical methods used and, therefore, recommended MRLs based on the sensitivity of the method. These values are "guidance MRLs" and should not be used to calculate the theoretical maximum daily intake of deltamethrin residues. The MRLs for cattle and chicken tissues were extended to sheep for muscle, liver, kidney and fat and salmon muscle tissue. The MRL for muscle tissue, milk and eggs is 30μg/kg; for liver and kidney, 50 μg/kg; and for fat, 500μg/kg.

Based on the daily intake of 300g muscle, 100g liver, 50 g kidney and fat, 1.5 kg milk and 100g eggs, and considering the MRL in muscle, milk and eggs set at two times the limit of quantification of the method; that the marker residue accounted of 4-20% of the total residues in liver, 3% of the total residues in kidney and 60% of the total residues in fat, the theoretical maximum daily intake of residues from veterinary drug use would be 150-250μg as deltamethrin equivalents.

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# DIHYDROSTREPTOMYCIN and STREPTOMYCIN

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**ADDENDUM**
**to the Dihydrostreptomycin and Streptomycin residue monographs**
**prepared by the 43rd and 48th meetings of the Committee**
**and published in FAO Food and Nutrition Papers 41/7, Rome 1995 and 41/10, Rome 1997**

## INTRODUCTION

The 43rd meeting of the Committee (JECFA, 1994) requested the following information on residues for evaluation in 1997.

1. An evaluation report or results of experimental studies on the metabolism of dihydrostreptomycin and streptomycin.
2. Data on residues of streptomycin and dihydrostreptomycin in eggs.
3. Results of studies to determine the relationship between the antimicrobial activity of the residues and their concentration, as measured by specific chemical methods.

Data were submitted at the 48th Meeting (JECFA, 1997) that satisfactorily covered the first two items. The sponsors provided some valuable information to support their analytical methods (Item 3) for the 48th Meeting but were only able to complete the work for the current Meeting. The data package supplied to the 52nd Meeting addresses Item 3 (Norbrook, 1998) and is the source for all the cited data in this monograph.

Two different analytical methods were used to measure residues of dihydrostreptomycin and streptomycin. The first of these methods was a microbiological method (a bioassay), which could not distinguish between the two compounds. The second method was an HPLC assay capable of measuring each compound separately. Data was submitted for the evaluation of the performance characteristics of the individual methods.

A specific requirement of the analytical methods requested by JECFA was that the relationship between the antimicrobial activity of the residues and their concentration, as measured by specific chemical methods should be determined. Such measurements were necessary, both in edible tissues fortified with dihydrostreptomycin and streptomycin, as well as in tissues obtained from animals treated with the compounds. Some of these incurred tissues should have residues above the limit of quantification of the methods. The animal studies, including the sampling and analytical procedures, were performed to GLP. The analytical methods are "in house" and do not appear to have any other accreditation.

## SUMMARY OF ANALYTICAL METHODS

### Sampling

In all the animal studies, samples weighing at least 200 g were collected. The samples were homogenised and divided into four sub-samples before storage in deep freeze conditions. It should be noted that homogenisation before storage could release enzymes which metabolise the antibiotics.

### Bioassay using *Bacillus subtilis*

The standard operating procedure for the bioassay method (Norbrook, 1998; SOM: MRD/DSS/010) and its validation in bovine tissues (Norbrook, 1998; VAL: MRD/DSS/010), ovine tissues (Norbrook, 1998; VAL: MRD/DSS/020) and porcine tissues Norbrook, 1998; VAL:MRD/DSS/030) was provided by the sponsor.

Ten grams of muscle, liver or kidney tissue or 7 g fat were homogenised in phosphate buffer, pH 2.0. A calibration or standard curve in the range 300 - 8000 µg/kg was obtained using blank samples fortified with equal amounts of pure

standards of dihydrostreptomycin and streptomycin dissolved in buffer. After vortex mixing, the mixture was centrifuged to remove the tissue debris and denatured proteins and the supernatant was collected and used for the microbiological assay. Monoethanolamine was added to 5 mL of supernatant to raise the pH. The assay uses *Bacillus subtilis* (probably ATCC 6633 but strain not given for any of the assays) as the test organism and penicillinase may be included to destroy any penicillins present in the extract.

The concentration in the test samples was calculated by interpolation with the standard curve. The assay did not cross-react with penicillins when penicillinase was used. The assay characteristics are summarised in Table 1.

**HPLC Assay**

The standard operating procedure for an HPLC method (Norbrook, 1998; SOM: CRD/DSS/010) and its validation in bovine tissues (Norbrook, 1998; VAL: CRD/DSS/010), ovine tissues (Norbrook, 1998; VAL: CRD/DSS/020) and porcine tissues (Norbrook, 1998; VAL:CRD/DSS/030) was provided by the sponsor. The sponsors have developed an HPLC method for the measurement of dihydrostreptomycin and streptomycin in muscle, liver and kidney of cattle, sheep and pigs; also for fat of cattle and sheep and for fat combined with skin for pigs. Tissues from untreated animals were used as blanks. Fortified samples for the calibration curves and validation procedures were prepared using 10 g of blank tissues with the addition of two times the amount of dihydrostreptomycin to streptomycin over the range of 400 – 6000 µg/kg for dihydrostreptomycin. Ten gram samples of blank tissue, fortified blank tissue and the test samples were deproteinised with perchloric acid and centrifuged. The liquid extracts were placed onto a small solid phase sulphonic acid resin column, washed with water and the dihydrostreptomycin and streptomycin eluted with phosphate buffer, pH 7.5. Water, perchloric acid and the ion pair concentrate were added to the eluate, this was mixed, filtered and retained for assay. The eluate was analysed by HPLC with post-column derivatisation and fluorescent detection.

The accuracy of the method was measured by using replicates at 400 and 5000 µg/kg spikes. The LOQ was determined as 400 µg/kg for dihydrostreptomycin and 200 µg/kg for streptomycin in all tissues. A recovery correction was not necessary because the standard curve was constructed using fortified material. No comparison of this curve with a pure standards only curve was made. The assay characteristics are summarised in Table 1.

The methods were suitable for measuring concentrations of dihydrostreptomycin and streptomycin in spiked muscle, liver, kidney and fat of cattle, sheep and pigs. The bioassay was suitable for measuring the sum of antimicrobial activity of both drugs in tissues. Although the penicillins did not interfere in the bioassay, other antibiotics may interfere. Thus the bioassay is best suited for the measurement of residues of the two drugs in those animals with a known veterinary treatment history. If the bioassay were to be used in a regulatory control system, any positives would have to be examined by more specific methods, e.g., the HPLC method.

**Table 1. Characteristics of the analytical methods for dihydrostreptomycin and streptomycin.**

| Criteria | Bioassay – Summary | HPLC assay – Summary |
|---|---|---|
| QA | In house | In house |
| Matrices | Muscle, liver, kidney, fat, fat/skin for pig, injection site | Muscle, liver, kidney, fat, fat/skin for pig, injection site |
| Accuracy at LOQ | See table 2 CVs 0.8 – 11.4% | See table 2 CVs 0 – 13.4% |
| Recovery | Used fortified standard curve. | Used fortified standard curve |
| Linearity | $r^2 \geq 0.991$ | $r^2 \geq 0.980$ |
| LOD | 200 µg/kg in liver<br>300 µg/kg other tissues. | 12 – 153 µg/kg |
| LOQ | 300 µg/kg | streptomycin 200 µg/kg<br>dihydrostreptomycin 400 µg/kg |
| Specificity from blank | Good | Good at LOQ |
| Specificity from related compounds. | Poor with certain antibiotics | No cross reaction with penicillin-G, gentamicin and lincomycin |

**Correlation of the results for the bioassay and HPLC assay.**

Fortified Samples.

The initial study reported to the 48th JECFA compared the results when sheep liver and kidney tissues were spiked with dihydrostreptomycin at 500 µg/kg and 1200 µg/kg and were measured using both assays. This experiment confirmed that the results for spiked samples obtained, if based on antimicrobial activity, were equivalent to results obtained from a specific chemical assay. The new studies used fortified blank tissue samples from cattle, sheep and pigs to produce the calibration curves for each assay. Both assays gave a good linear response over a wide range with $r^2$ values that were acceptable. The accuracy of the methods was checked using samples fortified with dihydrostreptomycin and streptomycin. A summary of the results is shown in Table 2.

**Table 2. Assay criteria using fortified cattle, sheep and pig tissues.**

| Species | Tissue | Method | | $r^2$ Experiment 1[a] | Accuracy (%) and CV (%) at LOQ* | | $r^2$ Experiment 2[b] | Accuracy (%) and CV (%) at LOQ* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Accuracy | CV | | Accuracy | CV |
| Cattle | Muscle | Bioassay | | >0.992 (7) | 96.9 | 4.7 | NA | 96.1 | 3.7 |
| | | HPLC | DS | 0.981 | 87.8 | 0 | 0.997 | 95.2 | 2.6 |
| | | | S | 0.984 | 92.7 | 2.5 | 0.989 | 110 | 5.3 |
| | Liver | Bioassay | | >0.992 (6) | 105 | 8.0 | NA | 101 | 7.0 |
| | | HPLC | DS | 0.984 | 88.0 | 0 | 0.995 | 100 | 12.0 |
| | | | S | 0.996 | 97.3 | 6.5 | 0.984 | 95.8 | 5.0 |
| | Kidney | Bioassay | | >0.994 (6) | 102 | 5.9 | NA | 99.8 | 3.8 |
| | | HPLC | DS | 0.983 | 96.6 | 5.8 | 0.992 | 96.4 | 6.0 |
| | | | S | 0.984 | 91.5 | 0 | 0.983 | 96.3 | 3.9 |
| | Fat | Bioassay | | >0.996 (7) | 105 | 4.2 | NA | 101 | 6.5 |
| | | HPLC | DS | 0.995 | 98.9 | 4.3 | 0.987 | 103 | 9.0 |
| | | | S | 0.989 | 106 | 5.4 | 0.984 | 104 | 4.4 |
| Sheep | Muscle | Bioassay | | >0.997 (6) | 96.0 | 5.1 | NA | 99.7 | 0.8 |
| | | HPLC | DS | 0.981 | 98.5 | 9.1 | 0.988 | 88.7 | 13.4 |
| | | | S | 0.983 | 88.1 | 3.5 | 0.984 | 109 | 5.8 |
| | Liver | Bioassay | | >0.995 (7) | 97.1 | 5.2 | NA | 94.1 | 7.6 |
| | | HPLC | DS | 0.996 | 113 | 6.7 | 0.997 | 97.5 | 3.9 |
| | | | S | 0.994 | 95.0 | 5.4 | 0.988 | 106 | 2.5 |
| | Kidney | Bioassay | | >0.992 (7) | 96.8 | 5.4 | NA | 103 | 4.4 |
| | | HPLC | DS | 0.984 | 90.7 | 4.7 | 0.992 | 97.3 | 2.9 |
| | | | S | 0.984 | 89.8 | 10.6 | 0.990 | 95.8 | 1.1 |
| | Fat | Bioassay | | >0.991 | 103 | 5.1 | NA | 88.8 | 2.5 |
| | | HPLC | DS | 0.981 | 91.5 | 3.8 | 0.991 | 104 | 4.4 |
| | | | S | 0.981 | 108 | 2.7 | 0.995 | 105 | 1.4 |

**Table 2 (continued). Assay criteria using fortified cattle, sheep and pig tissues.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pig | Muscle | Bioassay | | >0.993 (6) | 95.8 | 4.2 | NA | 100 | 4.7 |
| | | HPLC | DS | 0.981 | 87.5 | 5.1 | 0.981 | 115 | 6.6 |
| | | | S | 0.981 | 85.0 | 1.2 | 0.987 | 113 | 4.9 |
| | Liver | Bioassay | | >0.991 (6) | 105 | 3.5 | NA | 99.0 | 11.4 |
| | | HPLC | DS | 0.987 | 109 | 0 | 0.995 | 118 | 7.4 |
| | | | S | 0.981 | 110 | 1.6 | 0.992 | 113 | 2.3 |
| | Kidney | Bioassay | | >0.996 (6) | 103 | 8.8 | NA | 101 | 6.7 |
| | | HPLC | DS | 0.981 | 90.6 | 7.4 | 0.995 | 97.9 | 5.2 |
| | | | S | 0.981 | 97.9 | 5.2 | 0.995 | 107 | 3.1 |
| | Fat and | Bioassay | | >0.993 (6) | 100 | 3.8 | NA | 99.6 | 9.0 |
| | Skin | HPLC | DS | 0.981 | 100 | 4.0 | 0.994 | 94.2 | 11.6 |
| | | | S | 0.980 | 109 | 3.1 | 0.990 | 101 | 3.9 |

LOQ for HPLC method for Dihydrostreptomycin is 400 µg/kg; for Streptomycin it is 200 µg/kg. For the bioassay the LOQ is 300 µg/kg. [a] and [b] two separate experiments on different days.
Ranges: Dihydrostreptomycin it is 400 - 5000 µg/kg: for Streptomycin it is 200 - 2500 µg/kg. For the bioassay it is 300 - 8000 µg/kg. NA is not applicable.

Incurred Samples

Three new studies were carried out in which animals were treated with proprietary preparations of the two drugs.

1. Cattle were dosed IM with a combination of dihydrostreptomycin sulphate (10 mg/kg BW) and procaine penicillin (8 mg/kg BW). After 2 days the animals were sacrificed and tissues collected for assay.

2. Sheep were dosed IM with streptomycin sulphate (10 mg/kg BW). After 2 days the animals were sacrificed and tissues collected for assay.

3. Pigs were dosed IM with a combination of dihydrostreptomycin sulphate (5 mg/kg BW) and streptomycin sulphate (5 mg/kg BW). After 2 days the animals were sacrificed and tissues collected for assay.

The results for both assays are shown in Table 3. Although the withdrawal time of 2 days is short, there were no measurable residues in any of the muscle and fat tissues. Residues were found in liver, kidney and at the injection site. There was close agreement between the values for both methods.

**Table 3. Residues of dihydrostreptomycin and streptomycin (mg/kg) in tissues of treated farm animals.**

| Cattle | Muscle | Liver | Kidney | Fat | Injection Site |
|---|---|---|---|---|---|
| Bioassay | <0.3 | 1.13 ± 0.13 | 6.61 ± 0.72 | <0.3 | 1.70 ± 0.86 |
| HPLC | <0.4 | 1.49 ± 0.11 | 5.78 ± 2.28 | <0.4 | 1.71 ± 0.78 |
| **Sheep** | | | | | |
| Bioassay | <0.3 | 0.65 ± 0.24 | 0.91 ± 0.21 | <0.3 | 1.23 ± 0.47 |
| HPLC | <0.2 | 0.94 ± 0.46 | 0.89 ± 0.21 | <0.2 | 1.17 ± 0.30 |
| **Pigs** | | | | | |
| Bioassay | <0.3 | 1.21 ± 0.44 | 5.66 ± 1.70 | <0.3 | 1.61 ± 0.30 |
| HPLC | ND | 1.04 ± 0.35 | 5.12 ± 2.26 | ND | 1.71 ± 0.48 |

Each value is the mean ± SD for four animals. ND is not detectable for either drug.

## APPRAISAL

### MRL

The Committee had recommended temporary MRLs for streptomycin and dihyrostreptomycin in muscle, liver, kidney and fat of cattle, sheep, pigs and chickens; also for milk

The Committee considered that the sponsor had provided satisfactory answers to all the requests made at the forty-third meeting and, therefore, decided to delete the temporary status of the MRLs, except for milk. Taking into account the higher limit of quantification of the bioassay method compared with the HPLC method for streptomycin, the Committee recommended MRLs for muscle, liver and fat of 600 µg/kg, and for kidney, 1000 µg/kg in cattle, sheep, pigs and chickens. The temporary MRL for cattle milk is 200 µg/kg.

The ADI is equivalent to 0 – 3000 µg per 60 kg person. The theoretical maximum daily intake for all tissues and milk using the recommended MRLs is 620 µg and when milk is not included, 320 µg .

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# DORAMECTIN

**First draft prepared by**
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**ADDENDUM**
**to the doramectin residue monograph prepared by the 45th meetingof the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996**

## INTRODUCTION

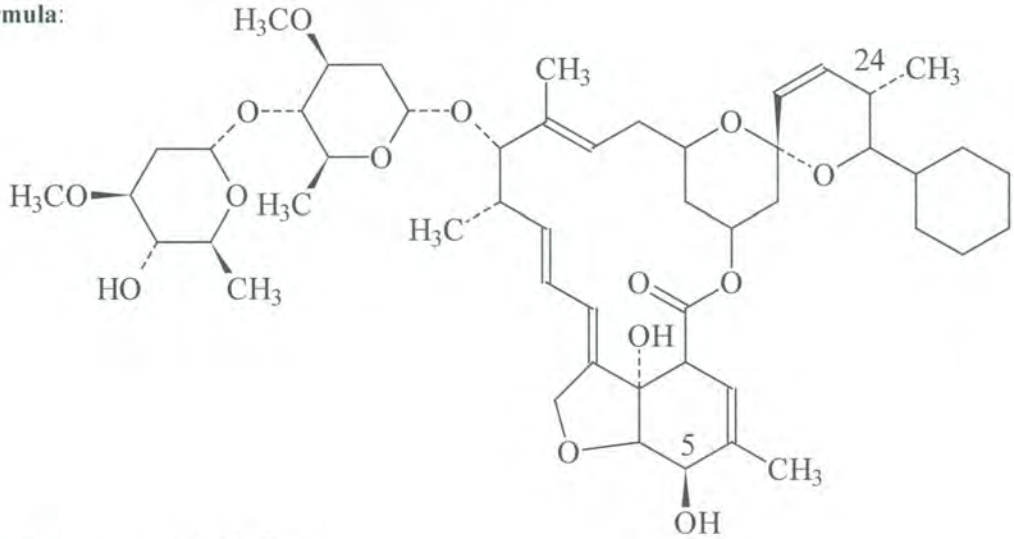
The use of doramectin as an ecto- and endoparasiticide for cattle was assessed at the 45th JECFA in 1995 (JECFA 1996a, 1996b, 1996c). An ADI and MRL were established. This review assesses the use of doramectin in pigs.

## IDENTITY

**Chemical name:** 25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl)avermectin $A_{1a}$

**Synonyms:** Doramectin; Dectomax; UK-67,994®

**Structural formula:**




**Molecular formula:** $C_{50}H_{74}O_{14}$

**Molecular weight:** 899.14

## OTHER INFORMATION ON IDENTITY AND PROPERTIES

**Pure active ingredient:** Doramectin

**Appearance:** White to light tan powder

**Melting point (average):** 160.5 - 162.2°C

**Solubility:**

| | |
|---|---|
| water | 0.0003 g/L at 22°C -25°C |
| acetonitrile | 33 g/L |
| methylene chloride | 530 g/L |

**Optical Rotation:** + 12.2°C (anhydrous)

**$UV_{max}$:** 244 nm

## RESIDUES IN FOOD AND THEIR EVALUATION

### CONDITIONS OF USE

General

Doramectin is a parasiticide for use in cattle and pigs.

Dosage

Doramectin is administered to pigs for the treatment of endo and ectoparasitic infections as a single intramuscular (IM) dose of 0.3 mg/kg BW. Doramectin is not intended for use in dairy cattle producing milk for human consumption.

## METABOLISM AND PHARMACOKINETIC STUDIES

### Pharmacokinetics

The plasma kinetics of doramectin were determined in eight pigs (4 male castrates and 4 females, each weighing approximately 40 kg) dosed IM with [$^3$H]-doramectin at 0.3 mg/kg BW using a prototype commercial formulation (75% sesame oil/25% ethyl oleate). The plasma concentrations were determined using liquid scintillation for quantification of total residues and chromatographic techniques for the quantification of unchanged doramectin. Data from this study are presented in Table 1.

**Table 1 Plasma concentration of total radio-labelled material and of doramectin in pigs treated IM with [$^3$H]-doramectin at 0.3 mg/kg BW.**

| | Equivalents of Doramectin (µg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day | 0.5 | 1 | 3 | 5 | 7 | 10 | 14 | 21 |
| Total [$^3$H] residues | 16 | 21 | 26 | 25 | 21 | 16 | 11 | 6 |
| Doramectin residues | 12 | 17 | 17 | 14 | 13 | 9 | 6 | <3 |

The apparent terminal half-lives of elimination from plasma of total [$^3$H]-labelled materials and unchanged doramectin were 7.7 and 6.4 days, respectively.

The drug dispersed from the injection site (see below) (Pfizer Inc, 1995a) and the main route of excretion in pigs was the faeces. After IM dosing, with [$^3$H]-doramectin at 0.3 mg/kg BW, 61 ± 20% of the dose was excreted in the faeces within 21 days and <1% of the dose was excreted into the urine (Pfizer, 1995a). The faeces are also the major route of excretion in cattle, dog and rat.

### Metabolism in animals

The biotransformation of doramectin was investigated in rats, dogs, pigs and cattle. Tissue distribution studies, discussed in detail below, showed that the highest concentrations of total residues were in liver and fat of treated cattle and pigs, with only traces detectable in muscle and kidney. For this reason, metabolite identification studies in pigs were limited to liver. The identity of doramectin metabolites was previously reviewed by the 45th JECFA in association with the use of doramectin in cattle. The methods employed for metabolite identification included gradient liquid chromatography with radiochemical

detection and fast atom bombardment mass spectrometry. A tritium label ($^3H$) was introduced into the doramectin molecule at the 5-position with high specificity (minimum 14.1 mCi/mg). The material used ranged in radiopurity from 95 to ~99.8%. The label was metabolically stable, since less than 1% was recovered as tritiated water from cattle faeces containing 87% of the dose (Pfizer Inc, 1994a).

The products of doramectin metabolism were similar in all species investigated. The metabolites were more polar than doramectin and were the result of O-demethylation in the distal saccharide ring (component C), of hydroxylation of the 24-methyl group (component B) and a combination of both of these biotransformations (component A). Table 2 shows the distribution of the metabolites (as a percentage of total radioactivity of the major radiolabelled component) in the liver and faeces of pigs, cattle, rats and dogs at 2, 3, 7 or 21 days post dose. Rats and dogs were dosed orally with 5 and 3.5 mg/kg BW respectively. Pigs were administered an IM dose at 0.3 mg/kg BW, cattle received a SC injection of 0.2 mg/kg BW.

**Table 2. Percentage of total radioactivity of the major radio-labelled components in the liver and faeces of pigs, cattle, rats and dogs at 2, 3, 7 or 21 days post dose**

| Tissue | Species | Day | % $^3H$ recovered | Component* | | | |
|---|---|---|---|---|---|---|---|
| | | | | A | B | C | UD |
| Liver | Pigs | 3[a] | 39 | ND | ND | 9 | 28 |
| Liver | Pigs | 7[aa] | 89 ± 8 | ND | ND | 20 ± 2 | 71 ± 4 |
| Liver | Cattle | 3[b] | 95 | 7 | ND | 9 | 70 |
| Liver | Cattle | 21[c] | 82 | 8 | 4.4 | 6.8 | 57.8 |
| Liver | Rat | | 37 | 2 | 3 | 12 | 18 |
| Liver | Dog | | 51 | ND | ND | 12 | 28 |
| Fat | Cattle | 21 | 74-91 | ND | ND | ND | 91[e] |
| Faeces | Pigs | | 72 | ND | 8 | 14 | 10 |
| Faeces | Cattle | | 75-82 | 4 | 5 | 14 | 24 |
| Faeces | Rat | | NC[d] | 16 | 14 | 19 | 22 |
| Faeces | Dog | | 46 | 4 | 5 | 8 | 6 |

* As identified above and in equivalents of [$^3H$]-doramectin. [a] Study CM-93-02, Pfizer Inc, 1994b;
[aa] Study using different extraction of liver tissue from Study CM-93-02, Pfizer 1994b;
[b] Study CM-92-01, Pfizer Inc, 1992a; [c] Study CM-93-01, Pfizer Inc, 1993
[d] NC = not calculated since this sample was used as a reference standard.
[e] 4.8-10.9% (mean 7.4%) of total residues were epi-doramectin. UD = unchanged doramectin; ND = not detected

Pigs

The metabolite profile of doramectin in pigs treated with [$^3H$]-doramectin was initially determined using subcutaneous (SC) administration of a 0.3 mg/kg BW dose in an aqueous micelle formulation to four male castrate pigs. One male was killed three days after dose administration and the liver collected for metabolite identification. The remaining animals were placed in metabolism cages and the faeces and urine collected at 24 hour intervals for 7 days. The samples containing the highest concentrations of radioactivity were selected for metabolite identification. In this study, 39% of the total radioactivity was extracted from liver (Pfizer, 1994b). The concentrations of the major radiolabelled components were confirmed by examination of liver and faeces samples collected from four animals (2 male and 2 female) weighing approximately 40 kg dosed with [$^3H$]-doramectin in the prototype commercial formulation (75:25 sesame oil:ethyl oleate) as an IM dose of 0.3 mg/kg BW. Animals were sacrificed seven days after treatment. Faeces were collected at 24-hour intervals until day 7 post-dose. The extraction of residues from the liver was improved over the earlier experiment, resulting in extraction of 89% of the label of which 71% of total residues (TR) was doramectin. Drug and metabolites were quantified using radiochemical

detection after separation by HPLC chromatography. The major drug-related component in pigs was unchanged doramectin, as in all species studied, (see Table 2) and bound residues constitute, at most, only a small fraction of the total. One metabolite (component C) was detected in porcine liver and faeces and component B was only detected in faeces. Component A was not detected in any pig sample, although this metabolite is found in treated cattle. The unchanged drug was the main component in pig faeces (Pfizer Inc, 1994b). In summary, the metabolism of doramectin is generally similar between the four species examined.

**Radiolabelled residue depletion studies**

General Comments

The studies were done in compliance with GLP. Total drug-related residues were measured by the use of tritium labelled doramectin. The radiochemical purity of [$^3$H]-doramectin used in these studies was ~ 99.8%. The [$^3$H] label was found to be metabolically stable in cattle since less than 1% of faecal radioactivity was recovered as tritiated water (Pfizer Inc, 1990, 1992a, 1994a).

Pigs

In the total residue depletion study, pigs weighing approximately 40 kg received a single IM dose of 0.3 mg/kg BW [$^3$H]-doramectin in the commercial prototype formulation described above. Two pigs of each sex were sacrificed at 7, 14, 21 and 28 days after dose administration and samples of muscle, liver, kidney and fat collected for analysis of total radioactivity and unchanged doramectin (Pfizer 1995b). Injection site samples were collected from these animals under a separate protocol and reported separately (Pfizer, 1995a). The concentrations of total doramectin residues, unchanged doramectin and the calculated unchanged drug to total residue ratios are presented in Table 3.

**Table 3. Radiolabelled residues and unchanged drug (µg/kg) in pig tissues after a single IM dose of 0.3 mg/kg BW [$^3$H]-doramectin[a]**

| Tissue | Mean Residue Concentration | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| Muscle | Total [$^3$H] residues (µg/kg) [b]<br>Unchanged drug[c] (µg/kg)<br>Unchanged drug (%) | 35 ± 7<br>7 ± 1<br>(20) | 19 ± 6<br><4<br>(<24) | 6 ± 1<br><3<br>n.e. | 4 ± 3<br><3<br>n.e. |
| Liver | Total [$^3$H] residues (µg/kg) ) [b]<br>Unchanged drug[c] (µg/kg)<br>Unchanged drug (%) | 186 ± 47<br>66 ± 15<br>(36) | 111 ± 20<br>37 ± 11<br>(34) | 46 ± 10<br>11 ± 3<br>(24) | 37 ± 20<br><7<br>(<229) |
| Kidney | Total [$^3$H] residues (µg/kg) ) [b]<br>Unchanged drug[c] (µg/kg)<br>Unchanged drug (%) | 79 ± 19<br>23 ± 7<br>(30) | 46 ± 7<br>11 ± 3<br>(23) | 17 ± 4<br>6 ± 2<br>(35) | 8 ± 3[d]<br>3 ± 1[d]<br>(45)[d] |
| Fat | Total [$^3$H] residues (µg/kg) ) [b]<br>Unchanged drug[c] (µg/kg)<br>Unchanged drug (%) | 412 ± 54<br>242 ± 22<br>(59) | 255 ± 42<br>113 ± 26<br>(44) | 90 ± 18<br>42 ± 14<br>(45) | 8 ± 33<br>30 ± 20<br>(49) |
| Injection Site (250 g) | Total [$^3$H] residues (µg/kg) ) [b]<br>Unchanged drug[c] (µg/kg)<br>Unchanged drug (%) | 5130<br>3660<br>(74) | 2510<br>2550<br>(102) | 1080<br>1030<br>(92) | 118*<br>35*<br>(32)* |

**a** = Study 1525N-60-90-011/012 (Pfizer, 1992b and 1995a) ; data are means ± SD from four animals at each time.
**b** = Expressed as doramectin equivalents
**c** =For calculations of the means 2.5 or 5 µg/kg, as appropriate, was substituted for those unchanged drug concentrations falling below the LOQ. A "less than" symbol (<) indicates that some data were below the LOQ (limit of quantification).
**d** - One sample of pig kidney on day 28 was apparently mislabelled and was actually an injection site sample. Therefore, this sample is not included in the mean calculations for kidney day 28. Individual data are provided in the study report.

n.e.= Not estimated because two or more individual samples were below the LOQ.
The results of the total residue depletion study presented in Table 3 clearly indicate that the liver and fat are most appropriate for selection as target tissues since the concentrations are the highest and the depletion from both tissues is slowest. Residues in kidney and muscle are low and close to the limit of quantification by day 28.

The ratio of unchanged drug to total residues was found to be generally constant over the measured withdrawal periods for each tissue. Combining the data from all measured withdrawal times (days 7, 14, 21 and 28) for each tissue, the approximate mean percentages of unchanged drug to total residues were calculated as follows: liver - 30%; fat - 50%; muscle - 20 %; kidney - 30%.

The calculated ratio of 30% for liver is considered by the sponsors to be inappropriately low. They argued that the assay methodology in place at the time this study was conducted was subsequently found to have deficiencies related to the variable extraction of unchanged drug from the liver and maybe from the other edible tissues. The liver samples from this study were not re-analysed after the method was improved. Evidence to support that the ratios of unchanged drug to total residues in liver were disproportionately low in this study are provided by inspection of the metabolic profile data generated in pig liver using radiochemical detection (Table 2, day 7). Using this methodology, it is clear that unchanged doramectin represents a much greater proportion of total residues than was detectable using the earlier, less robust HPLC method where incomplete residue extraction is probable.

Residues at the Injection Site

The injection site, consisting of fascia and underlying tissue, was removed and trimmed to approximately 250g (Pfizer, 1995a). The concentrations of total residues and unchanged drug were measured by similar methods used for the edible tissues. The results are shown in Table 4.

**Table 4. Residues (µg/kg) at the injection site of pigs.**

| Residue | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|
| Total Residues (TR) | 5100 ± 4400 | 2500 ± 1800 | 1100 ± 720 | 120± 38 |
| Doramectin (UD) | 3600 ± 3000 | 2600 ± 1900 | 1000 ± 850 | 35 ± 6 |
| % Doramectin in TR | 71% | 102% | 96% | 30% |

The residues at the injection site persist for at least 21 days but by day 28 they have fallen to much lower levels. In a study (see below) using non-radiolabelled doramectin, the results for the residues at the injection site yield much higher concentrations of doramectin and they are evenly distributed throughout a 500g sample. Thus the amount of residues in the tissue around the injection site in this labelled study could be double the amount found in the 250g sample taken for this work. The percentage of doramectin in the residue remaining at the injection is down to 30% and it is probable that the unidentified part of the residue is less active than doramectin.

**Depletion studies with unlabelled drug**

The depletion of the marker residue, doramectin, from edible tissues was evaluated in a depletion study conducted in pigs with a mean weight of 62 kg. Each pig received a single IM dose of doramectin in a commercial prototype formulation at a dosage of 375 µg/kg, which was 1.25x the recommended dose (Pfizer Inc, 1995b). Six animals, three of each sex, were evaluated at each time point. The injection site, consisting of a cylinder approximately 10 cm in diameter and 6 cm in depth, was removed and trimmed to approximately 500g, equivalent to the U.S. (500 g) requirement. The inner 300g portion, equivalent to the EU requirement, containing the injection site was trimmed from the 500g sample and homogenised; the outer portion was homogenised separately. One-half of the homogenised 300g sample was re-homogenised with one-half of the outer portion to yield a sample representing the 500g sample of the injection site. Two sub-samples were taken from each homogenate. The results of this study are presented in Table 5.

**Table 5.** **Depletion of doramectin (in µg/kg) from specified tissues of pigs treated with a single IM dose of 0.375 mg/kg BW of doramectin[a]**

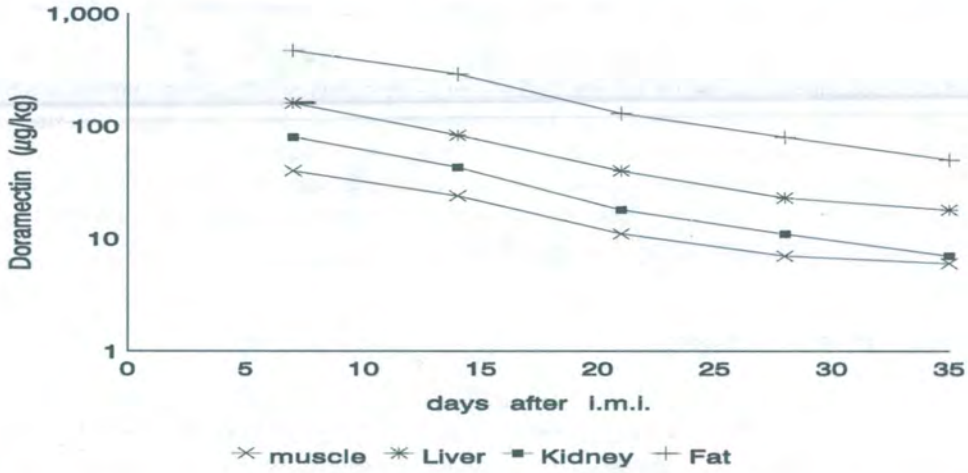
| Tissue | 7 days | 14 days | 21 days | 28 days | 35 days |
|---|---|---|---|---|---|
| Muscle | 40 ± 9 | 24 ± 8 | 11 ± 5 | <7 | <6 |
| Liver | 160 ± 30 | 83 ± 8 | 40 ± 20 | 23 ± 13 | 18 ± 8 |
| Kidney | 80 ± 20 | 43 ± 7 | 18 ± 7 | <13 | <7 |
| Fat | 470 ± 120 | 290 ± 40 | 130 ± 50 | 80 ± 50 | 50 ± 20 |
| I. Site (500g) | 9000 ± 6000 | 5000 ± 6000 | 900 ± 500 | 800 ± 600 | 70 ± 70 |
| I. Site (300g) | 7000 ± 4000 | 5000 ± 3000 | 900 ± 500 | 700 ± 500 | 160 ± 150 |

[a] Study 1521N-60-94-007. Data are means ± SD from 6 animals (3of each sex) at each time point.

[b] For calculation of the means, concentrations falling below the LLOQ of 2.5 µg/kg were included in the mean using a value of 2.5 µg/kg, and the mean value was preceded with a less than (<) symbol.

Concentrations of doramectin were highest in the liver, fat and injection sites. Although injection site samples showed a high degree of variability, this is not unexpected for this class of compounds. It is noteworthy that there was close agreement between the mean concentrations of residue in the 300 and 500 g sample, indicating that the drug was dispersed evenly throughout the 500g sample. Therefore it is probable that the residues at the injection site are spread over a larger volume than the sample collected in this study. Also the mean concentration of residues of doramectin (ca. 750 µg/kg) at 28 days are much higher than those observed in the radiolabelled study, in which 35µg/kg µg/kg doramectin and 118 µg/kg total residues, respectively, where found in a 250g core sample. This difference is not due to the 25% higher dose used in the cold study, particularly because the depletion of drug from the cold injection site was linear and relatively rapid, with a mean depletion half-life of approximately 4 days. Doramectin was shown to also deplete in a linear fashion from liver, fat, kidney and muscle, a shown in Figure 1, with depletion half-lives of 7.6 - 9.5 days.

**Figure 1.** **Depletion of doramectin from edible pig tissues following a 0.375 mg/kg BW IM dose.**




It is concluded, from both the total and marker residue depletion studies in pigs, that unchanged drug is the appropriate marker residue and liver and fat are the appropriate target tissues for the evaluation of doramectin residues in the edible tissues of pigs. Muscle and kidney are not considered to be suitable target tissues since concentrations of both total and unchanged residues are much lower and are, in fact, near the limit of quantification by 28 days post-treatment.

**Bound Residues/Bioavailability**

The results of the metabolism and total residue depletion studies conducted with [$^3$H]-doramectin indicate that doramectin residues are not tightly bound to tissues. The extractability of the total residues was measured in a pig liver, collected 7 days after dosing IM with radiolabelled doramectin. The liver was extracted using the proposed regulatory method and 93 ± 3% of the radioactivity was extracted with 9 ± 0.5% remaining in the pellet (Pfizer, 1997a). Unchanged doramectin comprises the majority (up to 71%) of doramectin related residues in liver (Pfizer Inc, 1994b)

**Choice of marker residue**

Examination of the results for the radiolabelled depletion studies indicates clearly that the parent drug, doramectin, is the best choice as the marker residue. However there are some minor difficulties in allocating the ratios of parent drug to total residues in tissues because the HPLC method used in the radiolabelled depletion study may not have recovered a significant proportion of the unchanged drug. This is addressed in detail in the later section on MRL.

## METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Doramectin concentration in pig tissues was determined by a validated HPLC method. The detection and quantification of doramectin at the µg/kg level was based on the extraction from tissue homogenate or fat and subsequent conversion to a chemically stable aromatic fluorescent derivative. The methods used for analysis of doramectin in pig tissues were substantially the same as those used for the quantification and confirmation of doramectin residues in cattle tissues and previously reviewed by JECFA (JECFA, 1996a). The HPLC method was specific for doramectin and showed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin. The proposed regulatory method was different from those used for determining residues of unchanged drug in the radiolabelled depletion studies. In particular the regulatory method used a much more exhaustive extraction procedure, whereas the mild extraction used in the radiolabelled studies was thought to only partially extract unchanged drug from liver tissue.

High Performance Liquid Chromatography (HPLC)

Liver, kidney, muscle and injection site samples were extracted by incubation at 55°C followed by re-homogenisation in the presence of extraction solvent. Fat samples were extracted using incubation at 55°C in hexane followed by homogenisation and re-partitioning into acetonitrile. The residues were derivatised by treatment with trifluoroacetic anhydride and triethylamine (minimising exposure to moisture), followed by treatment with methanolic ammonia to yield a fluorescent derivative that was stable to moisture. It was necessary to protect the derivative from light using amber coloured glass vials. Separation was effected using a reverse-phase $C_{18}$ HPLC column using acetonitrile, tetrahydrofuran and water in the mobile phase. The amount of doramectin in each sample was quantified with or without the use of an internal standard (UK-71,647). The assay, which was very similar to assays for related substances such as the avermectins, showed good sensitivity with a limit of quantification (LOQ) of 2.5 µg/kg in liver, kidney, muscle and injection site, and of 5 µg/kg in fat. Recoveries of doramectin from liver and fat were in excess of 80%. The coefficient of variation, was approximately10% (Pfizer Inc, 1995b, 1997b, 1998a and b)

High Performance Liquid Chromatography - Mass Spectrometry (LC-MS)

The presence of doramectin in pig liver and fat can be confirmed at trace (µg/kg) levels using LC-MS techniques. Doramectin was extracted from liver or fat using the same methods employed in the determinative HPLC method. Extracts from both tissues were then subjected to clean up by partitioning and re-partitioning of the analyte into acetonitrile and hexane or by elution from a solid phase extraction (SPE) column. The extract was then analysed by LC-MS/MS, using a

triple quadrupole mass spectrometer equipped with an ion spray HPLC interface. Daughter ions (m/z 145, 331, 593, and 899) of the doramectin ammonium adduct (m/z 916) generated by collision activated dissociation (CAD) were monitored. The analyses of tissues fortified with approximately 200 µg/kg doramectin were successfully carried out as was the analysis of tissues incurred with doramectin residues at concentrations programmed to be in the range of 81 and 184 µg/kg. The method was specific for the confirmatory identification of doramectin in tissue. No interference was found when ivermectin was analysed by this method (Pfizer Inc, 1997b).

Prior to the assay of study samples, a one-day mini-validation was performed of the existing pig tissue assay on the HPLC system to be used. For these tests, the batch (M03B) consisted of a duplicate curve, triplicate process standards, triplicate quality assurance standards, and control tissue. For the calibration standards, a l/C weighted linear least squares regression provided absolute errors of the mean back-calculated concentration of 14.2% or less at any level. The precision (for duplicate assays of the standards) ranged from ± 0.2% at the 50.3 µg/kg level to ± 3.6% at the 2.52 µg/kg level. For the quality assurance samples, the errors of the mean concentration found ranged from 1.7% for the 201 µg/kg pool to 5.1% for the 50.3 µg/kg samples. Intra-assay precision for the Quality Assurance samples (n = 3) varied from ± 0.5% to ± 1.4%. There were no peaks in the blank tissue pool samples co-eluting at the retention times of either the drug or the internal standard. The correlation coefficient, r, was 0.99888 for this batch. Though not stated the internal standard was probably UK-71,647.

HPLC Method used in Radiolabelled Depletion Studies.

A different method was used for the determination of the amount of unchanged drug in the radiolabelled depletion study (Pfizer, 1992b). The method used twice the amount of tissue and a much milder extraction procedure. Five gram muscle, liver or kidney samples were vortexed at room temperature with acetone/water and partitioned with isooctane. The isooctane layer was chromatographed on florisil. After elution, the analyte was derivatised and further purified on a silica column before HPLC. This method was assumed to have failed to extract the majority of the doramectin residue in liver. The sponsor's claim that the recovery of doramectin from muscle, kidney and fat was the same for both analytical methods was not substantiated by submission of experimental data. The sponsor did not analyse the incurred tissues by both methods and thus it is difficult to give an accurate figure for the true ratio of unchanged drug to total residues that are found in liver.

**Maximum residue limits (MRLs)**

The ADI established by the Committee was 0 - 0.5 µg/kg and is equivalent to 30 µg per day for a 60 kg person.

The values for the mean + 3SD for total residues (see table 3) and the theoretical maximum daily intake (TMDI) at day 28 are calculated in Table 6. There is no correction for the ratio of MR:TR. The theoretical maximum daily intake of residues is 22.4 µg, which is 70% of the ADI. Thus there is reassurance from the radio-depletion study that the total residues do not exceed the ADI at day 28. The main problem in the calculation of MRL is placing a value on the ratio of MR to TR. In the radiolabelled studies, the ratio is almost certainly too low because of the incomplete extraction of doramectin (the marker residue). However, whereas the regulatory method for MRL would appear to extract a much higher proportion of doramectin from the tissues, this method has not been applied to the radiolabelled study.

**Table 6. Theoretical maximum daily intake (TMDI) calculated on the total residues (TR) found 28 days after an IM dose of 0.3 mg/kg BW of [$^3$H]-Doramectin . Data from Table 3.**

| Tissue | TR Mean ± SD (µg/kg) | TR + 3SD (µg/kg) | Tissue consumed (kg) | TMDI (µg) |
|---|---|---|---|---|
| Muscle | 4 ± 3 | 13 | 0.3 | 4 |
| Liver | 37 ± 20 | 97 | 0.1 | 9.7 |
| Kidney | 8 ± 3 | 17 | 0.05 | 0.9 |
| Fat | 58 ± 33 | 157 | 0.05 | 7.8 |
| | | | **Total** | 22.4 µg |

In recommending MRLs the Committee took account of the following factors;

1 The drug is only intended for use in non-lactating cattle and pigs.
2. The marker residue (MR) is the parent drug doramectin.
3. The target tissues are fat and liver.
4. The total residues do not exceed the ADI at 28 days after dosing.
5. The Committee agreed to use the same estimated ratio MR:TR for residues as were used by the 45th Committee. The percentage of residues of parent drug to total residues in each tissue are 70% for muscle, 55% for liver, 75% for kidney and 80% for fat.
6. There are <10% bound residues.
7. No multiple or repeat doses are administered.
8. The LOQs of the analytical methods are 2.5 µg/kg for muscle, liver and kidney and 5 µg/kg for fat.
9. The possibility of harmonising the MRL for cattle and pigs.

The Committee recommended MRLs for pigs of 5 µg/kg in muscle, 100 µg/kg in liver, 30 µg/kg for kidney and 150 µg/kg in fat expressed as parent drug. Incorporating the factors given above the TMDI for each tissue and the total TMDI is calculated as shown in Table 7.

**Table7. Calculation of TMDI for pigs based on recommended MRL**

| Tissue (Wt ) | MRL (µg/kg) | µg |
|---|---|---|
| Muscle (300 g) | 5 | 2.1 (0.7) |
| Liver (100 g) | 100 | 18 (0.55) |
| Kidney (50 g) | 30 | 2 (0.75) |
| Fat (50 g) | 150 | 9.4 (0.8) |
| **Total TMDI** | | **29.5 µg** |

- the figure in paretheses indicate the % of MR to TR adopted at the 45Th JECFA

Repeat dosing has not been considered. The Committee also notes the high concentrations of residues at the injection sites.

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# ESTRADIOL-17β, PROGESTERONE AND TESTOSTERONE

**First draft prepared by**
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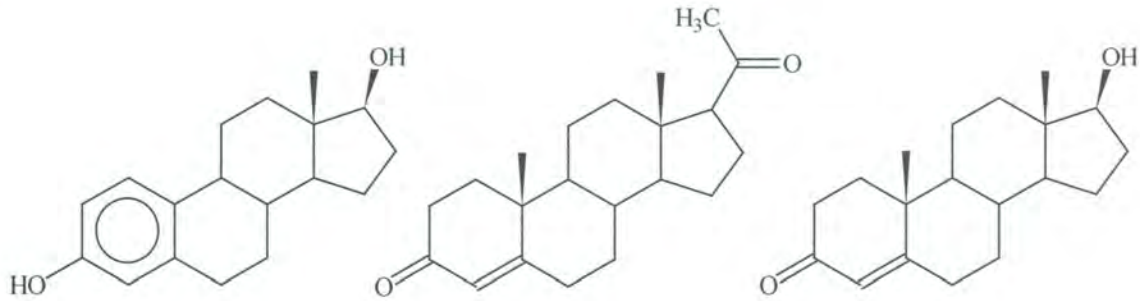
## IDENTITY

| | **Estradiol-17β** | **Progesterone** | **Testosterone** |
|---|---|---|---|
| **Chemical name:** | estra-1,3,5(10)-triene-3,17β-diol | pregn-4-ene-3,20-dione<br>Δ4-pregnene-3,20-dione | 17β-hydroxyandrost-4-en-3-one<br>Δ4-androsten-17β-ol-3-one |
| **Synonyms:** | estradiol-17β | Corpus luteum hormone<br>Luteohormone | trans-testosterone |

**Structural formula:**




| | | | |
|---|---|---|---|
| **Molecular formula:** | $C_{18}H_{24}O_2$ | $C_{21}H_{30}O_2$ | $C_{19}H_{28}O_2$ |
| Molecular weight: | 272.37 | 314.45 | 288.41 |
| Appearance: | White crystals or crystalline powder | Crystals | needles |
| Melting point: | 173-179° C | α-form: 127-131°C<br>β-form: 121°C | 155°C |
| Optical rotation: | $[\alpha]_D^{25}$ = +76 to +83° in dioxane | $[\alpha]_D^{25}$ = +172 to +182° (c=2 in dioxane) | $[\alpha]_D^{25}$ = +109° (c=4 in alcohol) |
| $UV_{max}$: | 225, 280 nm | 240 nm | 238 nm |
| Purity (USP grade): | 97-103% | >98% | >97% |

## DOSAGE

Estradiol-17β, alone or combinations with progesterone, testosterone or trenbolone acetate are given to animals to improve their rate of weight gain and feed efficiency. Administration is by subcutaneous implantation in the ear. The ear, along with any residual drug is discarded at slaughter. If estradiol-17β benzoate or testosterone propionate is used

instead of the un-esterified forms, the esters are rapidly hydrolysed in the animal after release from the implant. This report uses data obtained using the implants characterised in Table 1.

**Table 1 Composition of certain implants used for growth promotion and target animals for their use**

| Product Name | Composition of implants [mg/implant] | | | | | | Target Animals |
|---|---|---|---|---|---|---|---|
| | Estradiol | estradiol-benzoate | testosterone | testosterone propionate | progesterone | trenbolone-acetate | |
| Compudose | 24<br>45 | | | | | | cattle |
| Synovex S | | 20 | | | 200 | | steers |
| Synovex H | | 20 | | 200 | | | heifers |
| Synovex C | | 10 | | | 100 | | calves |
| Steer-oid | | 20 | | | 200 | | steers |
| Heifer-oid | | 20 | | 200 | | | heifers |
| Implix BM | 20 | | | | 200 | | steers |
| Implix BF | 20 | | 200 | | | | heifers |
| Torelor | 40 | | | | | 200 | steers |
| Revalor lactose | 20 | | | | | 140 | calves |
| Revalor G | 8 | | | | | 40 | steers |
| Revalor S | 24 | | | | | 120 | steers |
| Revalor H | 14 | | | | | 140 | heifers |
| Finaplix-S | | | | | | 140 | steers |

The use of Torelor as well as the concomitant or sequential use of Implix/Revalor is currently not authorised

## INTRODUCTION

The use of endogenous anabolic agents in farm animals, which has been reviewed (e.g., Weiert Velle, 1976), is summarised below.

### Estradiol-17β

Estradiol-17β is produced by the ovaries and the foetal placenta. In cows, production rates are very high in late pregnancy (several hundred mg every 24 hours). Plasma levels, which are present in the ng/L level in non-pregnant cows, are much higher (2-6 μg/L) in late pregnancy. Ruminants metabolise estradiol-17β to the 17α-isomer, which possesses very low estrogenic activity and is the major urinary metabolite. Biliary excretion is quantitatively the most important route for estradiol-17β.

Relative estrogenic potencies for estradiol-17β and its main metabolites in cattle have been estimated using the immature mouse uterus test and are shown in Table 2.

**Table 2. Relative estrogenic potencies for estradiol-17β and the main metabolites of in cattle**

| Estrogen | Subcutaneous route | Oral route |
|---|---|---|
| Estrone | 1 | 1 |
| Estradiol-17β | 3.41 | 2.01 |
| Estradiol-17α | 0.05 | 0.07 |

**Progesterone**

Progesterone is the main sex hormone produced by the corpus luteum and in cattle it is also responsible for the maintenance of pregnancy. Furthermore, it is an important precursor for the biosynthesis of androgens and corticosteroids and is also present in males and castrates. Conversion to androgens and excretion via faeces is an important route in ruminants.

**Testosterone**

The major source of testosterone is the testis. Small amounts are also secreted by the adrenals and the ovaries. Production rates in bulls are approximately 40-50 mg every 24 hours. Male cattle show a considerable diurnal variation in secretion. In the bovine male, testosterone is largely converted to the less active epitestosterone, which is mainly excreted in the faeces.

## RESIDUES IN ANIMALS AND THEIR EVALUATION

**Studies of the SYNOVEX® implants**

Studies with implants containing $^{14}C$ labelled hormones

Several early reports, starting about 1973, deal specifically with a description of the total radioactivity appearing in plasma, urine and faeces collected daily over a period of 14 days post implantation with $^{14}C$-labeled Synovex S and Synovex H preparations. Radioactivity was also determined in biopsy samples of selected muscle and fat tissues obtained three days post implantation, and in muscle, fat, liver and kidney obtained at slaughter 14 days post implantation (FDA/CVM NADA file 9-576, Vol. 3, p. 199; Vol. 4, p. 211; Vol. 4, p. 262; Vol. 4, p. 318). Four animals were implanted with different combinations of labelled and unlabelled steroid as shown in Table 3.

**Table 3. Composition of SYNOVEX® implants used in radioisotope studies in cattle**

| Animal | Amount of steroid implanted | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $^{14}C$-Estradiol benzoate | | Unlabelled estradiol benzoate | $^{14}C$-testosterone propionate | | Unlabelled testosterone propionate | $^{14}C$-progesterone | | Unlabelled progesterone |
| | mCi | mg | mg | mCi | mg | mg | mCi | mg | mg |
| Heifer A | | | 20 | 4.98 | 200 | | | | |
| Heifer B | 3.09 | 20 | | | | | | | 200 |
| Steer C | | | 20 | | | | 5.36 | 200 | |
| Steer D | 2.95 | 20 | | | | | | | 200 |

Table 4 summarises the recovery of radioactivity from urinary and faecal excreta form implanted animals, together with the radioactivity recovered from the implant. In general, the amount of radioactivity appearing in plasma was very low. The levels seen in the heifers were, on the average, lower than those seen in the steers, which could be explained by a lower release rate from the implants.

Analyses of biopsy samples removed three days after implantation showed that there was a lag period in the accumulation of hormones released from the implant site. Only radioactive progesterone could be detected in fat at this time. After 14 days, progesterone, of the three steroids, produced the majority of radioactive residues in all tissues. The smallest proportion was distributed to muscle, as shown in Table 5. The major route of excretion was via the faeces.

**Table 4.** **Recovery of radioactivity in urinary and faecal excreta and from the implants of implanted animals.**

| Animal | Labeled Molecule | Recovery of implanted dose (%) | | |
|---|---|---|---|---|
| | | Urinary excretion | Faecal excretion | Recovered from implant |
| Heifer A | Testosterone | 0.78 | 6.4 | 89 |
| Heifer B | Estradiol-17β | 3.4 | 5.4 | 79 |
| Steer C | Progesterone | 0.96 | 27 | 64 |
| Steer D | Estradiol-17β | 5.5 | 9.3 | 54 |

**Table 5** **Distribution of radioactivity in tissues of heifers and steers 14 days post implantation with radiolabelled hormones in the proportions of Synovex S and Synovex H**

| Tissue | | ng equivalents / kg of tissue | | | |
|---|---|---|---|---|---|
| | | Heifer | | Steer | |
| | | A | B | C | D |
| | | Testosterone propionate | Estradiol-17β | Progesterone | Estradiol-17β |
| Muscle- | Neck | 400 | 40 | 2500 | 30 |
| | Rump | 400 | 35 | 1900 | 40 |
| Liver | | 7000 | 660 | 54000 | 660 |
| Kidney | | 1700 | 680 | 16000 | 830 |
| Fat- | Neck | 2400 | 380 | 45000 | 400 |
| | Rump | 2600 | 380 | 45000 | 240 |

A significant fraction of the radioactivity was water-soluble and the metabolites responsible were probably conjugates of the respective hormones. The glucuronides appeared to be the predominant conjugates and were found in muscle, liver and kidney. Sulfate conjugates were also found in muscle, liver and kidney.

The radioactivity appearing in liver, kidney and muscle of Heifer $A_2$ which had received the implant with labelled testosterone propionate, was evenly distributed between the free and conjugated forms. In fat, the radioactivity appeared predominantly in the free form. In Heifer B, which received labelled estradiol in the implant, the unconjugated free estradiol fraction was predominant in fat and liver. The unconjugated fraction was only slightly higher than the conjugated fraction in muscle; in kidney the conjugated fraction was slightly higher than the unconjugated fraction. In Steer D, which had also received labelled estradiol in the implant, the unconjugated fractions dominated in fat and muscle. However, the conjugated fractions were slightly higher in liver and kidney.

The free steroid fraction predominated in the fat and muscle of Steer C, which had received labelled progesterone. In liver and kidney, the two fractions were more equally distributed with the unconjugated fraction still being slightly higher than the conjugated fraction. Unchanged progesterone was the main labelled compound in fat and was also the major component of the unconjugated fractions in muscle, liver and kidney. Between 10-20% of the total conjugated radioactivity was not hydrolysed by β-glucuronidase, sulfatase or solvolysis. The results are summarised in Table 6.

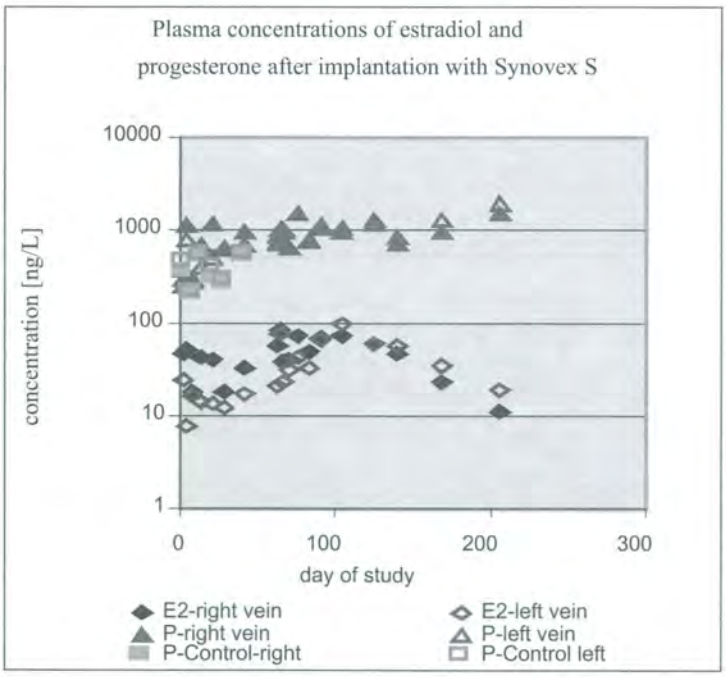
**Table 6. Distribution of radioactivity into water-soluble fraction ("conjugates") of treated cattle**

| Tissue | | Water soluble residues of $^{14}C$-testosterone propionate (%) | Water soluble residues of $^{14}C$ estradiol benzoate (%) | | Water soluble residues of $^{14}C$-progesterone (%) |
|---|---|---|---|---|---|
| | | Heifer A | Heifer B | Steer D | Steer C |
| Muscle | Neck | 50 | 40 | 35 | 21 |
| | Rump | 39 | 43 | 33 | 31 |
| Liver | | 56 | 27 | 57 | 48 |
| Kidney | | 56 | 63 | 66 | 41 |
| Fat | Neck | 13 | 10 | 18 | 4 |
| | Rump | 4 | 3 | 4 | 8 |

Effects of implantation / re-implantation of Synovex S on plasma levels in steers

Nine steers were treated with one Synovex implant in the right ear on study day 1 and re-implanted in the left ear on study day 63. Samples of blood were collected from the right and left jugular veins on various days after treatment and the concentrations of estradiol-17β and progesterone in plasma were determined (Lee and Massey, 1989). The outcomes of this study are illustrated in Figure 1. The number of sampling points was too limited to fully describe the kinetic and dynamic effects on plasma hormone levels resulting from the implantation. However, it seems that estrogen levels showed a distinct maximum at about 40 days after the second implantation (99.2 ng/L of estradiol in plasma from the left jugular vein on day 104 of the study). The highest level observed after the first implantation was observed on day 63 of the study, immediately before the re-implantation and was 57.8 ng/L. For progesterone the corresponding maxima were obtained on day 21 (1190 ng/L) and on day 206 (1910 ng/L).

**Figure 1. Plasma concentrations of estradiol and progesterone after implantation with SYNOVEX S**



Methods used for steroid quantification including methods validation

A radio-immunoassay method for the determination of estrone, estradiol-17β and progesterone was developed and progressively validated over several years (Kushinsky and Mirrasoul, 1979). The range of concentrations over which the assay was validated was consistent with the endogenous concentrations of hormones. The current review relies primarily on the progress report of the method by Kushinsky and Mirrasoul and on subsequent addenda to that report. The very sophisticated procedure, which has been described in full detail, starts with the extraction of the residues from tissues followed by removal of fat, separation of neutral and phenolic compounds, sulfatation, partitioning to separate progesterone from neutral hydroxy-steroids, and TLC to separate estrone from estradiol. When testosterone was later included in the procedure, hormone extracts were also purified by TLC prior to RIA analysis. Recovery was determined using $^{3}$H-labelled hormones (procedural recovery was 40-60%). A typical example of the results of the estimation of the procedural recovery is given in Table 7. From recovery experiments with fortified control samples the slopes of regression lines linking the concentration found to the concentration added can be calculated. It can be seen from the results given in Table 8 that the method slightly overestimates the concentrations of the free hormones with the exception of progesterone in muscle, liver and kidney, which are slightly underestimated.

**Table 7. Recovery (%) for the RIA analysis of estrone, estradiol-17β and progesterone**

| Tissue | Recovery (%) and Standard deviation (n=12) | | | | | |
|---|---|---|---|---|---|---|
| | Estrone | | Estradiol-17β | | Progesterone | |
| | Mean | SD | Mean | SD. | Mean | SD. |
| Muscle | 58.1 | 2.01 | 63.9 | 5.27 | 61.5 | 2.31 |
| Fat | 51.1 | 3.70 | 57.5 | 3.09 | 42.3 | 2.58 |

**Table 8. Performance factors for the RIA analysis of estrone, estradiol-17β and progesterone**

| Tissue | Working range and slope of regression line | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Estrone | | | Estradiol-17β | | | Progesterone | | |
| | Range | | Slope | Range | | Slope | Range | | Slope |
| Muscle | 2 | 27 | 1.11 | 0.94 | 13.5 | 1.14 | 650 | 2650 | 0.84 |
| Fat | 12 | 62 | 1.02 | 1.2 | 26 | 1.08 | 2800 | 18800 | 1.03 |
| Kidney | 10 | 60 | 1.17 | 17 | 117 | 1.19 | 770 | 4770 | 0.91 |
| Liver | 3 | 28 | 1.07 | 3 | 28 | 1.25 | 540 | 4540 | 0.95 |

Highly specific antisera were used for the radioimmunoassay. The RIA was typically conducted with 2 tubes for the unknown, 3 tubes per standard and 3 tubes for procedural controls. Examination of the details of the method description suggests that the procedure is unlikely to be capable of including the conjugated forms of the hormones in the determination of residues. Results were corrected for recovery. Procedural blanks were very low (1.2-1.4 picograms(pg)/test tube for estradiol, 1.9-2.5 pg/test tube for estrone, and 2.7-3.1 pg/test tube for progesterone). Using pooled tissue extracts, the RIA was further validated in a later study by demonstrating that nearly identical results were obtained using a GC-MS technique.

All analyses of incurred tissues were run as duplicates and, sometimes, as several replicates. The intra-assay variability with fortified tissues is concentration-dependent as seen in Figure 2.

In the process of further validation, tissues of six hormonally untreated finished steers were analysed in duplicate under intra-assay and inter-assay conditions, respectively. The results are compared in Figure 3.

**Figure 2.** **Variability intra-assay of sixfold estimates of steroid hormones in steer tissues**

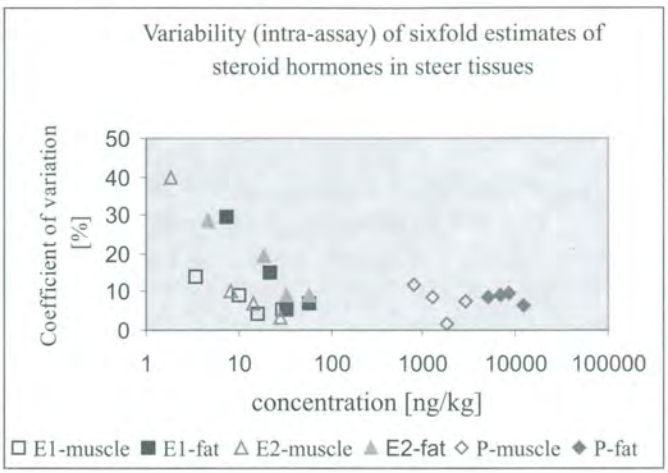
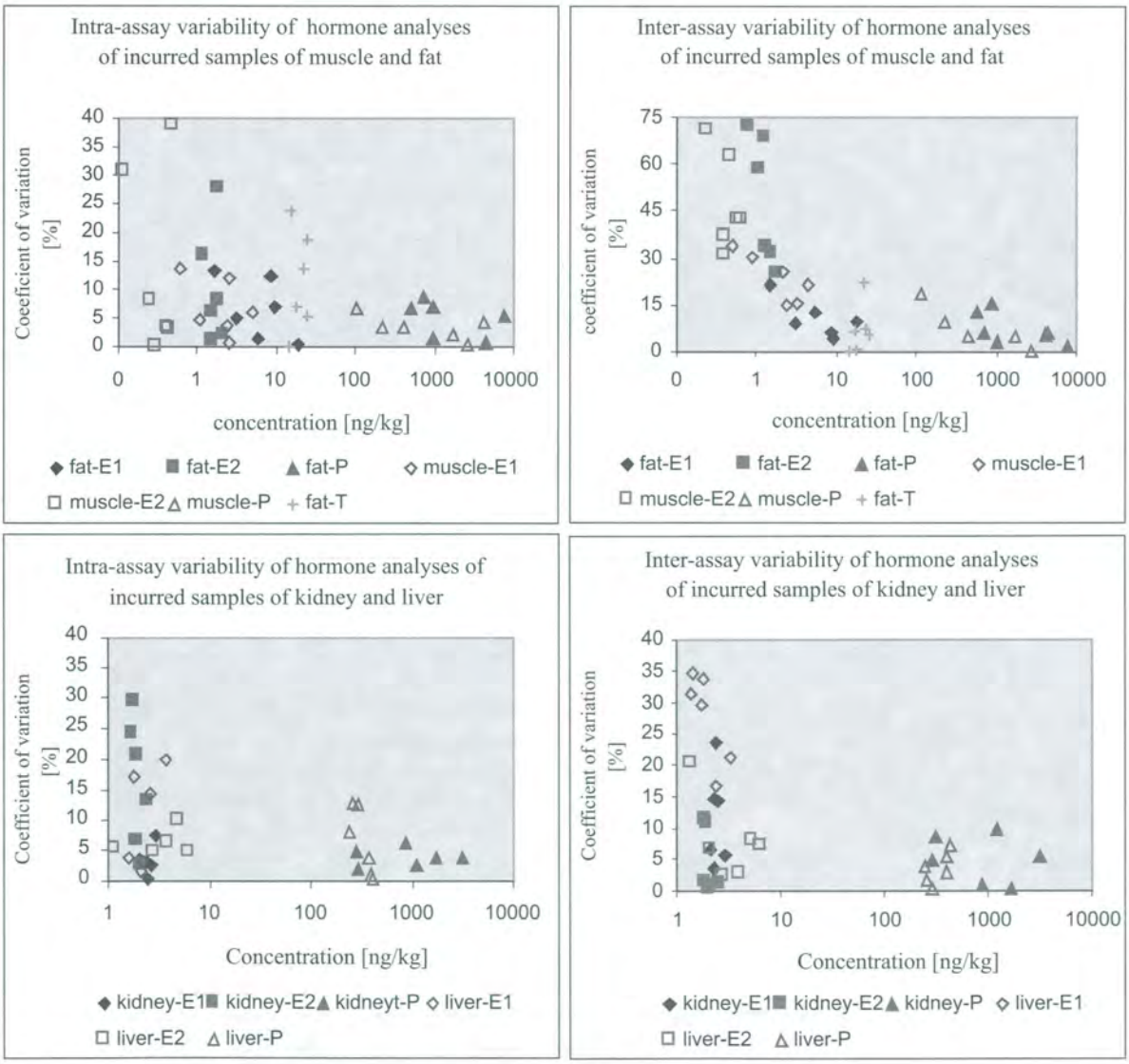



**Figure 3.** **Intra-assay variability of hormone analyses of incurred samples of muscle, fat, liver and kidney**



In all residue studies where this method was used all incurred tissues were analysed at least in duplicate. This database is excellently suited to investigate the precision of the method under routine conditions. The following figures 4a - 4c illustrate this performance characteristic as calculated by the current reviewer on the example of steer tissues and the three hormones progesterone, estradiol-17β and estrone.

**Figure 4. Precision of duplicate estimates of progesterone, estradiol-17β and estrone in steer tissues**

**Figure 4a**

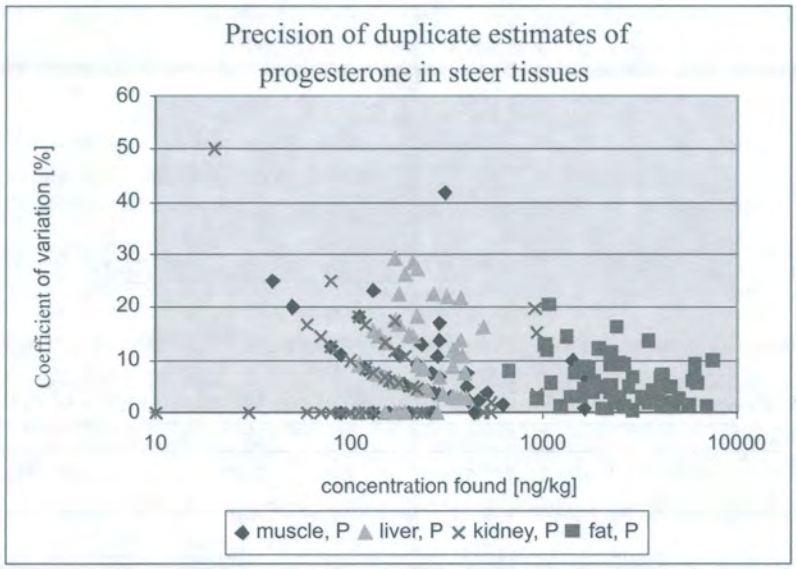



**Figure 4b**

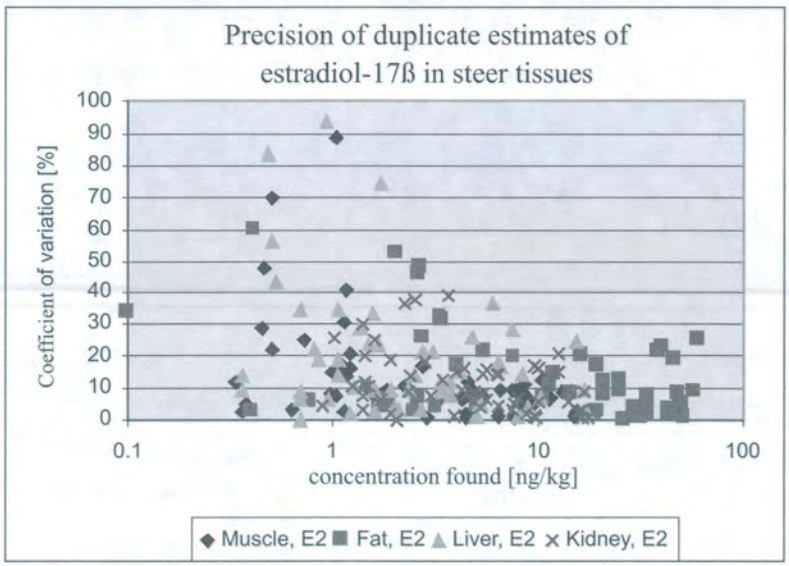
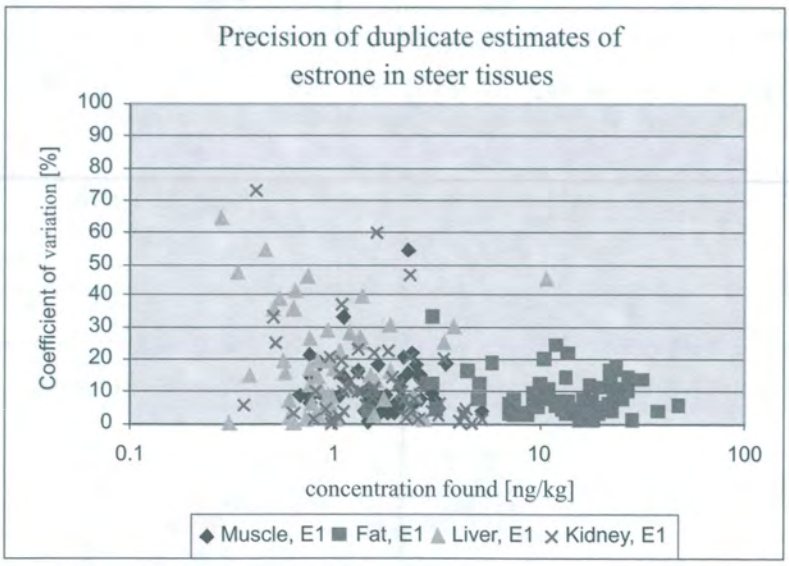



**Figure 4c**



The results obtained in a residue study with non-pregnant heifers were evaluated in the same way to estimate the precision of the method used. The results are shown in the following three Figures 5a - 5c for testosterone, estradiol-17β and estrone and the four edible tissues muscle, fat, liver and kidney

**Figure 5. Precision of duplicate estimates of testosterone, estradiol-17β and estrone in heifer tissues**

**Figure 5a**

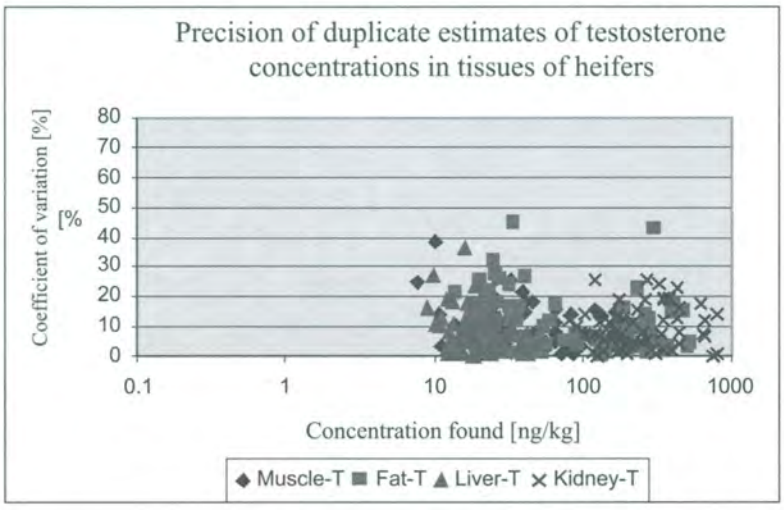



**Figure 5b**

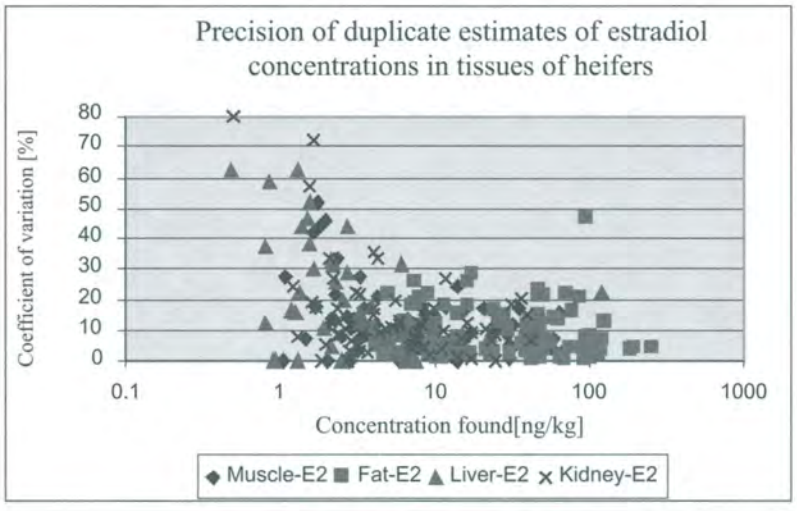
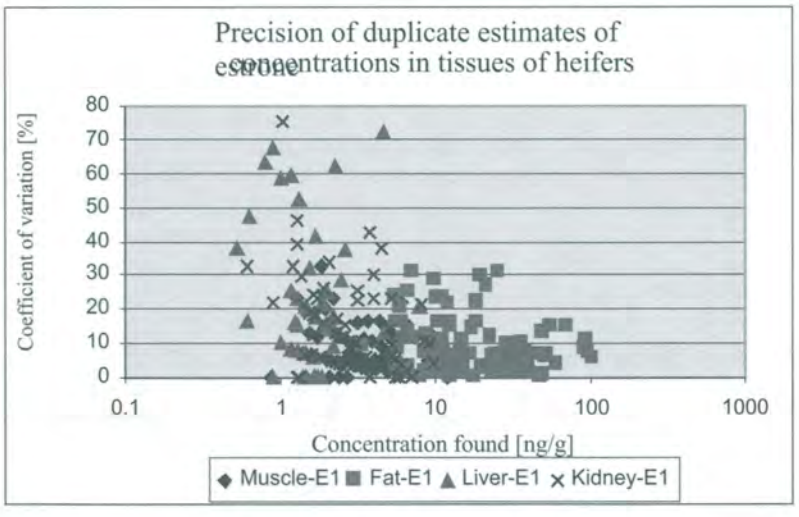



**Figure 5c**



**Information on residues**

Information obtained from studies of the analytical methodology

Some results of the method validation studies can be used as initial estimates of physiological levels of the three hormones in untreated steers. These data, obtained from six animals, are summarised in Table 9. The method was also used in a preliminary study to measure and to compare hormone concentrations in three pregnant heifers and in an untreated steer, as illustrated in Table 10.

**Table 9. Hormone concentrations in tissues of six hormonally untreated steers**

| Analytical parameter | Concentration (ng/kg) | | | | | |
|---|---|---|---|---|---|---|
| | Estrone | | Estradiol-17β | | Progesterone | |
| | Muscle | Fat | Muscle | Fat | Muscle | Fat |
| Range | 0.62-5.0 | 1.7-9.5 | 0.12-0.50 | 1.2-2.2 | 110-4200 | 530-7700 |
| Mean | 2.4 | 8.0 | 0.30 | 1.7 | 1570 | 2570 |
| Standard Deviation | 1.53 | 6.1 | 0.14 | 0.32 | 1650 | 2920 |
| Geometric Mean | 1.9 | 6.1 | 0.30 | 1.7 | 760 | 1530 |
| Median | 2.5 | 7.4 | 0.40 | 1.7 | 1080 | 970 |

**Table 10. Hormone concentrations (ng/kg) in tissues of pregnant heifers**

| Days pregnant | Hormone concentrations (ng/kg) in tissues of pregnant heifers | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Muscle | | | Fat | | | Kidney | | | Liver | | |
| | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P |
| 188 | 110 | 14.6 | 6350 | 1920 | 55.6 | 318000 | 207 | 280 | 7620 | 10.4 | 81.7 | 3180 |
| 243 | 458 | 44.9 | 6160 | 4880 | 139 | 106000 | 500 | 417 | 4090 | 258 | 630 | 2170 |
| 266 | 638 | 42.6 | 17800 | 5330 | 131 | 294000 | 602 | 484 | 6860 | 584 | 1590 | 4890 |
| | Hormone concentrations (ng/kg) in tissues of a steer | | | | | | | | | | | |
| | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P |
| | 1.8 | 0.97 | 624 | 10.2 | 1.4 | 3240 | 13 | 25.4 | 675 | 3.4 | 4.4 | 596 |

$E_1$ = Estrone, $E_2$ = Estradiol-17β, P = progesterone

Residue studies

**Residues in steers implanted with SYNOVEX S**

A residue study was conducted with 64 healthy, beef-type steers, weighing between 317.5 and 340.2 kg, which had not previously been implanted with any growth promoting substance (Study code IAS 1012-021.1.). The total duration of the study (minus acclimatisation period) was 120 days. Since all animals were slaughtered on day 120 and groups of animals were implanted on days 0, 30, 59, 90, 105, the time period between implantation and slaughter was 15, 30, 61, 90, and 120 days, respectively. Each treatment group initially comprised 8 animals and 21 animals served as a control. Some treatment groups consisted of 7 animals since two animals died and one developed chronic respiratory problems.

The statistical parameters describing the concentrations of hormones found in edible tissues were calculated from the individual results: number of animals sampled per control and treatment group, respectively, minimal concentration found, maximal concentration found, arithmetic mean and standard deviation, geometric mean and median. The results

of these calculations are summarised in Table 11 following the full discussion of residues. The means and standard deviations calculated from the raw data by the current reviewer sometimes slightly deviate from the results given in the original report. The current reviewer decided to uniformly present all data as results of duplicate estimates. The report of the authors of the studies use averages of a varying number of >2 of replicates if the same samples had been analysed more than twice and by different technicians (e.g., in order to determine the transferability of the method and to obtain an estimate of interlaboratory variability).

**Residues in nonpregnant heifers implanted with SYNOVEX H**

A study was conducted in which non-pregnant heifers were implanted with Synovex H Heifer finishing implant (study code IAS 1012-116.1.) (Kushinskyand Duffy, undated). The product contained 200 mg testosterone propionate and 20 mg estradiol benzoate and is recommended for use in heifers weighing 400 lbs. or more during the last 60 to 150 days of the fattening period. Animals were slaughtered 30, 60, 89 or 119 days after implantation and residues of estrone, estradiol, and testosterone were determined in fat, muscle, kidney and liver. Each sample was analysed in duplicate. Statistical parameters were calculated as described for the steer study. These data are given in Table 12 below.

**Residues in pregnant heifers implanted with SYNOVEX H**

It is stated in the dossier of the SYNOVEX implants (Kushinsky and Duffy, 1982) that pregnant heifers enter feedlots and are brought to slaughter in significant numbers and therefore consist of a significant part of the meat reaching the consuming public. A study was conducted in which tissues were obtained from pregnant heifers that were approximately 120, 180 or 240 days pregnant. Both the control animals the animals implanted 61, 90 or 120 days before slaughter were used. Fenprostalene was used for synchronisation of the estrous cycles to facilitate breeding of the animals by artificial insemination. Additional control animals, which were not synchronised, were also used. Statistical parameters were calculated as described for the steer study. These data are given in Table 13 below.

**Residues in calves implanted with SYNOVEX**

Thirty six suckling steer calves and 42 suckling heifer calves (purebred and crossbred Hereford) averaging 59.4 kg (range 34 to 94 kg) were implanted with Synovex-C implant, comprising 10 mg of estradiol benzoate plus 100 mg of progesterone ((Kushinsky and Duffy, 1983: Sheldon *et al.*, 1983). An equal number of animals served as non-implanted controls. Average daily growth rates prior to weaning were in the range of 0.59 to 0.64 kg/day. Calves were re-implanted 118 and 240 days later with steers receiving the Synovex S implant and heifers receiving the Synovex H implant. All implants were commercial products. The typical increase in average daily gain due to Synovex implants was apparent by 240 days.

Four to eight implanted and non-implanted calves of each sex were slaughtered at 61,119,241 301, 329 and 360 days after the initial implant was administered. Samples of edible tissues were analysed for concentrations of estradiol-17β, estrone and either progesterone or testosterone.

Generally, larger residues were found after each successive implantation. The residues of estrogens found after a single implantation were similar to those previously found for steers and heifers implanted with Synovex S and Synovex H, respectively (Study code IAS-1012-341.3). Results of residues in male calves are summarised in Tables 14 – 17, and residues in female calves are summarised in Tables 18 – 21.

**Table 11. Concentrations (ng/kg) of Estrone, Estradiol-17β and Progesterone in edible tissues of steers implanted with SYNOVEX-S**

| Tissue | Day after implantation | Concentrations (ng/kg) of estrone, estradiol-17β and progesterone in edible tissues of control steers and steers implanted with SYNOVEX-S | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Estrone | | | | | | | Estradiol-17β | | | | | | Progesterone | | | | | |
| | | n | Min. | Max. | Mean | S.D. | Geo. Mean | Median | Min. | Max. | Mean | S.D. | Geo. Mean | Median | Min. | Max. | mean | S.D. | Geo. Mean | Median |
| Fat | Control | 21 | 1.49 | 18.9 | 8.62 | 5.14 | 7.11 | 7.37 | 0.10 | 3.66 | 1.89 | 1.14 | 1.37 | 1.91 | 670 | 6280 | 2535 | 1762 | 2061 | 2290 |
| | 15 | 8 | 11.4 | 39.2 | 20.2 | 9.21 | 18.6 | 18.6 | 25.3 | 51.2 | 41.4 | 8.53 | 40.5 | 42.2 | 1430 | 4750 | 3196 | 1167 | 2979 | 3345 |
| | 30 | 8 | 3.04 | 25.8 | 14.4 | 7.71 | 12.1 | 14.7 | 5.13 | 61.5 | 28.6 | 21.8 | 20.8 | 26.0 | 1360 | 6400 | 3478 | 1641 | 3110 | 3725 |
| | 61 | 8 | 9.65 | 48.3 | 25.9 | 11.8 | 23.5 | 25.7 | 20.0 | 52.4 | 39.9 | 11.0 | 38.3 | 43.9 | 1680 | 5840 | 3528 | 1465 | 3260 | 3290 |
| | 90 | 7 | 13.2 | 27.8 | 18.0 | 5.43 | 17.4 | 16.3 | 12.2 | 48.3 | 26.8 | 11.7 | 24.7 | 25.6 | 1770 | 7710 | 4029 | 2444 | 3456 | 3350 |
| | 120 | 7 | 7.66 | 24.4 | 16.6 | 5.53 | 15.7 | 16.1 | 4.06 | 30.0 | 14.5 | 8.91 | 12.0 | 14.8 | 1490 | 4660 | 2657 | 1110 | 2482 | 2110 |
| Muscle | Control | 16 | 0.67 | 2.61 | 1.56 | 0.65 | 1.43 | 1.53 | 0.34 | 1.21 | 0.77 | 0.35 | 0.69 | 0.68 | 40 | 1410 | 271 | 327 | 188 | 150 |
| | 15 | 8 | 1.39 | 3.05 | 2.12 | 0.59 | 2.04 | 2.24 | 4.58 | 15.3 | 9.60 | 3.17 | 9.11 | 9.71 | 50 | 460 | 230 | 121 | 197 | 210 |
| | 30 | 8 | 0.75 | 3.51 | 1.98 | 0.85 | 1.81 | 2.04 | 1.00 | 15.3 | 8.27 | 5.14 | 6.01 | 8.74 | 160 | 310 | 227 | 52.5 | 222 | 220 |
| | 61 | 8 | 0.77 | 5.21 | 2.35 | 1.29 | 2.08 | 2.13 | 3.75 | 11.3 | 7.34 | 2.72 | 6.88 | 7.23 | 80 | 1650 | 419 | 507 | 282 | 260 |
| | 90 | 7 | 0.76 | 2.55 | 1.79 | 0.60 | 1.68 | 1.96 | 0.97 | 7.81 | 4.51 | 2.18 | 3.85 | 4.51 | 90 | 1670 | 439 | 573 | 250 | 180 |
| | 120 | 7 | 0.92 | 3.02 | 1.79 | 0.69 | 1.68 | 1.63 | 1.04 | 3.33 | 2.18 | 0.86 | 2.01 | 2.23 | 130 | 2460 | 583 | 836 | 342 | 270 |
| Kidney | Control | 18 | 0.18 | 2.34 | 1.03 | 0.50 | 0.90 | 1.02 | 0.55 | 2.78 | 1.7 | 0.6 | 1.55 | 1.53 | 10 | 530 | 161 | 141 | 111 | 135 |
| | 15 | 8 | 3.10 | 7.31 | 4.44 | 1.37 | 4.28 | 4.28 | 8.88 | 17.0 | 13.7 | 3.0 | 13.4 | 14 | 80 | 210 | 135 | 49 | 127 | 120 |
| | 30 | 8 | 1.20 | 4.39 | 2.55 | 1.23 | 2.30 | 2.18 | 1.42 | 17.6 | 9.45 | 5.4 | 7.6 | 9.7 | 70 | 150 | 114 | 31 | 110 | 120 |
| | 61 | 8 | 1.30 | 2.61 | 1.99 | 0.49 | 1.93 | 2.21 | 4.37 | 9.53 | 6.4 | 1.9 | 6.1 | 6.0 | 0 | 920 | 170 | 306 | NC | 80 |
| | 90 | 7 | 0.41 | 2.98 | 1.43 | 0.88 | 1.21 | 1.08 | 1.39 | 12.7 | 5.7 | 3.5 | 4.7 | 5.38 | 10 | 930 | 321 | 415 | 117 | 90 |
| | 120 | 7 | 0.63 | 2.56 | 1.70 | 0.64 | 1.57 | 1.56 | 2.37 | 6.39 | 4.2 | 1.3 | 4.0 | 3.91 | 60 | 540 | 173 | 172 | 128 | 90 |
| Liver | Control | 18 | 0.28 | 1.00 | 0.65 | 0.24 | 0.60 | 0.73 | 0.36 | 1.72 | 0.91 | 0.42 | 0.82 | 0.87 | 170 | 380 | 254 | 74 | 244 | 240 |
| | 15 | 8 | 1.32 | 3.85 | 2.04 | 0.84 | 1.91 | 1.73 | 1.92 | 8.05 | 5.36 | 2.04 | 4.93 | 5.51 | 130 | 240 | 175 | 34 | 172 | 175 |
| | 30 | 8 | 0.96 | 3.44 | 1.79 | 0.94 | 1.61 | 1.48 | 2.76 | 16.8 | 6.74 | 4.93 | 5.50 | 4.24 | 110 | 210 | 155 | 33 | 152 | 145 |
| | 61 | 8 | 0.54 | 10.9 | 2.09 | 3.57 | 1.11 | 0.79 | 0.7 | 17.4 | 4.51 | 5.48 | 2.83 | 2.42 | 280 | 490 | 349 | 68 | 343 | 340 |
| | 90 | 7 | 0.46 | 2.46 | 1.21 | 0.71 | 1.05 | 1.06 | 0.85 | 15.6 | 5.41 | 5.15 | 3.66 | 3.37 | 110 | 400 | 240 | 117 | 217 | 195 |
| | 120 | 7 | 0.50 | 0.93 | 0.72 | 0.19 | 0.70 | 0.64 | 0.69 | 2.07 | 1.39 | 0.42 | 1.32 | 1.4 | 180 | 370 | 272 | 81 | 262 | 245 |

n = number of specimens analysed; s.d. = standard deviation; geo. mean = geometric mean. NC = not calculated (see min. = zero)

**Table 12. Concentrations (ng/kg) of estrone, estradiol-17β and testosterone in edible tisssues of heifers implanted with SYNOVEX H**

| Tissue | Day after implantation | Concentrations (ng/kg) of estrone, estradiol-17β and testosterone in edible tissues of control heifers and heifers implanted with SYNOVEX-H | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Estrone | | | | | | | Estradiol-17β | | | | | | Testosterone | | | | | |
| | | n | Min. | Max. | Mean | S.D. | Geo. Mean | Median | Min. | Max. | Mean | S.D. | Geo. Mean | Median | Min. | Max. | Mean | S.D. | Geo. Mean | Median |
| Fat | Control | 35 | 2.11 | 31.7 | 10.9 | 5.94 | 9.54 | 10.4 | 3.93 | 71.5 | 13.4 | 14.1 | 10.1 | 9.19 | 14.5 | 44.7 | 25.9 | 6.94 | 25.1 | 23.9 |
| | 30 | 20 | 5.73 | 105 | 45.3 | 29.4 | 36.6 | 36.5 | 4.76 | 260 | 86.7 | 65.9 | 61.2 | 73.2 | 35.1 | 1113 | 359 | 228 | 274 | 290 |
| | 60 | 25 | 8.9 | 55.9 | 27.8 | 13.8 | 24.5 | 26.0 | 11.1 | 127 | 49.2 | 30.8 | 40.2 | 48.1 | 24.1 | 377 | 142 | 104 | 103 | 129 |
| | 89 | 10 | 14.8 | 70.2 | 35.3 | 17.5 | 31.7 | 29.0 | 10.5 | 118 | 62.3 | 34.9 | 51.3 | 54.3 | 26.6 | 203 | 115 | 69.8 | 91.2 | 132 |
| | 119 | 10 | 6.76 | 32.7 | 13.6 | 8.06 | 12.0 | 11.6 | 8.2 | 41.3 | 17.5 | 13.0 | 14.4 | 11.3 | 14.7 | 54.0 | 32.1 | 12.1 | 30.0 | 30.9 |
| Muscle | Control | 15 | 1.29 | 4.33 | 2.54 | 1.12 | 2.32 | 2.28 | 1.05 | 35.2 | 5.80 | 9.05 | 3.41 | 2.91 | 7.58 | 33.3 | 19.6 | 7.09 | 18.3 | 21.7 |
| | 30 | 20 | 0.88 | 12.8 | 6.42 | 3.68 | 5.28 | 5.27 | 2.14 | 65.8 | 33.2 | 19.6 | 24.8 | 30.5 | 11.3 | 183 | 102 | 48.1 | 85.6 | 101 |
| | 60 | 10 | 1.94 | 6.34 | 3.98 | 1.45 | 3.74 | 3.76 | 3.57 | 20.5 | 10.7 | 5.14 | 9.49 | 10.2 | 16.8 | 84.4 | 46.7 | 22.3 | 41.6 | 40.9 |
| | 89 | 10 | 2.92 | 12.3 | 6.40 | 3.38 | 5.67 | 4.77 | 2.39 | 16.8 | 10.1 | 4.44 | 8.96 | 10.4 | 20.9 | 135 | 58.7 | 34.2 | 50.6 | 51.9 |
| | 119 | 10 | 1.68 | 4.08 | 2.55 | 0.75 | 2.46 | 2.55 | 1.09 | 5.58 | 2.55 | 1.35 | 2.28 | 2.07 | 14.0 | 54.9 | 31.2 | 12.4 | 29.1 | 27.1 |
| Kidney | Control | 15 | 0.61 | 2.97 | 1.42 | 0.56 | 1.32 | 1.41 | 0.5 | 11 | 2.89 | 2.50 | 2.26 | 1.97 | 81 | 383 | 189 | 91.6 | 170 | 169 |
| | 30 | 10 | 2.15 | 9.7 | 5.87 | 2.27 | 5.45 | 5.37 | 4.82 | 42.5 | 23.6 | 11.9 | 20.2 | 23.0 | 161 | 793 | 451 | 201 | 407 | 431 |
| | 60 | 15 | 1.47 | 8.32 | 3.55 | 2.02 | 3.16 | 3.03 | 2.44 | 36.9 | 9.83 | 8.98 | 7.57 | 7.51 | 122 | 661 | 228 | 143 | 203 | 180 |
| | 89 | 10 | 2.35 | 6.55 | 4.23 | 1.49 | 3.99 | 3.96 | 2.42 | 16.4 | 8.88 | 4.83 | 7.62 | 7.72 | 106 | 816 | 371 | 267 | 292 | 256 |
| | 119 | 10 | 1.3 | 2.7 | 1.75 | 0.46 | 1.7 | 1.7 | 1.59 | 6.01 | 3.16 | 1.41 | 2.92 | 2.85 | 168 | 444 | 307 | 89.3 | 294 | 330 |
| Liver | Control | 10 | 0.63 | 4.57 | 1.7 | 1.15 | 1.44 | 1.39 | 0.48 | 3.89 | 1.54 | 1.12 | 1.23 | 1.00 | 9.98 | 15.5 | 12.9 | 1.96 | 12.8 | 13.3 |
| | 30 | 10 | 1.49 | 11.5 | 3.69 | 3.36 | 2.80 | 2.04 | 4.28 | 121 | 23.1 | 36.6 | 11.3 | 6.75 | 18.5 | 51.2 | 34.1 | 9.98 | 32.8 | 31.8 |
| | 60 | 10 | 0.53 | 2.66 | 1.49 | 0.76 | 1.30 | 1.44 | 0.8 | 7.62 | 3.21 | 2.36 | 2.51 | 2.25 | 8.85 | 19.5 | 15.7 | 3.34 | 15.3 | 16.3 |
| | 89 | 10 | 1.01 | 1.94 | 1.51 | 0.29 | 1.49 | 1.54 | 1.2 | 6.49 | 3.28 | 1.78 | 2.91 | 2.65 | 14 | 42.5 | 22.6 | 7.89 | 21.6 | 20.3 |
| | 119 | 10 | 0.89 | 1.68 | 1.33 | 0.22 | 1.31 | 1.35 | 0.93 | 2.21 | 1.48 | 0.42 | 1.43 | 1.36 | 10.8 | 26.9 | 16.1 | 5.26 | 15.5 | 14.9 |

n = number of specimens analysed; s.d = standard deviation; geo.mean = geometric mean

**Table 13. Concentrations of estrone, estradiol-17β and testosterone in edible tissues of pregnant heifers implanted with SYNOVEX H**

| Treatment group | days pregnant | n | min. | max. | mean | s.d. | geom. | median | min. | max. | mean | s.d. | geom. | median | min. | max. | mean | s.d. | geom. | median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Estrone in Fat (ng/kg) | | | | | | Estradiol-17 β in Fat (ng/kg) | | | | | | Testosterone in Fat (ng/kg) | | | | | |
| Unsynchronised control | 120 | 7 | 77.8 | 1964 | 780 | 641 | 526 | 811 | 11.5 | 52.7 | 30.9 | 13.9 | 28.0 | 31.8 | 228 | 530 | 406 | 101 | 394 | 414 |
| Synchronised controls | 120 | 10 | 213 | 3175 | 1283 | 885 | 1008 | 1132 | 13.7 | 77.5 | 42.1 | 19.8 | 37.8 | 38.8 | 411 | 1008 | 590 | 176 | 570 | 540 |
| 61 Days implanted | 120 | 5 | 44.5 | 877 | 421 | 352 | 273 | 300 | 43.8 | 140 | 82.9 | 37.6 | 76.3 | 79.6 | 446 | 942 | 751 | 198 | 727 | 803 |
| Synchronised controls | 180 | 11 | 983 | 4579 | 2717 | 1259 | 2417 | 2737 | 30.6 | 159 | 71.5 | 37.2 | 64.0 | 65.8 | 511 | 1000 | 751 | 174 | 732 | 731 |
| 61 Days implanted | 180 | 5 | 578 | 3448 | 1896 | 1290 | 1533 | 1299 | 62.7 | 208 | 123 | 58.9 | 112 | 136 | 701 | 1463 | 1047 | 274 | 1018 | 1012 |
| Synchronised controls | 240 | 4 | 1037 | 4663 | 2786 | 1497 | 2441 | 2723 | 23.7 | 108 | 67.5 | 34.6 | 59.1 | 69.0 | 445 | 1002 | 694 | 231 | 666 | 666 |
| 61 Days implanted | 240 | 4 | 2637 | 5980 | 4614 | 1513 | 4399 | 4920 | 95.4 | 152 | 136 | 27.2 | 134 | 149 | 981 | 1318 | 1195 | 158 | 1186 | 1240 |
| | | | Estrone in Muscle (ng/kg) | | | | | | Estradiol-17 β in Muscle (ng/kg) | | | | | | Testosterone in Muscle (ng/kg) | | | | | |
| Unsynchronised control | 120 | 8 | 13.9 | 462 | 203 | 170 | 127 | 154 | 4.3 | 31.6 | 15.6 | 11.6 | 11.9 | 11.3 | 126 | 547 | 302 | 163 | 266 | 247 |
| Synchronised controls | 120 | 10 | 37.7 | 299 | 156 | 79.3 | 133 | 173 | 5.12 | 21.3 | 13.2 | 5.21 | 12.2 | 12.8 | 174 | 500 | 267 | 101 | 252 | 230 |
| 61 Days implanted | 120 | 5 | 22.5 | 178 | 83.1 | 67.8 | 60.8 | 60.0 | 21 | 44.3 | 30.6 | 11.2 | 29.1 | 24.4 | 189 | 542 | 357 | 130 | 337 | 329 |
| Synchronised controls | 180 | 11 | 85.0 | 1115 | 482 | 301 | 387 | 422 | 9.82 | 57.8 | 27.3 | 14.3 | 24.1 | 24.0 | 232 | 557 | 343 | 117 | 327 | 312 |
| 61 Days implanted | 180 | 5 | 52.2 | 285 | 167 | 108 | 137 | 122 | 13.7 | 35.0 | 24.7 | 7.88 | 23.6 | 23.4 | 291 | 479 | 356 | 81.4 | 349 | 309 |
| Synchronised controls | 240 | 4 | 208 | 774 | 523 | 240 | 470 | 555 | 9.54 | 45.8 | 32.7 | 16.1 | 28.0 | 37.8 | 202 | 630 | 418 | 107 | 386 | 421 |
| 61 Days implanted | 240 | 4 | 196 | 435 | 353 | 109 | 338 | 391 | 15.0 | 33 | 26.3 | 8.16 | 25.2 | 28.7 | 251 | 457 | 370 | 89.0 | 361 | 387 |
| | | | Estrone in Liver (ng/kg) | | | | | | Estradiol-17 β in Liver (ng/kg) | | | | | | Testosterone in Liver (ng/kg) | | | | | |
| Unsynchronised control | 120 | 8 | 1.3 | 94.3 | 29.7 | 33.0 | 13.1 | 18.5 | 3.99 | 139 | 58.4 | 49.9 | 34.3 | 49.9 | 20.9 | 56.8 | 38.9 | 13.2 | 36.9 | 36.7 |
| Synchronised controls | 120 | 10 | 6.5 | 53.2 | 18.2 | 15.1 | 14.2 | 12.1 | 26.7 | 271 | 82.5 | 75.9 | 61.17 | 53.1 | 40.9 | 73.3 | 52.8 | 10.1 | 52.0 | 50.3 |
| 61 Days implanted | 120 | 5 | 3.01 | 7.84 | 5.52 | 2.19 | 5.141 | 6.03 | 10.4 | 27.7 | 18.7 | 7.38 | 17.6 | 16.0 | 28.9 | 46.2 | 37.6 | 6.94 | 37.1 | 39.4 |
| Synchronised controls | 180 | 11 | 32.5 | 320 | 115 | 82.6 | 94.1 | 91 | 73 | 925 | 380 | 280 | 299 | 242 | 99.7 | 147 | 121 | 19.4 | 120 | 117 |
| 61 Days implanted | 180 | 5 | 8.95 | 44.7 | 23.4 | 13.7 | 20.3 | 19.1 | 22.7 | 100 | 50.4 | 33.6 | 42.4 | 34.7 | 49.7 | 71.65 | 60.6 | 7.96 | 60.2 | 60 |
| Synchronised controls | 240 | 4 | 66.3 | 196 | 145 | 55.4 | 135 | 159 | 526 | 1396 | 1027 | 365 | 967 | 1094 | 207 | 353 | 273 | 70.1 | 266 | 266 |
| 61 days implanted | 240 | 4 | 8.52 | 95.8 | 34.5 | 41.5 | 20.8 | 16.8 | 50.7 | 438 | 198 | 173 | 145 | 152 | 81.9 | 102 | 90.2 | 8.75 | 89.9 | 88.6 |
| | | | Estrone in Kidney (ng/kg) | | | | | | Estradiol-17β in Kidney (ng/kg) | | | | | | Testosterone in Kidney (ng/kg) | | | | | |
| Unsynchronised control | 120 | 5 | 49.4 | 101 | 84.2 | 21.0 | 81.6 | 87.1 | 63.8 | 191 | 127 | 46.3 | 119 | 120 | 1371 | 2373 | 1930 | 453 | 1885 | 2086 |
| Synchronised controls | 120 | 10 | 31.1 | 268 | 85.3 | 69.4 | 69.9 | 60.7 | 68.8 | 258 | 118 | 58.6 | 108 | 99.6 | 1185 | 2250 | 1513 | 331 | 1484 | 1413 |
| 61 Days implanted | 120 | 5 | 11.8 | 66.6 | 29.7 | 23.1 | 23.8 | 17.4 | 30.9 | 138 | 62.3 | 44.8 | 52.3 | 43.2 | 1276 | 2344 | 1856 | 426 | 1814 | 1808 |
| Synchronised controls | 180 | 6 | 37.5 | 304 | 166 | 94.0 | 139 | 151.3 | 90.9 | 354 | 230 | 99.9 | 208 | 259 | 2010 | 5644 | 3505 | 1537 | 3248 | 2805 |
| 61 Days implanted | 180 | 5 | 9.35 | 110 | 59.4 | 36.6 | 45.9 | 64.7 | 39.4 | 184 | 126 | 58.2 | 111 | 140 | 1579 | 2813 | 1974 | 510.3 | 1927 | 1751 |
| Synchronised controls | 240 | 4 | 86.2 | 186 | 142 | 41.2 | 137 | 148 | 163 | 352 | 274 | 84.8 | 263 | 291 | 1632 | 6801 | 4014 | 2269 | 3503 | 3812 |
| 61 Days implanted | 240 | 4 | 117 | 271 | 214 | 67 | 204 | 234 | 165 | 407 | 318 | 107 | 301 | 351 | 1782 | 4311 | 2914 | 1057 | 2773 | 2782 |

**Table 14. Hormone concentrations (ng/kg) in muscle of calves (steers) implanted with SYNOVEX C and S**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A | 5 | 1.35 | 4.36 | 2.37 | 1.18 | 2.18 | 2.05 | 0.56 | 1.47 | 0.94 | 0.36 | 0.78 | 0.78 | 2.63 |
| E2 | | 5 | 10.7 | 12.2 | 11.5 | 0.58 | 11.4 | 11.7 | 0.72 | 1.44 | 1.11 | 0.30 | 1.08 | 1.01 | 11.5 |
| P | | 5 | 134 | 1336 | 764 | 463 | 597 | 863 | 49.1 | 548 | 292 | 191 | 223 | 318 | 2.72 |
| E1 | B | 5 | 1.36 | 3.53 | 2.17 | 0.93 | 2.02 | 1.88 | 0.32 | 1.09 | 0.57 | 0.31 | 0.55 | 0.49 | 3.84 |
| E2 | | 5 | 3.75 | 6.87 | 5.67 | 1.23 | 5.54 | 5.84 | 0.36 | 0.73 | 0.51 | 0.14 | 0.50 | 0.47 | 12.4 |
| P | | 5 | 128 | 602 | 313 | 199 | 265 | 242 | 121 | 860 | 404 | 325 | 298 | 302 | 0.8 |
| E1 | C | 5 | 1.32 | 4.08 | 2.72 | 1.10 | 2.52 | 2.78 | 1.13 | 2.31 | 1.73 | 0.55 | 2.31 | 1.93 | 1.44 |
| E2 | | 5 | 2.78 | 13.4 | 7.33 | 4.38 | 6.29 | 5.52 | 1.89 | 2.72 | 2.21 | 0.32 | 2.20 | 2.18 | 2.53 |
| P | | 5 | 328 | 804 | 517 | 239 | 476 | 370 | 356 | 946 | 563 | 226 | 532 | 507 | 0.73 |
| E1 | D | 5 | 2.82 | 6.68 | 4.42 | 1.57 | 4.2 | 4.53 | 0.63 | 1.4 | 0.93 | 0.34 | 1.18 | 0.79 | 5.73 |
| E2 | | 5 | 15.1 | 71.4 | 29.6 | 23.5 | 24.7 | 21.75 | 0.75 | 8.79 | 2.91 | 3.36 | 1.89 | 1.3 | 16.7 |
| P | | 5 | 297 | 414 | 355 | 43.3 | 352 | 353 | 148 | 2887 | 886 | 1129 | 528 | 438 | 0.81 |
| E1 | E | 5 | 1.63 | 3.77 | 2.77 | 0.88 | 2.66 | 2.53 | 1.28 | 2.41 | 1.95 | 0.48 | 1.28 | 2.22 | 1.14 |
| E2 | | 5 | 6.9 | 14.8 | 9.72 | 2.97 | 9.41 | 9.06 | 0.81 | 1.84 | 1.23 | 0.43 | 1.17 | 1.02 | 8.88 |
| P | | 5 | 275 | 1335 | 541 | 452 | 440 | 331 | 305 | 1009 | 607 | 279 | 557 | 544 | 0.61 |
| E1 | F | 4 | 1.08 | 6.01 | 3.29 | 2.53 | 2.48 | 3.04 | 0.69 | 3.55 | 1.61 | 1.20 | 0.72 | 1.14 | 2.67 |
| E2 | | 4 | 3.77 | 9.78 | 6.23 | 2.60 | 5.85 | 5.68 | 0.52 | 3.64 | 1.69 | 1.44 | 1.22 | 0.97 | 5.86 |
| P | | 4 | 357 | 1461 | 777 | 477 | 681 | 645 | 1339 | 3642 | 2413 | 889 | 2277 | 2511 | 0.26 |

**Table 15. Hormone concentrations (ng/kg) in fat of calves (steers) implanted with SYNOVEX C and S**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A | 5 | 16.3 | 36.3 | 22.0 | 8.59 | 20.8 | 16.9 | 1.66 | 8.85 | 5.36 | 3.15 | 6.37 | 6.37 | 2.65 |
| E2 | | 5 | 35.3 | 47.6 | 38.6 | 5.14 | 38.3 | 36.2 | 2.85 | 4.39 | 3.3 | 0.64 | 3.3 | 3.1 | 11.7 |
| P | | 5 | 3860 | 15500 | 8616 | 4938 | 7545 | 6200 | 2630 | 12650 | 7340 | 3686 | 6502 | 7780 | 0.80 |
| E1 | B | 5 | 2.37 | 36.9 | 16.8 | 13.7 | 11.5 | 15.9 | 0.97 | 19.5 | 6.10 | 7.73 | 2.63 | 2.63 | 6.0 |
| E2 | | 5 | 2.37 | 35.6 | 24.0 | 14.2 | 17.2 | 30.1 | 1.92 | 22.9 | 6.73 | 9.03 | 4.08 | 3 | 10.0 |
| P | | 5 | 5030 | 12720 | 8192 | 2924 | 7799 | 7840 | 3550 | 1.41e5 | 34230 | 59775 | 12478 | 8720 | 0.90 |
| E1 | C | 5 | 5.02 | 27.1 | 14.7 | 10.0 | 11.8 | 11.4 | 2.92 | 7.25 | 4.83 | 1.99 | 7.25 | 3.85 | 2.96 |
| E2 | | 5 | 7.59 | 43.3 | 24.8 | 16.8 | 19.7 | 21.15 | 2.38 | 4.74 | 3.28 | 1.07 | 3.15 | 2.6 | 8.13 |
| P | | 5 | 6910 | 41750 | 16458 | 14402 | 13051 | 11900 | 3350 | 64600 | 19244 | 25771 | 10375 | 9780 | 1.22 |
| E1 | D | 5 | 22.2 | 61.4 | 40.0 | 14.7 | 37.8 | 38.5 | 1.93 | 5.2 | 3.09 | 1.40 | 2.11 | 2.35 | 16.4 |
| E2 | | 5 | 34.8 | 64.2 | 52.6 | 12.8 | 51.2 | 57.0 | 1.68 | 4.52 | 2.88 | 1.12 | 2.71 | 2.7 | 21.1 |
| P | | 5 | 4030 | 10390 | 7924 | 2526 | 7530 | 7980 | 2350 | 7670 | 5234 | 2317 | 4774 | 5150 | 1.55 |
| E1 | E | 5 | 10.7 | 35.2 | 19.8 | 9.4 | 18.2 | 18.7 | 2.47 | 7.77 | 5.07 | 1.98 | 4.15 | 4.96 | 4.06 |
| E2 | | 5 | 18.0 | 58.6 | 32.6 | 17.0 | 29.5 | 22.9 | 2.56 | 4.71 | 3.16 | 0.90 | 3.07 | 2.75 | 8.32 |
| P | | 5 | 4890 | 8950 | 6504 | 1676 | 6342 | 5690 | 3290 | 8450 | 6383 | 2386 | 5986 | 6895 | 0.83 |
| E1 | F | 4 | 6.44 | 23.3 | 15.6 | 8.01 | 13.8 | 16.42 | 1.9 | 7.09 | 3.64 | 2.104 | 1.9 | 3.1 | 5.30 |
| E2 | | 4 | 14.1 | 27.9 | 19.4 | 6.38 | 18.7 | 17.88 | 2.87 | 10.8 | 5.81 | 3.4 | 5.08 | 4.59 | 3.89 |
| P | | 4 | 3600 | 11240 | 6638 | 3536 | 5967 | 5855 | 4900 | 16400 | 9008 | 4647 | 8181 | 6680 | 0.88 |

**A** = treatment group: implanted day slaughtered day 61 **B** = treatment group: implanted day 0 slaughtered day 119
**C** = treatment group: implanted day 0 implanted day 118 slaughtered day 241
**D** = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 301
**E** = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 329
**F** = treatment group: implanted day 0 implanted day 118 implanted day 240 slaughtered day 360
Testosterone was not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

**Table 16. Hormone concentrations (ng/kg) in liver of calves (steers) implanted with SYNOVEX C and S**

| Analyte | Treat ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A | 5 | 1.87 | 2.99 | 2.43 | 0.45 | 2.39 | 2.38 | 1.58 | 2.22 | 1.78 | 0.27 | 1.58 | 1.66 | 1.43 |
| E2 | | 5 | 2.04 | 3.65 | 2.91 | 0.65 | 2.85 | 3.07 | 1.05 | 3.64 | 1.78 | 1.05 | 1.61 | 1.43 | 2.15 |
| P | | 5 | 61.9 | 100 | 74.4 | 15.1 | 73.3 | 68.4 | 47.7 | 113 | 68.1 | 25.7 | 65.0 | 59.95 | 1.14 |
| E1 | B | 5 | 0.62 | 1.51 | 1.15 | 0.39 | 1.09 | 1.22 | 0.88 | 1.23 | 1.06 | 0.16 | 1.12 | 1.12 | 1.09 |
| E2 | | 5 | 1.86 | 2.62 | 2.05 | 0.32 | 2.03 | 1.93 | 1.31 | 1.97 | 1.57 | 0.26 | 1.55 | 1.56 | 1.24 |
| P | | 5 | 136 | 161 | 142 | 10.3 | 142 | 139 | 74.0 | 133 | 104 | 23.8 | 102 | 111 | 1.25 |
| E1 | C | 5 | 1.17 | 2.62 | 1.76 | 0.59 | 1.68 | 1.61 | 1.13 | 2.49 | 1.69 | 0.54 | 1.65 | 1.65 | 0.98 |
| E2 | | 5 | 2.99 | 6.32 | 4.23 | 1.31 | 4.08 | 3.99 | 2.43 | 4.38 | 3.45 | 0.79 | 3.37 | 3.24 | 1.23 |
| P | | 5 | 131 | 239 | 166 | 44.3 | 161 | 158 | 72.6 | 115 | 93.3 | 19.4 | 91.6 | 102.5 | 1.54 |
| E1 | D | 5 | 1.18 | 7.16 | 2.72 | 2.54 | 2.09 | 1.38 | 0.9 | 2.26 | 1.27 | 0.57 | 1.2 | 1.03 | 1.34 |
| E2 | | 5 | 2.88 | 28.2 | 8.69 | 10.9 | 5.57 | 4 | 1.75 | 3.39 | 2.48 | 0.67 | 2.40 | 2.66 | 1.50 |
| P | | 5 | 125 | 320 | 225 | 78.1 | 213 | 247 | 110 | 189 | 151 | 30.8 | 149 | 147 | 1.68 |
| E1 | E | 5 | 1.51 | 6.66 | 2.88 | 2.13 | 2.46 | 2.07 | 0.97 | 1.74 | 1.26 | 0.31 | 0.97 | 1.19 | 1.74 |
| E2 | | 5 | 3.03 | 8.03 | 4.90 | 1.90 | 4.64 | 4.32 | 1.66 | 1.89 | 1.77 | 0.09 | 1.77 | 1.76 | 2.45 |
| P | | 5 | 144 | 233 | 201 | 35.1 | 198 | 206 | 113 | 191 | 147 | 31.8 | 142 | 133 | 1.55 |
| E1 | F | 4 | 2.06 | 2.57 | 2.37 | 0.27 | 2.36 | 2.49 | 1.43 | 1.96 | 1.72 | 0.20 | 1.43 | 1.76 | 1.41 |
| E2 | | 4 | 3.06 | 5.31 | 4.28 | 1.14 | 4.17 | 4.47 | 2.9 | 4.14 | 3.62 | 0.45 | 3.6 | 3.71 | 1.20 |
| P | | 4 | 150 | 207 | 185 | 30.3 | 183 | 197 | 116 | 164 | 128 | 22.1 | 130 | 128 | 1.60 |

**Table 17. Hormone concentrations (ng/kg) in kidney of calves (steers) implanted with SYNOVEX C and S**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A | 5 | 2.96 | 4.36 | 3.67 | 0.51 | 3.64 | 3.79 | 0.7 | 1.7 | 1.05 | 0.40 | 0.7 | 0.92 | 4.12 |
| E2 | | 5 | 5.63 | 7.28 | 6.54 | 0.61 | 6.52 | 6.64 | 0.28 | 2.45 | 1.46 | 0.8 | 1.19 | 1.36 | 4.88 |
| P | | 5 | 168 | 1473 | 777 | 522 | 609 | 700 | 182 | 807 | 375 | 265 | 315 | 217 | 3.22 |
| E1 | B | 5 | 1.87 | 3.32 | 2.66 | 0.55 | 2.61 | 2.75 | 0.68 | 1.78 | 1.03 | 0.45 | 1.02 | 0.97 | 2.84 |
| E2 | | 5 | 2.7 | 6.81 | 4.98 | 1.62 | 4.74 | 4.78 | 0.82 | 2.73 | 1.28 | 0.82 | 1.14 | 0.91 | 5.25 |
| P | | 5 | 121 | 609 | 353 | 222 | 296 | 246.5 | 135 | 1264 | 481 | 460 | 349 | 286 | 0.86 |
| E1 | C | 5 | 3.43 | 4.81 | 4.21 | 0.52 | 4.18 | 4.33 | 2.26 | 3.53 | 2.92 | 0.48 | 2.26 | 2.8 | 1.55 |
| E2 | | 5 | 2.55 | 10.0 | 5.65 | 3.15 | 4.91 | 6.36 | 1.36 | 3.76 | 2.09 | 0.97 | 1.95 | 1.62 | 3.92 |
| P | | 5 | 297 | 515 | 388 | 94.4 | 379 | 344 | 213 | 900 | 453 | 264 | 403 | 361 | 0.95 |
| E1 | D | 5 | 4.45 | 7.81 | 5.73 | 1.51 | 5.58 | 4.99 | 0.64 | 2.5 | 1.20 | 0.77 | 0.64 | 0.89 | 5.61 |
| E2 | | 5 | 13.9 | 24.9 | 17.8 | 4.39 | 17.4 | 17.45 | 2.39 | 16.8 | 5.64 | 6.25 | 4.04 | 2.9 | 6.02 |
| P | | 5 | 170 | 295 | 225 | 57.5 | 219 | 209 | 79.7 | 2402 | 733 | 951 | 396 | 359 | 0.58 |
| E1 | E | 5 | 1.9 | 6.09 | 4.27 | 1.63 | 3.96 | 4.94 | 1.17 | 3.57 | 2.41 | 0.98 | 3.13 | 2.42 | 2.04 |
| E2 | | 5 | 5.94 | 12.2 | 8.41 | 2.54 | 8.12 | 7.49 | 1.91 | 7.81 | 3.29 | 2.54 | 2.77 | 2.09 | 3.58 |
| P | | 5 | 126 | 1047 | 358 | 389 | 255 | 199 | 203 | 803 | 387 | 239 | 342 | 289 | 0.69 |
| E1 | F | 4 | 2.51 | 4.84 | 3.91 | 1.02 | 3.79 | 4.135 | 2.26 | 3.39 | 2.55 | 0.48 | 2.26 | 2.38 | 1.74 |
| E2 | | 4 | 4.73 | 8.4 | 6.82 | 1.57 | 6.67 | 7.08 | 1.87 | 4.33 | 2.77 | 0.92 | 2.67 | 2.62 | 2.70 |
| P | | 4 | 327 | 792 | 492 | 206 | 464 | 424 | 619 | 2303 | 1336 | 667 | 1200 | 1456 | 0.29 |

**A** = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
**C** = treatment group: implanted day 0, implanted day 118, slaughtered day 241
**D** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
**E** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
**F** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
Testosterone was not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

**Table 18. Hormone concentrations (ng/kg) in muscle of calves (heifers) implanted with SYNOVEX C and H**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A* | 5 | 1.71 | 2.52 | 2.08 | 0.35 | 2.06 | 2.07 | 0.66 | 1.15 | 0.91 | 0.20 | 1.06 | 0.89 | 2.33 |
| E2 | | 5 | 6.15 | 22.9 | 13.0 | 6.14 | 11.9 | 12.3 | 0.96 | 1.91 | 1.42 | 0.39 | 1.37 | 1.51 | 8.11 |
| P | | 5 | 311 | 1489 | 698 | 487 | 587 | 447 | 109 | 916 | 435 | 392 | 298 | 182 | 2.46 |
| E1 | B* | 5 | 2.74 | 4.6 | 3.84 | 0.71 | 3.78 | 4 | 2.25 | 5.25 | 3.07 | 1.26 | 2.25 | 2.45 | 1.63 |
| E2 | | 5 | 3.23 | 14.1 | 7.32 | 4.38 | 6.38 | 5.57 | 0.8 | 1.43 | 1.14 | 0.24 | 1.12 | 1.16 | 4.80 |
| P | | 5 | 209 | 744 | 423 | 215 | 380 | 453 | 96.1 | 1089 | 540 | 373 | 412 | 581 | 0.78 |
| E1 | C** | 5 | 2.49 | 4.04 | 3.33 | 0.60 | 3.28 | 3.48 | 2.36 | 3.31 | 2.61 | 0.40 | 2.38 | 2.49 | 1.40 |
| E2 | | 5 | 6.17 | 11.7 | 8.42 | 2.25 | 8.19 | 7.86 | 2.15 | 9.01 | 3.68 | 2.99 | 3.06 | 2.41 | 3.26 |
| T | | 5 | 29.7 | 53.6 | 38.7 | 10.1 | 37.7 | 33.6 | 15 | 22.1 | 17.5 | 2.75 | 17.3 | 16.8 | 2 |
| E1 | D** | 5 | 3.31 | 15.0 | 6.96 | 4.97 | 5.80 | 4.36 | 1.29 | 2.28 | 1.68 | 0.41 | 1.94 | 1.48 | 2.95 |
| E2 | | 5 | 13.6 | 97.9 | 38.0 | 34.5 | 29.3 | 23.05 | 1.38 | 6.37 | 3.42 | 2.03 | 2.95 | 2.74 | 8.41 |
| T | | 5 | 77.1 | 546 | 196 | 197 | 147 | 115 | 18.2 | 52.1 | 37.4 | 12.4 | 35.4 | 38.2 | 3.01 |
| E1 | E** | 5 | 2.05 | 4.08 | 2.60 | 0.87 | 2.50 | 2.08 | 0.9 | 1.77 | 1.39 | 0.33 | 1.49 | 1.49 | 1.40 |
| E2 | | 5 | 2.64 | 17.6 | 9.23 | 5.38 | 7.84 | 9.21 | 1.19 | 7.01 | 2.97 | 2.35 | 2.42 | 2.03 | 4.54 |
| T | | 5 | 13.2 | 66.6 | 43.1 | 20.1 | 39.6 | 52.3 | 14.2 | 27.2 | 21.3 | 5.61 | 20.6 | 21.7 | 2.41 |
| E1 | F** | 5 | 0.65 | 3.32 | 1.88 | 1.17 | 1.54 | 2.01 | 1.35 | 3.46 | 2.91 | 0.89 | 3.46 | 3.35 | 0.6 |
| E2 | | 5 | 1.2 | 10.7 | 3.67 | 3.98 | 2.6 | 2.06 | 1.93 | 17.3 | 9.51 | 6.03 | 7.54 | 8.1 | 0.25 |
| T | | 5 | 23.4 | 75.7 | 47.0 | 19.2 | 43.8 | 46.1 | 38.9 | 90.2 | 64.5 | 20.2 | 61.9 | 59.2 | 0.78 |

**Table 19. Hormone concentrations (ng/kg) in fat of calves (heifers) implanted with SYNOVEX C and H**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A* | 5 | 16.9 | 23.9 | 20.2 | 3.08 | 20 | 20.1 | 2.24 | 6.11 | 3.88 | 1.87 | 6.11 | 3 | 6.7 |
| E2 | | 5 | 24.5 | 68.5 | 41.2 | 16.6 | 38.8 | 39.1 | 1.58 | 3.91 | 2.43 | 0.92 | 2.31 | 2.39 | 16.3 |
| P | | 5 | 5180 | 14450 | 8554 | 3706 | 7992 | 6740 | 2090 | 12750 | 5624 | 4141 | 4681 | 4820 | 1.40 |
| E1 | B* | 5 | 15.6 | 52.7 | 30.7 | 13.8 | 28.3 | 28.15 | 1.25 | 6.19 | 3.72 | 2.2 | 6.19 | 3.23 | 8.72 |
| E2 | | 5 | 11.0 | 86.5 | 38.5 | 30.6 | 29.7 | 28.1 | 2.25 | 4.31 | 2.944 | 0.84 | 2.86 | 2.88 | 9.76 |
| P | | 5 | 5710 | 14250 | 8510 | 3445 | 8043 | 6760 | 6520 | 24800 | 12974 | 6989 | 11730 | 11950 | 0.57 |
| E1 | C** | 5 | 8.9 | 25.0 | 18.4 | 6.47 | 17.3 | 20.2 | 2.59 | 24.7 | 9.92 | 8.94 | 24.7 | 7.07 | 2.86 |
| E2 | | 5 | 14.6 | 45.1 | 30.2 | 12.1 | 28.1 | 27.3 | 4.52 | 7.99 | 5.58 | 1.38 | 5.46 | 5.25 | 5.2 |
| T | | 5 | 87.2 | 159 | 126 | 31.5 | 123 | 119 | 23.7 | 43.2 | 30.3 | 7.73 | 29.6 | 27.6 | 4.30 |
| E1 | D** | 5 | 18.5 | 98.7 | 47.0 | 34.5 | 37.9 | 30 | 3.11 | 7.02 | 5.28 | 1.58 | 6.18 | 5.85 | 5.13 |
| E2 | | 5 | 27 | 141 | 74.5 | 42.8 | 64.6 | 75.7 | 3.25 | 10.03 | 6.19 | 3.38 | 5.465 | 4.77 | 15.9 |
| T | | 5 | 162 | 552 | 337 | 173 | 301 | 280 | 20.1 | 136 | 69.4 | 49.4 | 54.9 | 52.3 | 5.35 |
| E1 | E** | 5 | 13.0 | 28.9 | 19.6 | 6.30 | 18.9 | 17.0 | 4.86 | 11.8 | 7.88 | 2.51 | 6.87 | 7.73 | 2.19 |
| E2 | | 5 | 21.4 | 70.8 | 38.0 | 19.0 | 34.9 | 32.4 | 3.56 | 24.7 | 9.19 | 9.04 | 6.70 | 4.32 | 7.49 |
| T | | 5 | 84.1 | 316 | 203 | 91.1 | 185 | 193 | 52.4 | 103 | 81.7 | 22.3 | 79.1 | 86.2 | 2.24 |
| E1 | F** | 5 | 17.9 | 43.2 | 27.8 | 9.52 | 26.6 | 27.7 | 7.71 | 20.8 | 13.9 | 4.85 | 20.8 | 13.5 | 2.05 |
| E2 | | 5 | 24.7 | 63.25 | 41.9 | 16.1 | 39.3 | 44 | 4.58 | 7.28 | 5.36 | 1.11 | 5.28 | 4.94 | 8.91 |
| T | | 5 | 171 | 329 | 216 | 64.3 | 209 | 194.5 | 19.1 | 72.4 | 46.1 | 19.4 | 42.3 | 46.7 | 4.17 |

**A** = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
**C** = treatment group: implanted day 0, implanted day 118, slaughtered day 241
**D** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
**E** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
**F** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
* =Testosterone not measured ** = progesterone not measured. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

**Table 20. Hormone concentrations (ng/kg) in liver of calves (heifers) implanted with SYNOVEX C and H**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S.D. | Geo-Mean | Median | |
| E1 | A* | 5 | 0.64 | 3.02 | 1.45 | 0.93 | 1.26 | 1.07 | 0.55 | 0.8 | 0.64 | 0.10 | 0.62 | 0.62 | 1.73 |
| E2 | | 5 | 1.52 | 11.6 | 4.46 | 4.12 | 3.33 | 3.63 | 1.16 | 1.73 | 1.43 | 0.22 | 1.42 | 1.48 | 2.45 |
| P | | 5 | 84.3 | 174 | 117 | 41.8 | 111 | 88.7 | 63.4 | 103 | 79.9 | 18.8 | 78.2 | 72.7 | 1.22 |
| E1 | B* | 5 | 1.21 | 4.22 | 2.27 | 1.15 | 2.08 | 2.04 | 0.59 | 1.73 | 1.30 | 0.52 | 0.59 | 1.61 | 1.27 |
| E2 | | 5 | 1.55 | 5.46 | 3.46 | 1.53 | 3.17 | 3.58 | 1.19 | 2.11 | 1.71 | 0.33 | 1.69 | 1.8 | 1.99 |
| P | | 5 | 127 | 298 | 227 | 77.2 | 215 | 274 | 153 | 271 | 196 | 44.3 | 193 | 188 | 1.46 |
| E1 | C** | 5 | 1.15 | 2.2 | 1.60 | 0.4 | 1.56 | 1.63 | 1.1 | 1.38 | 1.22 | 0.138 | 1.11 | 1.15 | 1.42 |
| E2 | | 5 | 2.58 | 5.13 | 3.92 | 1.07 | 3.80 | 3.56 | 1.66 | 5.12 | 3.15 | 1.45 | 2.88 | 3.1 | 1.15 |
| T | | 5 | 20.9 | 35.4 | 29.8 | 6.44 | 29.2 | 32.15 | 15.3 | 26.3 | 19.9 | 4.08 | 19.6 | 19.15 | 1.68 |
| E1 | D** | 5 | 2.08 | 7 | 3.44 | 2.05 | 3.08 | 2.89 | 1.66 | 2.28 | 1.88 | 0.26 | 1.75 | 1.75 | 1.65 |
| E2 | | 5 | 7.82 | 33.4 | 15.0 | 10.6 | 12.8 | 12.6 | 7.08 | 8.22 | 7.56 | 0.41 | 7.56 | 7.51 | 1.68 |
| T | | 5 | 21.6 | 35.9 | 28.6 | 5.26 | 28.2 | 28.7 | 14.1 | 19.6 | 17.6 | 2.17 | 17.5 | 18 | 1.59 |
| E1 | E** | 5 | 0.69 | 1.43 | 1.12 | 0.28 | 1.09 | 1.22 | 0.81 | 1.19 | 1.02 | 0.155 | 0.94 | 1.03 | 1.18 |
| E2 | | 5 | 1.69 | 4.16 | 2.84 | 0.90 | 2.72 | 2.63 | 1.62 | 2.92 | 2.38 | 0.49 | 2.34 | 2.45 | 1.07 |
| T | | 5 | 18.1 | 33.7 | 24.1 | 6.45 | 23.5 | 24 | 16.9 | 25.1 | 19.4 | 3.35 | 19.2 | 17.8 | 1.35 |
| E1 | F** | 5 | 1.38 | 3.37 | 2.34 | 0.96 | 2.19 | 1.9 | 1.33 | 2.2 | 1.67 | 0.33 | 2.2 | 1.62 | 1.17 |
| E2 | | 5 | 3.19 | 6.69 | 4.90 | 1.61 | 4.68 | 5.26 | 1.26 | 2.65 | 1.91 | 0.51 | 1.86 | 1.83 | 2.87 |
| T | | 5 | 43.2 | 57.6 | 47.6 | 5.74 | 47.3 | 46 | 21.15 | 42.6 | 33.3 | 8.51 | 32.3 | 35.5 | 1.30 |

**Table 21. Hormone concentrations (ng/kg) in kidney of calves (heifers) implanted with SYNOVEX C and H**

| Analyte | Treat-ment | n | Statistics of treatment groups | | | | | | Statistics of control groups | | | | | | Ratio: implant/ control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | S.D. | Geo-Mean | Median | Min. | Max. | Mean | S D. | Geo-Mean | Median | |
| E1 | A* | 5 | 1.64 | 4.35 | 3.04 | 1.01 | 2.89 | 3.17 | 0.63 | 1.08 | 0.83 | 0.179 | 0.92 | 0.82 | 3.87 |
| E2 | | 5 | 5.1 | 13.4 | 7.86 | 3.21 | 7.43 | 6.9 | 0.99 | 1.99 | 1.38 | 0.39 | 1.34 | 1.22 | 5.66 |
| P | | 5 | 249 | 993 | 548 | 296 | 487 | 485 | 112 | 800 | 399 | 295 | 310 | 288 | 1.68 |
| E1 | B* | 5 | 2.66 | 5.11 | 3.65 | 1.07 | 3.53 | 3.1 | 1.38 | 2.26 | 1.67 | 0.36 | 1.38 | 1.5 | 2.07 |
| E2 | | 5 | 3.09 | 15.3 | 7.56 | 4.87 | 6.45 | 5.85 | 1.25 | 2.69 | 1.96 | 0.52 | 1.90 | 1.98 | 2.95 |
| P | | 5 | 189 | 1371 | 533 | 481 | 411 | 373 | 143 | 1080 | 558 | 406 | 409 | 684 | 0.54 |
| E1 | C** | 5 | 3.24 | 5.47 | 4.38 | 0.98 | 4.29 | 3.99 | 2.5 | 3.45 | 2.91 | 0.44 | 2.85 | 2.85 | 1.4 |
| E2 | | 5 | 6.11 | 13.6 | 9.61 | 3.04 | 9.22 | 9.4 | 2.19 | 3.7 | 2.76 | 0.59 | 2.72 | 2.61 | 3.60 |
| T | | 5 | 142 | 445 | 249 | 122 | 228 | 214 | 125 | 255 | 199 | 59.3 | 191 | 214 | 1.00 |
| E1 | D** | 5 | 4.35 | 10.1 | 6.52 | 2.15 | 6.27 | 5.83 | 1.98 | 5.27 | 2.94 | 1.36 | 1.98 | 2.45 | 2.38 |
| E2 | | 5 | 14.6 | 28.3 | 20.7 | 5.42 | 20.2 | 18.7 | 5.72 | 8.54 | 7.13 | 1.07 | 7.06 | 6.85 | 2.73 |
| T | | 5 | 356 | 746 | 521 | 158 | 503 | 488 | 216 | 417 | 358 | 82.7 | 349 | 391 | 1.25 |
| E1 | E** | 5 | 1.28 | 5.39 | 2.94 | 1.56 | 2.63 | 2.69 | 1.06 | 5.9 | 2.68 | 1.94 | 1.6 | 1.83 | 1.47 |
| E2 | | 5 | 2.24 | 15.6 | 7.55 | 5.41 | 5.95 | 7.35 | 1.53 | 5.15 | 2.46 | 1.53 | 2.19 | 1.83 | 4.02 |
| T | | 5 | 136 | 464 | 299 | 133 | 274 | 276 | 217 | 686 | 391 | 189 | 358 | 390 | 0.71 |
| E1 | F** | 5 | 1.97 | 6.06 | 3.18 | 1.65 | 2.92 | 2.75 | 0.7 | 1.64 | 1.08 | 0.43 | 1.64 | 0.86 | 3.20 |
| E2 | | 5 | 6.54 | 16.5 | 9.71 | 3.88 | 9.21 | 8.47 | 1.64 | 2.43 | 2.02 | 0.32 | 2.00 | 1.93 | 4.39 |
| T | | 5 | 278 | 1033 | 489 | 313 | 430 | 396 | 178 | 804 | 508 | 232 | 454 | 552 | 0.72 |

**A** = treatment group: implanted day 0, slaughtered day 61 **B** = treatment group: implanted day 0, slaughtered day 119.
**C** = treatment group: implanted day 0, implanted day 118, slaughtered day 241
**D** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 301
**E** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 329
**F** = treatment group: implanted day 0, implanted day 118, implanted day 240, slaughtered day 360
* =Testosterone not measured ** = progesterone not measured . n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

**Studies in support of STEER-oid™**

A 1982 tissue residue study was submitted to FDA under NADA 110-315. The study includes:

- data on the validation of radioimmunoassay
- results of tissue analyses conducted with muscle and fat samples obtained from 8 untreated steers and from 8 steers treated with STEER-oid™ (20 mg estradiol benzoate and 200 mg progesterone).

The investigators found no statistically significant differences between control animals and animals treated for 15 and 30 days with STEER-oid implants.

Analytical method

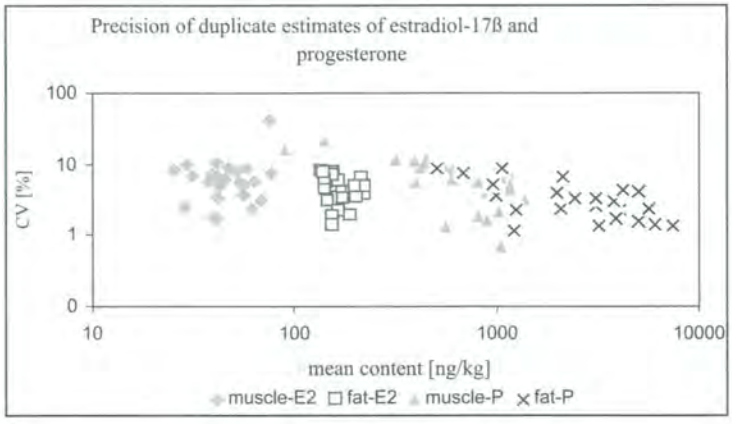
The results of the validation of the fully described analytical procedure (Marcus *et al.*, undated) are shown in Table 22. The sensitivity was sufficient to measure a difference of analytes as shown in Table 22. Recoveries averaged 77% in both muscle and fat. Recoveries were determined individually for every sample analysed. All results were corrected for recovery. The precision of the method was also tested. All samples were run in duplicate. The Coefficients of Variation (%CV) of duplicate estimates of the tissue hormone concentrations of the animals used in the study are shown in Table 22. . All samples were run in duplicate. The precision of the estimates was slightly dependent on the concentration of the analyte. This is shown in Figure 6. Cross-reactivity of other substances in the assay was also determined. There was a 0.6% cross-reactivity of Δ5-pregnenolone in the progesterone assay. Estradiol-17α (35%), estrone (32%) and estratriol (6%) all demonstrated some cross-reactivity in the estradiol-17ß assay. According to the authors, estradiol-17α is the only cross-reactive steroid that would elute with estradiol-17β during column chromatography of the crude extracts. The procedure has not been developed to include conjugates of the hormones in the determinations.

**Table 22. Performance characteristics of the analytical method used to determine concentrations of hormones in steers treated with STEER-oid**

| **Matrix** | **Muscle** | | | | **Fat** | | | |
|---|---|---|---|---|---|---|---|---|
| | Sensitivity | | %CV (n=24) | | Sensitivity | | %CV (n=24) | |
| **Analyte** | LOQ (ng/kg) | difference* (ng/kg) | median | 95th percentile | LOQ (ng/kg) | difference* (ng/kg) | median | 95th percentile |
| Progesterone | 430 | 500 | 5.7 | 15.0 | 1930 | 500 | 3.1 | 8.3 |
| Estradiol-17ß | 26 | 5 | 5.9 | 10.3 | 109 | 10 | 4.5 | 7.5 |

* = 'difference' defines the difference in the concentration of the analyte in two separate tissue samples that can be reliably differentiated and quantified.

**Figure 6. Precision duplicate estimates of estradiol-17β and progesterone**



Residues in tissues

Concentration of hormones in control steers and in steers implanted with STEER-oid are given in Tables 23 and 24 showing the percentage increase in hormone concentrations due to treatment. Figure 7 shows the results of the residue study. Since conjugates were not measured and residue data for liver and kidney were not obtained the study probably significantly underestimates the concentrations of the hormones in edible tissues.

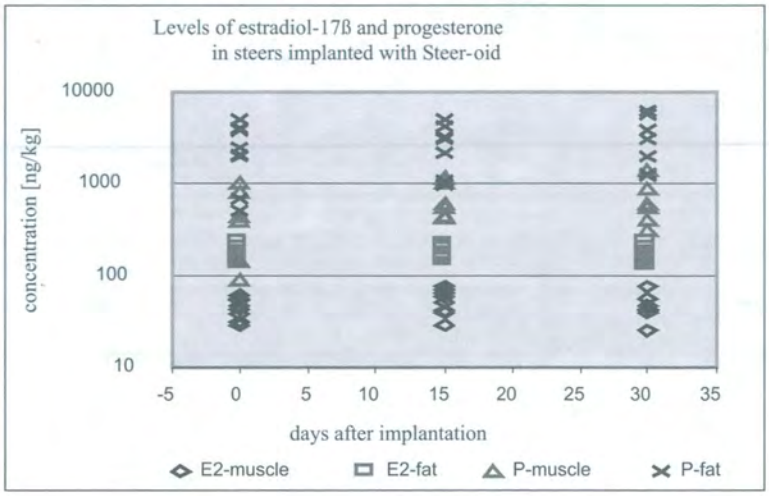
**Table 23. Concentration of hormones in steers implanted with STEER-oid**

| Tissue | Analyte | Days after implantation | n | Hormone concentration (ng/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Range | | Mean | S.D. | Geometric Mean | Median |
| | | | | Min. | Max. | | | | |
| Muscle | Progesterone | Control | 8 | 90 | 1025 | 564 | 347 | 430 | 620 |
| | | 15 | 8 | 415 | 1160 | 799 | 326 | 737 | 820 |
| | | 30 | 8 | 315 | 1380 | 769 | 370 | 689 | 742 |
| Fat | | Control | 8 | 500 | 5045 | 2568 | 1616 | 2004 | 2265 |
| | | 15 | 8 | 955 | 5055 | 2586 | 1569 | 2144 | 2585 |
| | | 30 | 8 | 1210 | 7510 | 3816 | 2371 | 3117 | 3458 |
| Muscle | Estradiol-17β | Control | 8 | 28.5 | 61.0 | 44.5 | 11.7 | 43.1 | 44.3 |
| | | 15 | 8 | 29.0 | 76.0 | 53.6 | 16.2 | 51.3 | 55.3 |
| | | 30 | 8 | 25.5 | 77.0 | 46.2 | 15.1 | 44.2 | 41.8 |
| Fat | | Control | 8 | 137.5 | 224.0 | 166.8 | 29.9 | 164.7 | 154.0 |
| | | 15 | 8 | 143.5 | 217.5 | 172.3 | 28.7 | 170.3 | 161.0 |
| | | 30 | 8 | 131.0 | 225.5 | 165.2 | 32.2 | 162.7 | 156.8 |

**Table 24. Percentage increase in hormone concentrations in steers due to treatment**

| Tissue | Analyte | Days after implantation | Basis for the calculations | | |
|---|---|---|---|---|---|
| | | | Arithmetic Mean | Geometric Mean | Median |
| Muscle | Progesterone | 15 | 42 | 71 | 32 |
| | | 30 | 36 | 60 | 20 |
| Fat | | 15 | 0.7 | 7 | 14 |
| | | 30 | 49 | 56 | 53 |
| Muscle | Estradiol-17β | 15 | 21 | 19 | 25 |
| | | 30 | 3.8 | 2.5 | -5.6 |
| Fat | | 15 | 3.3 | 3.4 | 4.5 |
| | | 30 | -1.0 | -1.2 | 1.8 |

**Figure 7. Estradiol-17β and progesterone levels in steers implanted with STEER-oid**



**Studies in support of the HEIFER-oid™ implant**

A 1983 tissue residue study was submitted to FDA under NADA 135-906 was evaluated. The study includes:

- data on the validation of a fully described radioimmunoassay procedures for testosterone and estradiol, respectively, in muscle and fat,
- results of tissue analyses conducted with muscle and fat samples obtained from 8 untreated heifers and from 8 heifers treated by ear implant with HEIFER-oid™ (200 mg testosterone propionate and 20 mg estradiol-benzoate) for 15 and 30 days, respectively (Marcus, undated).

Analytical method

The assays were validated with respect to sensitivity and recovery, linearity and parallelism of the calibration curve, and cross-reactivity with other steroids.

The sensitivity was sufficient to measure a difference of between 5 and 20 ng/kg at level between 37 and 274 ng/kg, as shown in Table 25. Recoveries of testosterone and estradiol were not significantly different, averaging about 74% in muscle and fat. Excellent linearity (logit $B/B_0$ vs. log dose) was demonstrated throughout the experimental working range. Concentrations found in serial dilutions of sample extracts paralleled the calibration curve. Cross-reactivity was tested with 17 steroids. The substances exhibiting the strongest cross-reactivities in the testosterone assay were Δ4-androstanediol (0.9%), 5α-androstan-3β,17β-diol (3%) and dihydrotestosterone (66%). The cross-reactivity of dihydrotestosterone was minimised through chromatographic separation on celite columns. Estradiol-17α (6%), estratriol (0.4%) and testosterone (0.3%) all demonstrated some cross-reactivity in the estradiol-17ß assay. According to the authors, estradiol-17α is the only cross-reactive steroid that would elute with estradiol-17β during column chromatography of the crude extracts. The procedure has not been developed to include conjugates of the hormones in the determinations.

Recovery was estimated with $^3$H-labeled steroids. All results were corrected for recovery. All samples were run in duplicate. The coefficient of variation of duplicate estimates of the tissue hormone concentrations of the animals used in the study is shown in Table 25. The method was not designed to include conjugates of the hormones in the determinations of residues.

**Table 25. Performance characteristics of the analytical method used to determine concentrations of hormones in steers treated with Steer-oid**

| **Matrix:** | **Muscle** | | | | **Fat** | | | |
|---|---|---|---|---|---|---|---|---|
| | Sensitivity | | %CV (n=24) | | Sensitivity | | %CV (n=24) | |
| **Analyte** | LOQ (ng/kg) | Difference* (ng/kg) | Median | 95$^{th}$ Percentile | LOQ (ng/kg) | Difference* (ng/kg) | Median | 95$^{th}$ Percentile |
| Testosterone | 104 | 5 | 3.9 | 9.8 | 274 | 10-20 | 3.5 | 8.7 |
| Estradiol-17ß | 37 | 5 | 6.6 | 19.0 | 154 | 10 | 4.8 | 11.9 |

* = 'difference' defines the difference in the concentration of the analyte in two separate tissue samples that can be reliably differentiated and quantified

Residues in tissues

The investigators concluded that small but statistically insignificant differences were observed between 8 untreated heifers and 8 treated by ear implant with Heifer-oid for 15 days and 8 others treated for 30 days with the same product. Statistical parameters are given in the below Table 26.

The increases in the concentrations of the two hormones were in fact very low. However, since conjugates were not measured and residue data for liver and kidney were not obtained, the study probably significantly underestimates the concentrations of the hormones in edible tissues.

**Table 26. Concentration of hormones in control animals and in steers implanted with the HEIFER-oid™ implant**

| Tissue | Analyte | Days after implantation | n | Hormone concentration (ng/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Range | | Mean | S.D. | Geometric Mean | Median |
| | | | | Min. | Max. | | | | |
| Muscle | Testosterone | Control | 8 | 56.5 | 186.5 | 95.6 | 25.1 | 92.4 | 102.0 |
| | | 15 | 8 | 129.5 | 311.5 | 108.3 | 20.9 | 106.3 | 114.3 |
| | | 30 | 8 | 71.5 | 203.5 | 106.4 | 22.6 | 103.9 | 107.8 |
| Fat | | Control | 8 | 132.5 | 377.5 | 246.7 | 46.3 | 242.9 | 238.3 |
| | | 15 | 8 | 61.5 | 224.0 | 277.4 | 55.3 | 272.7 | 272.0 |
| | | 30 | 8 | 139.5 | 333.0 | 271.3 | 44.1 | 268.2 | 258.3 |
| Muscle | Estradiol-17β | Control | 8 | 13.5 | 58.5 | 34.9 | 15.8 | 31.3 | 37.5 |
| | | 15 | 8 | 10.5 | 71.0 | 39.8 | 19.7 | 34.5 | 42.0 |
| | | 30 | 8 | 24.0 | 76.0 | 42.6 | 17.9 | 39.6 | 38.0 |
| Fat | | Control | 8 | 46.5 | 165.5 | 96.1 | 38.7 | 89.1 | 92.5 |
| | | 15 | 8 | 70.5 | 173.5 | 110.2 | 38.1 | 104.5 | 108.5 |
| | | 30 | 8 | 60.0 | 213.5 | 118.6 | 50.2 | 109.6 | 116.0 |

**Studies conducted in support of the COMPUDOSE implants.**

COMPUDOSE implants containing either 24 mg or 45 mg of estradiol-17β provide a continuous release of the hormone of 1.25 to 2.5 μg/hour for either 200 days or 400 days at least. Excretion rates of estrogen in female cattle, for comparison, range from 7.8 μg/hour to 31.8 μg/hour in cycling heifers, depending upon the phase of the cycle, and range from 300 μg/hour during the second trimester of pregnancy to 3400 μg/hour within 15 days of parturition.

In cattle, 84% of the estradiol-17β is converted into the non-estrogenic metabolite estradiol-17α which is excreted as either the glucuronide or the sulfate conjugates by the liver (FAO, a).

Analytical Methodology

Fat tissues are extracted with hexane and chlorobutane. Liver, kidney and muscle tissues are extracted with 80/20 acetonitrile/methanol and the sulfate and glucuronide conjugates hydrolysed enzymatically. Extracts are purified by liquid-liquid partitioning and by column chromatography. Estradiol is separated from estrone by chromatography on Sephadex LH 20. The determinative step is a radioimmunoassay method using specific antisera.

The range of procedural recoveries was high and inconsistent in some studies. Extraction of all samples was monitored for individual recovery based on the recovery of an added radiolabelled standard. If the radiolabel recovery indicated high process losses, the samples were re-analysed. It is surprising that no adjustment of the results for recovery was undertaken prior to statistical analyses in this work. The transferability of the method to an independently operated second laboratory has been demonstrated (Sieck and Turner. 1981).

The limit of detection (LOD) is reported to be approximately 5 ng/kg. Below LOD results are typically reported as < 5ng/kg or an arbitrarily assigned value, of up to 5, throughout most residue studies (FAO, b).

Estrogens in kidney fat of untreated slaughter steers

The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers were determined in a study with steers from seven geographical locations within the U.S.A. (2 from Alabama, 1 from each of Oklahoma, Texas, Indiana, Idaho, and Nebraska) (Frank *et al.*,

undated). Results are shown in Figure 8 and Table 27. Analytical results were corrected for recovery, determined with added isotopically labelled hormone. The levels found are possibly heterogeneous across location and/or breed and/or carcass weights. There seems to exist a linear relationship between estrone and estradiol levels, which is influenced by unknown factors (see Figure 8, where the data are labelled according to the seven regions). Information on animal characteristics was too limited to investigate possible factors.

**Figure 8.** **The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers from seven geographical locations within the U.S.A.**

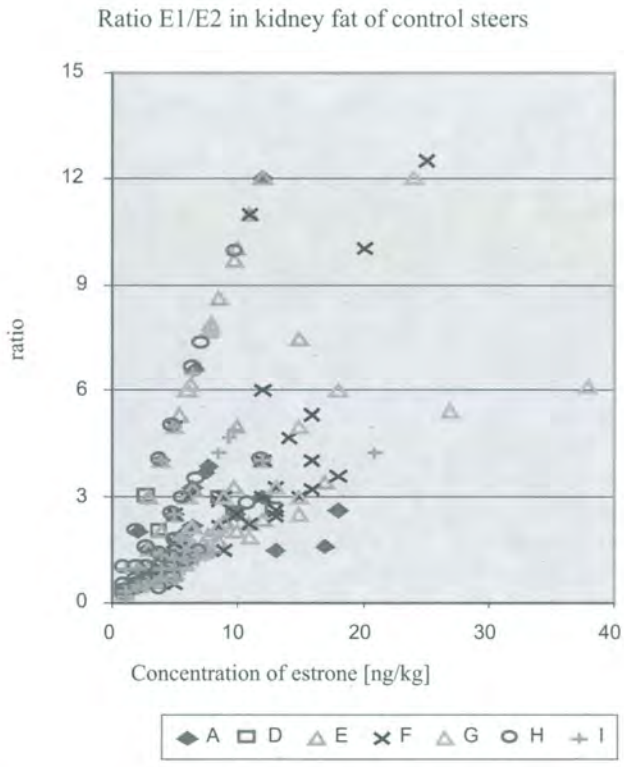



**Table 27.** **The effects of location, breed, carcass weight and federal grade on distribution functions of endogenous estradiol-17β and estrone in kidney fat of slaughter steers from seven geographical locations within the U.S.A.**

| Statistical parameter | Concentration (ng/kg) | | Ratio $E_1/E_2$ |
|---|---|---|---|
| | Estrone | Estradiol | |
| n | 307 | 306 | |
| minimum | 1.0 | 1.0 | |
| maximum | 38 | 11 | |
| mean | 6.5 | 3.6 | 1.8 |
| standard deviation | 4.78 | 1.71 | 2.8 |
| geometric mean | 5.2 | 3.1 | 1.7 |
| median | 5.0 | 4.0 | 1.3 |
| $75^{th}$ percentile | 7.8 | 5.0 | 1.6 |
| $90^{th}$ percentile | 12 | 5.0 | 2.5 |
| $95^{th}$ percentile | 15 | 6 | 2.5 |
| $99^{th}$ percentile | 25 | 9.0 | 2.8 |

Effect of implant withdrawal on hormone levels in steers

205 steers at three different geographic locations were implanted with COMPUDOSE®. After approximately 100 days perirenal fat samples were removed from 10 steers from two of the two locations (20 total) by biopsy prior to removal of the implant. For the remaining steers, implants were removed 24 hours prior to sampling of kidney fat. Samples were analysed for estradiol-17β and estrone. Twentyfour hours after implant removal the $E_1$ and $E_2\beta$ levels had returned to the levels found in untreated steers. Results are shown in Table 28 (Sieck *et al.*, undated).

**Table 28. Concentration of estradiol-17β and estrone in steers from 3 different geographical locations in the U.S.A. implanted with COMPUDOSE®**

| Location | n | Withdrawal [hours] | Hormone concentration (ng/kg) | |
|---|---|---|---|---|
| | | | Estradiol-17β | Estrone |
| | | | Mean ± Standard Deviation | Mean ± Standard Deviation |
| Colorado | 68 | 24 | 8.1 ± 3.9 | 5.3 ± 1.03 |
| Georgia | 10 | 0 | 12.9 ± 6.1 | 11.4 ± 4.1 |
| | 58 | 24 | 7.8 ± 3..9 | 5.1 ± 0.5 |
| Idaho | 10 | 0 | 12.2 ± 5.3 | 8.0 ± 1.8 |
| | 59 | 24 | 6.3 ± 2.3 | 5.0 ± 0.08 |

Residue Studies

*Study APH 216A:*

33 Angus and Hereford steers were implanted for 70-180 days with estradiol-17β Compudose implants. Several withdrawal times were observed prior to slaughter. The implants released approximately 70 µg/animal/day. Edible tissues were collected from treated animals and from 20 untreated control animals. All tissues were analysed for estradiol-17ß and estrone.

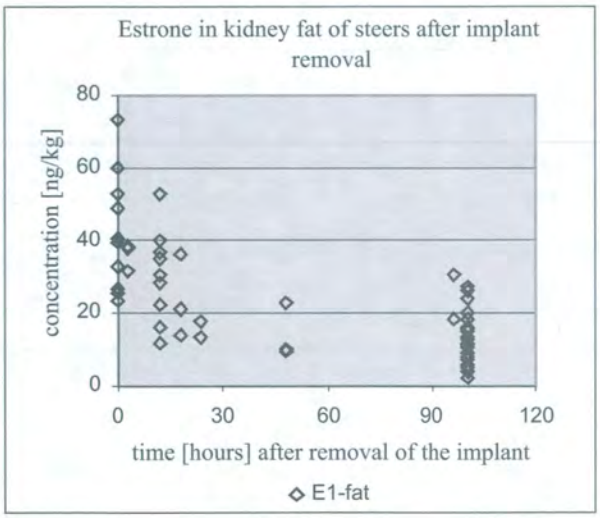
***Study APH-216B***

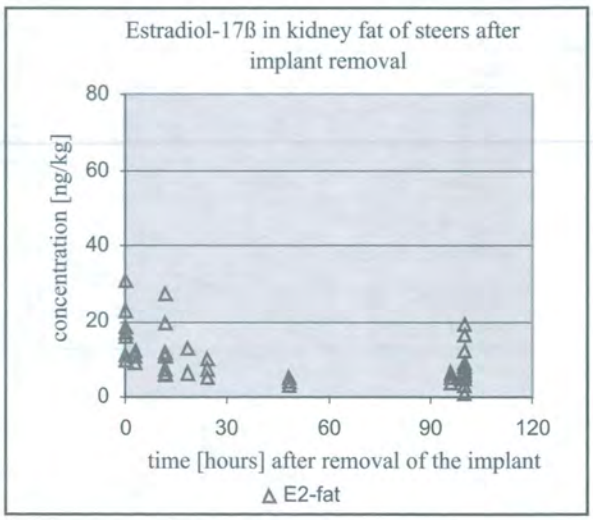
12 animals were implanted for 85 days. Implants were removed and samples were taken 0 and 12 hours after withdrawal of the implants (6 animals per group). These animals were used to provide replacement data, which was lost when the tissues of 12 animals in the first study were contaminated upon sampling.

***Study APH-216E***

Eight additional animals were implanted for 69-70 days to provide further information on the effects of a withdrawal time. The Figures 9a and 9b show, using fat data as an example, the rapid time-dependent decrease of hormone level following implant withdrawal (FAO, c).

**Figure 9. Decrease of hormone levels in fat following the withdrawal of implant in Study APH-216E**



Data for the combined studies described above is shown in Table 29. The 100-hour group appears as control in the study protocol and as 100 group in the annex with the raw data. This latter designation was originally done to facilitate line printer plots of the data. The 100-hour animals were, in fact, control animals

**Table 29. Effect of implantation of steers with COMPUDOSE implants and of implant removal on hormone concentrations in edible tissues**

| Tissue | Kidney fat | | | | Kidney | | | | Liver | | | | Muscle | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hours after implant removal | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| Hormone | Estrone | | Estradiol-17β | | Estrone | | Estradiol-17β | | Estrone | | Estradiol-17β | | Estrone | | Estradiol-17β | |
| N | 10 | 24 | 10 | 24 | 10 | 29 | 10 | 24 | 10 | 27 | 10 | 26 | 6 | 20 | 6 | 20 |
| | Hormone concentration (ng/kg) | | | | | | | | | | | | | | | |
| Minimum | 23.3 | 2.1 | 9.6 | 1.3 | 10.0 | 1.2 | 12.0 | 1.5 | 4.4 | 1.9 | 1.9 | 1.9 | 3.9 | 1.1 | 2.1 | 2.4 |
| Maximum | 73.3 | 27.3 | 31.1 | 19.1 | 42.1 | 38.2 | 45.9 | 23.7 | 57.9 | 22.6 | 40.4 | 17.9 | 20.6 | 22.9 | 11.7 | 28.9 |
| Mean [1] | 39.4 | 12.2 | 19.6 | 7.5 | 24.5 | 10.4 | 28.4 | 10.1 | 18.7 | 9.5 | 17.5 | 6.7 | 10.2 | 8.8 | 7.3 | 11.4 |
| S.D. | 18.4 | 6.9 | 6.4 | 4.0 | 9.5 | 6.7 | 9.0 | 5.8 | 15.4 | 5.5 | 15.1 | 3.3 | 6.6 | 5.7 | 3.7 | 6.3 |
| Geometric Mean | 39.6 | 10.4 | 18.6 | 6.6 | 22.7 | 8.6 | 26.9 | 8.5 | 14.6 | 8.0 | 11.4 | 5.9 | 8.5 | 6.9 | 6.3 | 9.7 |
| Median | 39.5 | 10.9 | 20.9 | 6.3 | 24.2 | 9.1 | 29.9 | 9.4 | 15.1 | 8.3 | 9.5 | 5.9 | 9.1 | 8.4 | 7.4 | 10.4 |
| Mean [2] | 34.8 | 10.5 | 15.3 | 6.8 | 19.2 | 7.9 | 21.1 | 6.7 | 8.8 | 6.5 | 10 | 4 | 10.4 | 4.8 | 3.4 | 5.8 |
| Factor[3] | 1.1 | 1.2 | 1.3 | 1.1 | 1.3 | 1.3 | 1.3 | 1.5 | 2.1 | 1.5 | 1.8 | 1.7 | 1.0 | 1.8 | 2.1 | 2.0 |

[1] basis: corrected results [2] basis: uncorrected results [3] = $mean_{corr.}/mean_{uncorr.}$

*Study APH-216 C*

In a further study, 4 steers weighing approximately 715 pounds were implanted for 90 to 147 days. Biopsy samples of kidney fat were collected at six withdrawal times after implant removal. The results are given in Table.30.

**Table 30. Estrogens in kidney fat of steers implanted with COMPUDOSE as a function of the time after implant withdrawal**

| Withdrawal time: (hours) | 0 | | 12 | | 24 | | 36 | | 72 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration (ng/kg) | | | | | | | | | |
| | $E_1$ | $E_2$ | $E_1$ | $E_2$ | $E_1$ | $E_2$ | $E_1$ | $E_2$ | $E_1$ | $E_2$ |
| n | 4 | 4 | 12 | 12 | 23 | 23 | 29 | 29 | 9 | 9 |
| Minimum | 8.9 | <LOQ | 5.0 | 1.0 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Maximum | 50.5 | 8.8 | 21.0 | 6.1 | 16.0 | 5.7 | 17.0 | 7.8 | 20.0 | 25.0 |
| Mean | 23.5 | 6.5 | 10.5 | <LOQ | 7.8 | <LOQ | 5.6 | <LOQ | 10.6 | 7.1 |
| S.D. | 18.5 | 2.1 | 5.3 | 1.7 | 3.9 | 1.6 | <LOQ | <LOQ | 6.2 | 7.5 |
| Geometric Mean | 19.1 | 6.2 | 9.4 | <LOQ | 6.7 | <LOQ | <LOQ | <LOQ | 8.8 | <LOQ |
| Median | 17.3 | 6.6 | 9.1 | <LOQ | 8.4 | <LOQ | <LOQ | <LOQ | 9.3 | <LOQ |

Effects of overdosing of steers

Eight of 16 Hereford steers weighing approximately 850 lbs received four implants per animal, delivering an estimated total of 644 μg of estradiol-17β daily per animal. The remaining animals served as controls. Kidney fat tissue samples (biopsy) were taken 49-50 days after implantation. The estrone levels found in treated animals ranged from 32 to 60 ng/kg. The range of concentrations in control animals ranged from 5-6.5 ng/kg (Linsey *et al.*, undated).

Estradiol-17α in steer tissues

Tissues were collected from untreated steers, from steers having implants in place and from steers from which the implants had been removed 12 hours prior to slaughter. Tissues were analysed by RIA with an antiserum exhibiting cross-reactivities to estradiol-17α of 12, 4, and 1% by estrone, estradiol-17β and estriol, respectively. The RIA used with chromatographic fraction enriched in estradiol-17α. Residues in liver and kidney appear in Table 31 (Hendrix and Franks, undated): Concentrations in muscle and kidney fat were below the limit of detection (ca. 5 ng/kg) at all times.

**Table 31. Concentration of estradiol-17α in steers with COMPUDOSE implants and in controls.**

| Experiment | Liver | | Kidney | |
|---|---|---|---|---|
| | Concentration (ng/kg) | | | |
| Control | <10 | | <10 | 12.5 |
| Zero withdrawal time | 35 | 71.4 | 57.8 | 53.3 |
| 12 hours withdrawal time | <10 | <10 | 15.1 | <10 |

Residues in implanted Heifers

**Estrogen concentrations in kidney fat of cycling and pregnant heifers**

For the Compudose studies, kidney fat was collected at the slaughterhouse from 14 cycling and 12 pregnant heifers and assayed for estrone and estradiol-17β. Results are shown in Table 32.

Mature Hereford heifers weighing approximately 800 pounds (363.2 kg) were divided into one control group (12 animals) and one group (6 animals) which was implanted with a single COMPUDOSE 200 implant (24 mg estradiol-17β) and slaughtered 84 days after implantation. Hormone concentrations were determined in muscle, liver tissues, kidney and perirenal fat (Sieck *et al.*, undated, b). Results are summarised in Table 33.

**Table 32. Estrogen concentration in kidney fat from 14 cycling and 12 pregnant heifers (ng/kg)**

| Reproduction status | n | Estrone | | | Estradiol-17β | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Min. | Max. | Mean | Min. | Max. |
| Cycling | 14 | 44 | 5 | 176 | 10 | 5 | 28 |
| Pregnant | | | | | | | |
| 1st trimester | 3 | 40 | 11 | 100 | 5 | 5 | 6 |
| 2nd trimester | 4 | 964 | 380 | 1920 | 22 | 5 | 50 |
| 3rd trimester | 3 | 3870 | 750 | 8200 | 163 | 22 | 440 |

**Table 33. Comparison of estrogen levels in mature Hereford heifers comprising one control group of 12 animals and one group of 6 animals implanted with a single COMPUDOSE 200 implant.**

| Tissue | Group | n | Concentration of hormones (ng/kg) | |
|---|---|---|---|---|
| | | | Estrone | Estradiol-17β |
| | | | Mean ± SD | Mean ± SD |
| Lean muscle | Control | 11 | 5.9 ± 1.8 | 7.1 ± 3.3 |
| | 0 –hour | 6 | 6.4 ± 2.2 | 5.8 ± 1.1 |
| Liver | Control | 12 | 7.5 ± 4.8 | 8.2 ± 4 |
| | 0-hour | 6 | 6.7 ± 2.4 | 7.3 ± 2.5 |
| Kidney | Control | 11 | 6.5 ± 3 | 8.5 ± 4 |
| | 0-hour | 6 | 10.2 ± 6.4 | 26.3 ± 11.9 |
| Perirenal fat | Control | 12 | 5.3 ± 1.1 | 5.6 ± 1.1 |
| | 0-hour | 6 | 9.9 ± 6.7 | 9.3 ± 4.7 |

Residues in implanted bull calves

Sixty Holstein intact bull calves with an initial average weight of approximately 42.7 kg were divided into one control group and two treatment groups of 20 animals each. The implanted groups were slaughtered 56 days after implantation with no removal of the implant in one group and removal of the implant 24 hours prior to slaughter in the second treatment group. Estrogen levels were measured in kidney, lean, kidney fat, and liver of control animals and treated animals. The results of the experiment are shown in Table 34. Implant withdrawal of 24 hours were sufficient to bring the increased estrogen concentrations down to control levels (Sieck *et al.*, undated, c).

**Table 34 Estrogens in tissues of bull calves implanted with COMPUDOSE**

| Concentration of hormone (ng/kg) | Tissue and time after implant removal | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Muscle | | | Liver | | | Kidney | | | Perirenal fat | | |
| | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 |
| **Estrone** ( n =) | 17 | 17 | 16 | 17 | 16 | 16 | 17 | 17 | 16 | 16 | 17 | 15 |
| Minimum | 5.0 | 5.0 | 5.0 | 5.0 | 9.6 | 5.0 | 5.0 | 20.1 | 4.8 | 7.6 | 9.9 | 5.0 |
| Maximum | 36.5 | 23.3 | 16.6 | 15.0 | 34.6 | 18.4 | 19.1 | 59.9 | 15.4 | 25.0 | 60.8 | 19.8 |
| Mean | 10.8 | 11.7 | 8.0 | 9.1 | 22.3 | 9.2 | 9.8 | 34.7 | 9.6 | 13.9 | 24.6 | 10.4 |
| Standard Deviation | 8.71 | 5.72 | 4.11 | 3.56 | 8.85 | 4.38 | 4.55 | 9.77 | 3.66 | 4.88 | 12.07 | 4.85 |
| Geometric mean | 8.6 | 10.4 | 7.2 | 8.5 | 20.4 | 8.3 | 8.8 | 33.5 | 8.9 | 13.1 | 22.4 | 9.3 |
| Median | 7.0 | 11.4 | 5.3 | 9.1 | 22.8 | 8.5 | 8.4 | 34.5 | 9.7 | 13.2 | 23.3 | 10.4 |
| **Estradiol 17β** (n=) | 17 | 17 | 16 | 17 | 17 | 16 | 17 | 17 | 16 | 16 | 17 | 15 |
| Minimum | 5.0 | 7.6 | 5.0 | 5.0 | 13.6 | 5.0 | 5.0 | 22.8 | 5.6 | 5.0 | 16.5 | 5.0 |
| Maximum | 17.1 | 30.5 | 10.1 | 29.4 | 56.4 | 31.6 | 24.6 | 86.9 | 20.4 | 63.9 | 56.9 | 12.7 |
| Mean | 6.8 | 18.4 | 6.1 | 14.7 | 32.0 | 16.0 | 10.6 | 57.0 | 10.9 | 11.2 | 38.5 | 7.1 |
| Standard Deviation | 3.45 | 5.60 | 1.70 | 5.76 | 11.23 | 8.33 | 5.28 | 18.08 | 4.20 | 14.94 | 10.94 | 2.87 |
| Geometric Mean | 6.2 | 17.5 | 5.9 | 13.6 | 30.1 | 13.6 | 9.5 | 53.7 | 10.2 | 7.7 | 36.8 | 6.7 |
| Median | 5.0 | 18.8 | 5.3 | 14.0 | 31.3 | 15.7 | 10.5 | 55.8 | 9.9 | 5.3 | 39.9 | 5.2 |

C = control, 0 = zero hours withdrawal, 24 = 24 hours withdrawal

Residues in implanted bulls

A study was conducted to determine the tissue estrogen levels resulting from treatment of bulls with COMPUDOSE (containing 45 mg estradiol-17β per implant). Twelve Hereford bulls weighing approximately 385 kg were used in the study. Six animals were implanted; the other six served as a control. Both groups were slaughtered 63 days after

implantation. Muscle, liver, kidney and perirenal fat were analysed for estrogens and results of the study are shown in Table 35. The daily weight gain was higher than observed in steers and heifers and the corresponding increases in estrogen levels were low in muscle and liver (Decker and Turner, undated).

**Table 35. Levels of estrone and estradiol-17β in Hereford bulls implanted with COMPUDOSE**

| Tissue: | Muscle | | | | Liver | | | | Kidney | | | | Perirenal fat | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment group | C | I | C | I | C | I | C | I | C | I | C | I | C | I | C | I |
| Hormone: | Estrone | | Estradiol | | Estrone | | Estradiol | | Estrone | | Estradiol | | Estrone | | Estradiol | |
| Parameter | Concentration (ng/kg) | | | | | | | | | | | | | | | |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Minimum | 5 | 5 | 5 | 5.6 | 5 | 5 | 5 | 5 | 5.3 | 8.6 | 5.6 | 9.4 | 5 | 14.7 | 6.2 | 13.3 |
| Maximum | 12.0 | 9.0 | 9.4 | 12.3 | 7.9 | 11.8 | 12.5 | 38.5 | 12.8 | 23.8 | 15.3 | 25.9 | 25.0 | 41.8 | 11.7 | 28.5 |
| Mean | 7.7 | 6.9 | 6.3 | 8.5 | 6.0 | 6.9 | 8.5 | 16.6 | 8.7 | 15.1 | 10.0 | 19.9 | 15.3 | 29.6 | 9.1 | 20.2 |
| Standard Dev. | 3.1 | 1.9 | 2.0 | 2.7 | 1.2 | 2.6 | 3.2 | 12.4 | 3.0 | 5.4 | 3.4 | 6.2 | 8.2 | 10.4 | 2.0 | 6.2 |
| Geometric Mean | 7.2 | 6.6 | 6.0 | 8.1 | 5.9 | 6.6 | 7.9 | 12.9 | 8.3 | 14.4 | 9.5 | 18.9 | 13.3 | 27.8 | 8.9 | 19.4 |
| Median | 7.2 | 6.8 | 5 | 8.0 | 5.5 | 6.1 | 8.7 | 15.3 | 8.5 | 13.6 | 9.7 | 20.9 | 13.6 | 33.6 | 9.0 | 19.1 |

C = control group, I = Implanted group

Residues in implanted Zebu steers

A tissue residue study was carried out with 12 Brazilian Zebu steers comprising 3 groups of 4 control animals, 4 animals implanted and slaughtered without prior implant removal and 4 animals slaughtered after 24 hours withdrawal of the implants. COMPUDOSE implants containing 45 mg estradiol-17β were used. The period during which the animals were implanted is not given in the report (Sieck and Turer, undated). Results are summarised in Table 36.

**Table 36. Estrogen levels in Zebu steers implanted with COMPUDOSE**

| Tissue: | Perirenal fat | | | | | | Liver | | | | | | Muscle | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment:** | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 | C | 0 | 24 |
| **Hormone:** | Estrone | | | Estradiol-17β | | | Estrone | | | Estradiol-17β | | | Estrone | | | Estradiol-17β | | |
| **Parameter** | Concentration (ng/kg) | | | | | | | | | | | | | | | | | |
| Minimum | <5 | <5 | <5 | <5 | 5.9 | <5 | 6.3 | 7.4 | <5 | <5 | 9.5 | 6.8 | 13.8 | <5 | <5 | 8.4 | <5 | <5 |
| Maximum | 6.5 | 11.4 | <5 | <5 | 20.0 | <5 | 13.9 | 16.6 | 17.3 | 34.5 | 20.4 | 15.5 | 28.8 | 16.3 | 20.0 | 27.5 | 12.0 | 6.4 |
| Median | 5.3 | 7.65 | <5 | <5 | 6.65 | <5 | 8.0 | 10.6 | 10.4 | 8.1 | 13.9 | 10.3 | 16.8 | 9.75 | 9.45 | 8.6 | 5.45 | 5.65 |

C = control, 0 = 0 h withdrawal, 24 = 24 h withdrawal

**Effects of Trenbolone-containing Implants on Estrogen Levels in Tissues of Treated Animals**

Estradiol benzoate/progesterone implants and trenbolone implants are approved for use in steers for improved feed efficiency. The concomitant use of estradiol implants and of trenbolone implants (Implix/Revalor) was investigated in one study, however, it does not represent an authorised usage. All the remaining studies reported here have been conducted with products containing the substances in fixed combinations.

**Studies using Finaplix® implants in heifers**

Methodological aspects

Free steroids were extracted from crude tissue extracts with toluene:ether (7:3); conjugated steroids were extracted by the same mixture following hydrolysis with glucuronidase/sulfatase (*helix pomatia*). A further cleanup-step was performed on reverse phase C-18 cartridges. The steroids were then separated by HPLC and quantified by RIA. The analytical detection limits for free estradiol-17β, conjugated estradiol-17β and free estrone in tissues were 18%, 25% and 27%, respectively. Recoveries of free estradiol-17β and conjugated estradiol-17β were 30-40% and 25-30%, respectively. Recovery of the extraction was estimated using $^{3}$H-estradiol-17β (free hormone) and $^{3}$H-testosterone glucuronide (conjugated hormone) and results of recovery experiments are summarised in Table 37.

During the study, it was observed that, at certain times, the heifers were in estrus. Where deviations in estradiol levels were found, when compared with the mean estradiol levels of the group, the phase of the menstrual cycle may be one of the reasons

**Table 37. The recoveries of estradiol-17β and estrone using Finaplix® implants in heifers**

| Analyte | muscle (n=9) | | liver (n=9) | | Kidney (n=9) | |
|---|---|---|---|---|---|---|
| | level (ng/kg) | CV (%) | level (ng/kg) | CV (%) | Level (ng/kg) | CV (%) |
| Free estradiol-17β | 23<br>13 | 20<br>36 | 21<br>5 | 24<br>68 | 48<br>6 | 14<br>99 |
| Conjugated estradiol-17β | | | 127<br>21 | 9<br>40 | 46<br>15 | 17<br>43 |
| Free estrone | | | 15<br>107 | 52<br>12 | | |

Residue study in heifers

Twenty-four heifers (average age 1.5 years; 270-295 kg BW) were implanted with Finaplix® (300 mg trenbolone acetate). Groups of 6 animals were slaughtered at 15, 30, 60, and 75 days after implantation, respectively (Arts *et al.*, 1986a). Unfortunately, there was no control group in the study. Only the results of the determination of estrogen concentrations are considered in this review. The concentrations of free and conjugated estradiol-17β in muscle, liver, kidney, fat, and blood plasma were determined. Free estrone was only determined in liver and fat. The method was not sensitive enough to reliably measure the endogenous hormone levels.

No clear trend can be identified from the results of the study because of the insensitivity of the method, the high variability of the results, the small group sizes and absence of untreated controls. There is possibly a trend in direction of decreasing estrogen levels in liver and fat. Table 38 shows the individual estrogens and the median of the sum of all three compounds measured (n=6).

**Table 38. Effects of implantation with FINAPLIX® on the median estrogen levels in tissues of heifers**

| Days Withdrawal | Tissue: | Muscle | Liver | Kidney | Fat |
|---|---|---|---|---|---|
| | Analyte | Concentration (ng/kg) | | | |
| 15 | $E_2$, free | 15.5* | 17.0* | 12.5* | 47.5 |
| | $E_2$, conjugated | 11.0* | 88.5 | 26.5 | 14.0* |
| | $E_1$, free | | 29.5 | | 33 |
| | Sum of estrogens | 36.0 | 137.5 | 38.5 | 89.0 |
| 30 | $E_2$, free | 22.5 | 27.0 | 11.5* | 40.5 |
| | $E_2$, conjugated | 22.0* | 88.0 | 15.5* | 14.5* |
| | $E_1$, free | | 24.5* | | 54.5 |
| | Sum of estrogens | 44.5 | 129.0 | 31.5 | 112.0 |
| 60 | $E_2$, free | 7.0* | 22.5 | 20.5 | 25.5 |
| | $E_2$, conjugated | 3.0* | 40.0 | 17.5* | 13.0* |
| | $E_1$, free | | 22 | | 34.5 |
| | Sum of estrogens | 9.5 | 86.5 | 38.0 | 73.5 |
| 75 | $E_2$, free | 9.5* | 18.5 | 30.5 | 8.0* |
| | $E_2$, conjugated | 11.5* | 23.5* | 40.5 | 0.0* |
| | $E_1$, free | | 18* | | 38 |
| | Sum of estrogens | 28.5 | 62.5 | 75.5 | 45.0 |

* = values are below the LOQ for the given analyte, $E_2$ = estradiol-17β

**Studies using Torelor® implants**

Twenty four steers (average age 2.5 years; 354-459 kg BW) were treated with Torelor® (200 mg trenbolone acetate, 40 mg estradiol-17ß); 6 other animals served as a control group. Groups of six treated animals were slaughtered at 15, 30, 60, and 75 days, respectively, following the time of implantation. Two control animals were slaughtered at 15, 30, and 60 days following implantation of the treated animals. Tissue and blood samples were taken and analysed using the method already described above. Blood plasma levels were monitored over all time frames, with groups slaughtered on the same day after implantation (Arts *et al.,*1986b). The results are shown in Table 39.

In a further study with TORELOR®, 24 steers (approximately 430 kg; 2 years old) were implanted twice with an intermediate period of 60 days (Arts *et al.,* 1986c). One group of six steers was slaughtered 60 days after the first implantation. Three other groups of six animals each were re-implanted on day 60 and slaughtered on days 15, 30, and 60, respectively, after the second implantation. The results of this work are shown in Table 40.

**Studies in veal calves using Implix BM, Implix BF and Revalor lactose**

Studies in veal calves using Implix BM, Implix BF and Revalor lactose have been reported (Roberts and Cameron, 1986). The report contains no data on method validation. Friesian calves (39 male and 39 female), three weeks old at implant, were divided into three groups for treatment. Group 1 animals, comprised of 21 males and 21 females, were dosed with Implix BM (males) and Implix BF (females), respectively, on day 0 of the study. On day 50 the animals were dosed with Revalor lactose. Group 2, comprised of 12 animals of each sex, were dosed with Revalor lactose on day 50. Group 3, comprised of 6 animals of each sex, served as untreated controls. Calves were killed at various intervals. Results are summarised in Table 41

**Table 39** **Estrogen concentrations (ng/kg) in steers implanted with TORELOR®**

| Treatment group | Tissue | N | Min | Max | Mean | S.D. | Geomean | Median | |
|---|---|---|---|---|---|---|---|---|---|
| Control | Muscle | 6 | 13.0 | 46.0 | 22.5 | 12.4 | 20.3 | 17.5 | free $E_2$ |
| | | 6 | 0.0 | 25.0 | 9.3 | 8.4 | 0.7 | 8.0 | conjugated $E_2$ |
| | | 6 | 15.0 | 51.0 | 31.8 | 13.2 | 29.4 | 31.5 | total $E_2$ |
| | Liver | 6 | 14.0 | 31.0 | 20.5 | 6.9 | 19.6 | 17.5 | free $E_2$ |
| | | 6 | 24.0 | 51.0 | 34.5 | 9.2 | 33.6 | 33.5 | conjugated $E_2$ |
| | | 6 | 43.0 | 66.0 | 55.0 | 9.2 | 54.4 | 54.0 | total $E_2$ |
| | | 5 | 12.0 | 22.0 | 16.4 | 4.0 | 16.0 | 17.0 | estrone |
| | Kidney | 6 | 12.0 | 20.0 | 15.5 | 3.4 | 15.2 | 14.5 | free $E_2$ |
| | | 6 | 35.0 | 47.0 | 41.0 | 4.1 | 40.8 | 41.5 | conjugated $E_2$ |
| | | 6 | 47.0 | 67.0 | 56.5 | 7.1 | 56.1 | 56.5 | total $E_2$ |
| | Fat | 6 | 2.0 | 22.0 | 13.7 | 7.4 | 10.8 | 15.5 | free $E_2$ |
| | | 6 | 0.0 | 17.0 | 9.7 | 7.9 | 0.1 | 12.5 | conjugated $E_2$ |
| | | 6 | 2.0 | 39.0 | 23.3 | 14.7 | 16.1 | 29.5 | total $E_2$ |
| | | 6 | 7.0 | 31.0 | 24.0 | 9.6 | 21.5 | 28.5 | estrone |
| 15 days implant | Muscle | 6 | 12.0 | 100.0 | 40.2 | 31.1 | 32.4 | 34.5 | free $E_2$ |
| | | 6 | 1.0 | 38.0 | 14.3 | 13.7 | 8.4 | 10.5 | conjugated $E_2$ |
| | | 6 | 22.0 | 138.0 | 54.5 | 43.0 | 44.6 | 40.0 | total $E_2$ |
| | Liver | 6 | 8.0 | 68.0 | 40.2 | 24.8 | 30.5 | 50.0 | free $E_2$ |
| | | 6 | 39.0 | 710.0 | 296.3 | 252.6 | 186.7 | 276.5 | conjugated $E_2$ |
| | | 6 | 50.0 | 756.0 | 336.5 | 268.6 | 220.0 | 337.5 | total $E_2$ |
| | | 6 | 11.0 | 116.0 | 53.2 | 37.1 | 41.2 | 53.5 | estrone |
| | Kidney | 6 | 35.0 | 66.0 | 53.2 | 10.3 | 52.2 | 53.5 | free $E_2$ |
| | | 6 | 67.0 | 146.0 | 98.7 | 31.0 | 94.8 | 91.0 | conjugated $E_2$ |
| | | 6 | 121.0 | 212.0 | 151.8 | 37.1 | 148.3 | 140.0 | total $E_2$ |
| | Fat | 6 | 94.0 | 141.0 | 119.2 | 17.2 | 118.1 | 120.0 | free $E_2$ |
| | | 6 | 0.0 | 18.0 | 13.7 | 6.8 | 1.0 | 15.5 | conjugated $E_2$ |
| | | 6 | 112.0 | 157.0 | 132.8 | 18.4 | 131.8 | 133.5 | total $E_2$ |
| | | 6 | | | | | | | estrone |
| 30 days implant | Muscle | 6 | 15.0 | 82.0 | 38.7 | 24.4 | 32.9 | 36.0 | free $E_2$ |
| | | 6 | 2.0 | 44.0 | 20.0 | 15.8 | 12.7 | 20.0 | conjugated $E_2$ |
| | | 6 | 33.0 | 126.0 | 58.7 | 34.4 | 52.6 | 47.0 | total $E_2$ |
| | Liver | 6 | 5.0 | 148.0 | 61.5 | 48.2 | 41.9 | 60.5 | free $E_2$ |
| | | 6 | 37.0 | 525.0 | 306.0 | 191.7 | 226.6 | 339.0 | conjugated $E_2$ |
| | | 6 | 42.0 | 586.0 | 367.5 | 195.5 | 284.6 | 384.5 | total $E_2$ |
| | | 6 | 13.0 | 81.0 | 59.5 | 24.6 | 51.7 | 62.5 | estrone |
| | Kidney | 6 | 42.0 | 68.0 | 53.0 | 10.2 | 52.2 | 53.0 | free $E_2$ |
| | | 6 | 77.0 | 171.0 | 117.2 | 36.2 | 112.5 | 115.5 | conjugated $E_2$ |
| | | 6 | 119.0 | 239.0 | 170.2 | 42.6 | 165.8 | 172.0 | total $E_2$ |
| | Fat | 6 | 68.0 | 176.0 | 122.0 | 40.9 | 116.1 | 115.0 | free $E_2$ |
| | | 6 | 0.0 | 22.0 | 12.7 | 10.3 | 0.7 | 15.5 | conjugated $E_2$ |
| | | 6 | 90.0 | 196.0 | 134.7 | 44.4 | 129.0 | 115.5 | total $E_2$ |
| | | 6 | 76.0 | 110.0 | 92.5 | 12.2 | 91.8 | 91.0 | estrone |

**Table 39 continued. Estrogen concentrations (ng/kg) in steers implanted with TORELOR®**

| Treatment group | Tissue | N | Min | Max | Mean | S.D. | Geomean | Median | |
|---|---|---|---|---|---|---|---|---|---|
| 60 days implant | Muscle | 6 | 16.0 | 56.0 | 37.2 | 14.7 | 34.4 | 38.0 | free $E_2$ |
| | | 6 | 0.0 | 13.0 | 8.2 | 4.5 | 0.7 | 9.0 | conjugated $E_2$ |
| | | 6 | 27.0 | 65.0 | 45.3 | 15.2 | 43.1 | 47.0 | total $E_2$ |
| | Liver | 6 | 13.0 | 41.0 | 25.2 | 9.3 | 23.7 | 23.5 | free $E_2$ |
| | | 6 | 77.0 | 955.0 | 295.3 | 333.1 | 198.9 | 167.0 | conjugated $E_2$ |
| | | 6 | 101.0 | 996.0 | 320.5 | 340.6 | 228.0 | 188.0 | total $E_2$ |
| | | 6 | 20.0 | 139.0 | 57.0 | 42.4 | 47.1 | 49.5 | estrone |
| | Kidney | 6 | 20.0 | 94.0 | 50.8 | 28.6 | 44.4 | 41.5 | free $E_2$ |
| | | 6 | 11.0 | 380.0 | 111.3 | 140.2 | 55.1 | 66.5 | conjugated $E_2$ |
| | | 6 | 31.0 | 474.0 | 162.2 | 166.2 | 106.6 | 105.5 | total $E_2$ |
| | Fat | 6 | 74.0 | 180.0 | 125.5 | 46.5 | 118.2 | 118.0 | free $E_2$ |
| | | 6 | 2.0 | 57.0 | 23.7 | 18.2 | 16.5 | 21.0 | conjugated $E_2$ |
| | | 6 | 97.0 | 201.0 | 149.2 | 45.5 | 143.1 | 150.5 | total $E_2$ |
| | | 6 | 59.0 | 106.0 | 84.5 | 16.2 | 83.1 | 88.0 | estrone |
| 75 days implant | Muscle | 6 | 8.0 | 47.0 | 27.3 | 16.5 | 22.5 | 27.0 | free $E_2$ |
| | | 6 | 6.0 | 16.0 | 11.0 | 3.5 | 10.5 | 11.0 | conjugated $E_2$ |
| | | 6 | 17.0 | 59.0 | 38.3 | 17.8 | 34.7 | 35.0 | total $E_2$ |
| | Liver | 6 | 13.0 | 27.0 | 20.3 | 4.6 | 19.9 | 20.5 | free $E_2$ |
| | | 6 | 26.0 | 375.0 | 98.5 | 136.8 | 57.9 | 36.5 | conjugated $E_2$ |
| | | 6 | 48.0 | 402.0 | 118.8 | 140.3 | 81.8 | 55.5 | total $E_2$ |
| | | 6 | 13.0 | 132.0 | 36.2 | 47.1 | 23.6 | 18.0 | estrone |
| | Kidney | 6 | 21.0 | 126.0 | 73.8 | 43.1 | 61.6 | 71.5 | free $E_2$ |
| | | 6 | 32.0 | 383.0 | 133.3 | 129.1 | 96.2 | 90.0 | conjugated $E_2$ |
| | | 6 | 53.0 | 497.0 | 207.2 | 162.7 | 160.1 | 161.5 | total $E_2$ |
| | Fat | 6 | 32.0 | 175.0 | 83.7 | 60.0 | 67.1 | 61.5 | free $E_2$ |
| | | 6 | 0.0 | 18.0 | 8.5 | 9.3 | 0.0 | 8.0 | conjugated $E_2$ |
| | | 6 | 32.0 | 191.0 | 92.2 | 65.8 | 74.4 | 65.0 | total $E_2$ |
| | | 6 | 38.0 | 109.0 | 68.5 | 26.2 | 64.2 | 74.0 | estrone |

. n = number of specimens analysed; s.d =standard deviation; geo.mean =geometric mean

**Table 40. Estrogens in plasma and tissues of steers implanted with TORELOR®**

| Time | Tissue | Muscle | | | Liver | | | | Kidney | | | Fat | | | | Plasma | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Substance | Free $E_2$ | Conjug. $E_2$ | Total $E_2$ | Free $E_2$ | Conjug. $E_2$ | Total $E_2$ | $E_1$ | Free $E_2$ | Conjug. $E_2$ | Total $E_2$ | Free $E_2$ | Conjug. $E_2$ | Total $E_2$ | $E_1$ | Free $E_2$ | Conjug. $E_2$ | Total $E_2$ |
| | | Concentration (ng/kg) | | | | | | | | | | | | | | | | |
| Day 60 | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | | 6 |
| | Minimum | 14 | 0 | 15 | 10 | 22 | 32 | 5 | 49 | 90 | 139 | 73 | 1 | 77 | 39 | 4 | | 4 |
| | Maximum | 45 | 3 | 48 | 81 | 651 | 732 | 94 | 84 | 244 | 307 | 177 | 10 | 180 | 69 | 18 | | 18 |
| | Mean | 30 | 1 | 31 | 41.8 | 279 | 321 | 35.6 | 65.6 | 175 | 240 | 124 | 4.4 | 129 | 54.8 | 11 | | 11 |
| | Stand. Dev. | 11.9 | 1.22 | 12.5 | 26.9 | 240 | 267 | 34.4 | 12.5 | 61.9 | 69.8 | 38.5 | 3.36 | 37.3 | 11.2 | 5.70 | | 5.70 |
| | Geom.Mean | 27.9 | | 28.8 | 33.7 | 174 | 211 | 23.9 | 64.6 | 165 | 231 | 119 | 3.44 | 124 | 53.8 | 9.64 | | 9.64 |
| | Median | 30 | 1 | 31 | 44 | 257 | 301 | 22 | 65 | 197 | 262 | 128 | 4 | 132 | 53 | 11 | | 11 |
| Day 75 | n | 5 | 5 | 6 | 5 | 5 | 6 | 5 | 5 | 5 | 6 | 5 | 5 | 6 | 5 | 5 | | 6 |
| | Minimum | 25 | 0 | 0 | 3 | 52 | 0 | 11 | 46 | 60 | 0 | 105 | 0 | 0 | 35 | 6 | | 0 |
| | Maximum | 36 | 2 | 38 | 51 | 491 | 542 | 56 | 117 | 295 | 412 | 184 | 10 | 184 | 97 | 16 | | 16 |
| | Mean | 30.3 | 0.5 | 24.6 | 24 | 205 | 183 | 25.5 | 73.3 | 164 | 190 | 130 | 2.75 | 106 | 65.8 | 9.25 | | 7.4 |
| | Stand. Dev. | 4.79 | 1 | 14.6 | 20.7 | 195 | 213 | 20.9 | 30.6 | 97.7 | 154 | 37.1 | 4.86 | 67.5 | 25.7 | 4.57 | | 5.73 |
| | Geom.Mean | 30.0 | | | 15.6 | 149 | | 20.5 | 69.0 | 141 | | 127 | | | 61.7 | 8.56 | | |
| | Median | 30 | 0 | 28 | 21 | 139 | 139 | 17.5 | 65 | 151 | 200 | 116 | 0.5 | 106 | 65.5 | 7.5 | | 7 |
| Day 90 | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 3 | 6 |
| | Minimum | 73 | 5 | 110 | 9 | 101 | 122 | 14 | 73 | 131 | 204 | 164 | 2 | 169 | 46 | 10 | 4 | 10 |
| | Maximum | 135 | 43 | 177 | 98 | 1074 | 1172 | 194 | 137 | 303 | 422 | 401 | 15 | 416 | 119 | 126 | 9 | 135 |
| | Mean | 100 | 29.4 | 130 | 37.6 | 328 | 366 | 57 | 109 | 239 | 348 | 284 | 8.2 | 292 | 88.6 | 46.2 | 5.67 | 49.6 |
| | Stand. Dev. | 24 | 16.5 | 27.6 | 37.1 | 418 | 452 | 77.0 | 30.7 | 72.0 | 88.1 | 88.7 | 4.97 | 92.1 | 30.0 | 49.3 | 2.89 | 52.7 |
| | Geom.Mean | 98.1 | 23.2 | 128 | 25.4 | 209 | 240 | 33.1 | 105 | 229 | 337 | 272 | 6.7 | 280 | 83.9 | 28.9 | 5.24 | 30.8 |
| | Median | 105 | 37 | 126 | 21 | 161 | 177 | 27 | 127 | 277 | 381 | 290 | 9 | 292 | 92 | 20 | 4 | 24 |
| Day 120 | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 |
| | Minimum | 48 | 19 | 94 | 10 | 47 | 63 | 14 | 56 | 110 | 166 | 111 | 1 | 112 | 65 | 9 | 11 | 19 |
| | Maximum | 128 | 50 | 178 | 25 | 193 | 207 | 57 | 106 | 211 | 303 | 293 | 8 | 298 | 109 | 19 | 19 | 29 |
| | Mean | 86.6 | 41.6 | 128 | 17 | 102 | 119 | 28.4 | 83.2 | 155 | 239 | 194 | 4 | 198 | 80.6 | 13.2 | 14.5 | 24.8 |
| | Stand. Dev. | 29.8 | 12.7 | 34.3 | 5.74 | 59.6 | 60.0 | 17.9 | 19.9 | 39.1 | 53.8 | 75.3 | 2.74 | 77.5 | 19.2 | 4.38 | 3.70 | 4.49 |
| | Geom.Mean | 82.3 | 39.4 | 125 | 16.2 | 89.4 | 108 | 24.6 | 81.1 | 152 | 234 | 182 | 3.17 | 186 | 78.9 | 12.6 | 14.2 | 24.5 |
| | Median | 83 | 46 | 121 | 16 | 85 | 110 | 18 | 92 | 153 | 223 | 160 | 4 | 164 | 70 | 13 | 14 | 27 |

**Table 41.** **Concentration (ng/kg) of estradiol-17β, estradiol-17α, progesterone and testosterone in tissues of control and calves implanted with Implix BM, Implix BF and Revalor lactose**

| Analyte | Day | Muscle | | | Liver* | | | Liver | | | Kidney | | | Fat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor |
| Estradiol in males | 15 | | | 36.1 | | | 954 | | | 154 | | | 85 | | | 91.9 |
| | 30 | 7.3 | | 48.3 | 574 | | 682 | 32.1 | | 75.6 | 13 | | 125 | 11.8 | | 38.3 |
| | 50 | | | 60.3 | | | 824 | | | 193 | | | 134 | | | 95.6 |
| | 65 | | 94.4 | 114 | | 1105 | 1300 | 53.3 | 139 | 179 | | 154 | 208 | | 96.4 | 159 |
| | 80 | 20.2 | 117 | 106 | 685 | 1323 | 1284 | | 160 | 206 | 9.2 | 177 | 268 | 23.1 | 60 | 122 |
| | 100 | | 112 | 172 | | 666 | 2334 | | 106 | 226 | | 172 | 250 | | 50.9 | 206 |
| Progesterone in males | 15 | | | 606 | | | | | | 599 | | | 994 | | | 5407 |
| | 30 | 901 | | 597 | | | | 749 | | 924 | 4066 | | 2798 | 1598 | | 6520 |
| | 50 | | | 772 | | | | | | 771 | | | 1409 | | | 8664 |
| | 65 | | | 268 | | | | | | 20245 | | | 1582 | | | 15534 |
| | 80 | 409 | | 482 | | | | 1855 | | 633 | 1022 | | 1221 | 2662 | | 6933 |
| | 100 | | | 500 | | | | | | 442 | | | 1460 | | | 7996 |
| | | Control | Revalor only | Implix BM, Revalor | Control | Revalor Only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor | Control | Revalor only | Implix BM, Revalor |
| Estradiol in females | 15 | | | 106.5 | | | 1790 | | | 512 | | | 249 | | | 75.2 |
| | 30 | 5.6 | | 88.5 | 601 | | 703 | 32.4 | | 72.6 | 17 | | 170 | 15.2 | | 171 |
| | 50 | | | 88.6 | | | 698 | | | 95.4 | | | 146 | | | 119 |
| | 65 | | 84.9 | 156.4 | | 875 | 1721 | | 79.6 | 271 | | 144 | 163 | | 16.1 | 139 |
| | 80 | 14.6 | 100.7 | 155.4 | 589 | 1114 | 1560 | 23.4 | 148 | 149 | 18.5 | 198 | 451 | 18.8 | 129 | 132 |
| Testosterone in females | 15 | | | 360 | | | | | | 196 | | | 588 | | | 1027 |
| | 30 | 6.1 | | 246 | | | | 108 | | 66.4 | 95.5 | | 564 | 22.2 | | 1259 |
| | 50 | | | 226 | | | | | | 71.2 | | | 515 | | | 750 |
| | 65 | | | 188 | | | | | | 79.4 | | | 708 | | | 587 |
| | 80 | 8.5 | | 179 | | | | 101 | | 72.2 | 238 | | 637 | 77.2 | | 722 |

- = estradiol-17 α
-

**Studies using REVALOR implants**

Therre are several types of Revalor® implants available. These are listed below

- Revalor® -G for feedlot steers: The recommended dosage, one implant, contains 40 mg trenbolone acetate and 8 mg estradiol in two pellets
- Revalor® -S for feedlot steers; one implant contains 120 mg trenbolone acetate and 24 mg estradiol in six pellets.
- Revalor-H® for heifers: One implant contains 7 pellets (140 mg trenbolone acetate and 14 mg estradiol-17β) for use in heifers fed in confinement (feedlot).

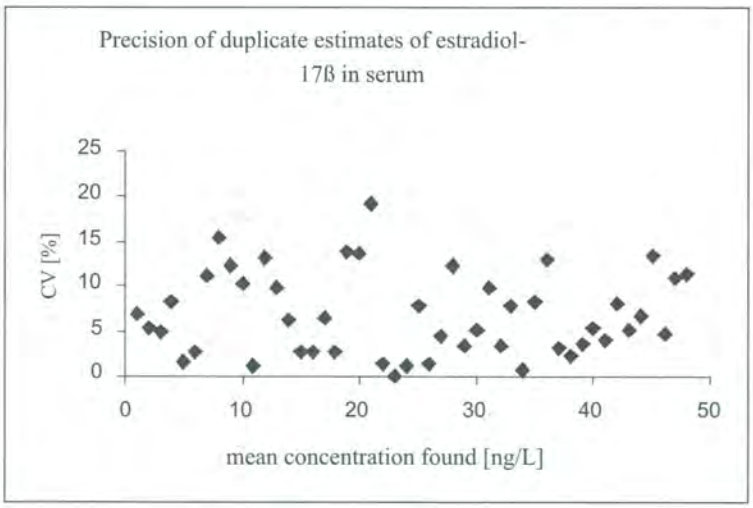
In a study by Wray (1986), twelve steer calves of primarily Angus, Herford and various continental breeds (initial average weight 563 pounds on day five of the study, and 788 on the last day) were used as controls (4 animals) or treated by implantation (8 animals). Blood samples were taken twice from each animal on days -1, 0, 14, 28, 42, 56, 70, 84, 98, 112 and serum was obtained for analyses. The analyses were performed by Clemson University. The report concluded: "While hardly any significant differences were found between the treatments when looking at each collection date individually, there was a significant difference between the control and treated animals over all time in both $E_2$-ß and T".

Method characteristics

Serum extracts were cleaned by $C_{18}$ reverse phase columns prior to RIA. All samples were corrected for recovery. Accuracy was determined as the percent recovery using fortified tissue and averaged 103.5. However, efficiency of extraction of $^3$H-$E_2$ (mean recovery from a subset of serum samples in each of the assays) was 87%. Inter-assay variation was 13% and intra-assay variation using replicates of all experimental samples > LOQ was 7%. No information was provided to indicate that conjugates are included in the residue determinations.

Each serum sample was assayed in duplicate using. The coefficient of variation, expressed as a percentage, of duplicate estimates calculated from these results was 6.2 for the median and 13.8 for the 95$^{th}$ percentile, respectively (n=47). Results are plotted in Figure 10.

**Figure 10. Precision of duplicate estimates of estradiol-17β in serum analyses following REVALOR® implantation**




Residues in plasma and tissues

Figure 11 shows the plasma levels determined at various times after Revalor® implantation. The results obtained with some of the individual animals suggest that the concentrations in serum reach a maximum after approximately 50 days. This conclusion is also supported if the median (or the mean, or the geometric mean) of the results obtained from all

animals is calculated and the results are plotted instead of the individual data. Table 42 shows data on the estradiol concentrations in the tissues of animals from this study.

**Figure 11. Estradiol concentrations in the serum of steers implanted with REVALOR**

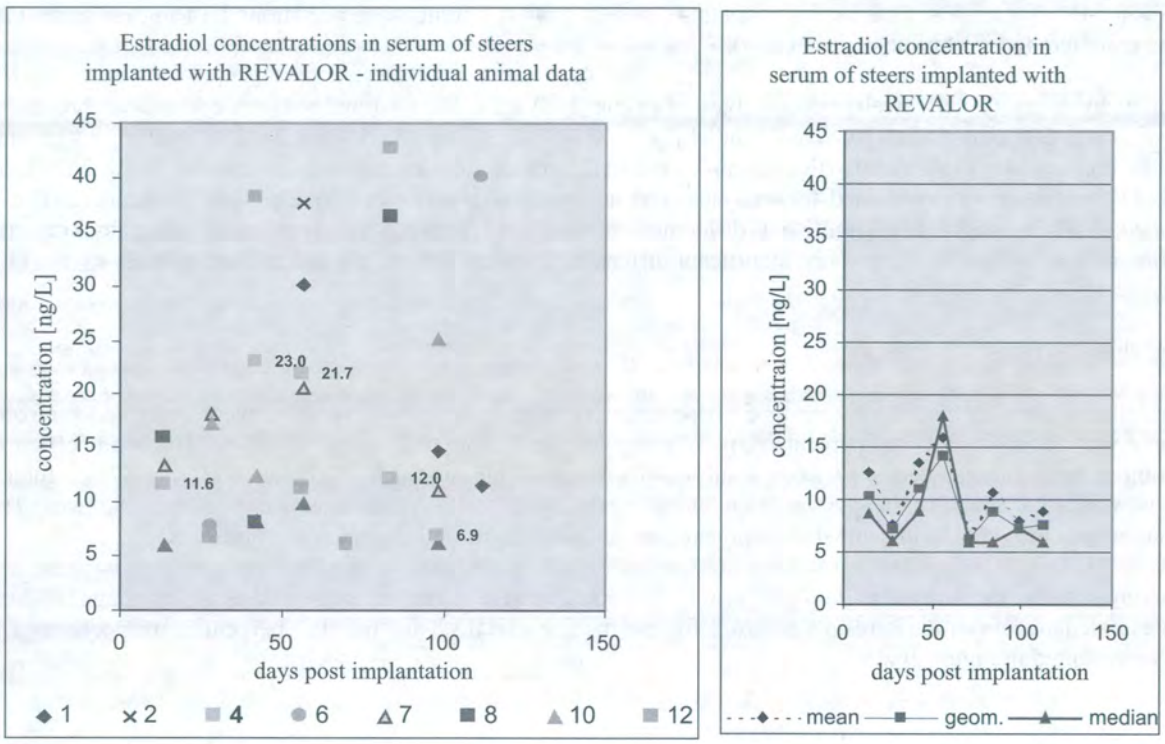



**Table 42 Estradiol in tissues of steers implanted with estradiol-17ß/trenbolone acetate (REVALOR)**

| Withdrawal time | Concentration (ng/kg) | | | |
|---|---|---|---|---|
| | Muscle | Fat | Liver | Kidney |
| Control | 2.1 | 6.6 | 0.0 | 21.2 |
| Day 15 | 4.2 | 17.9 | 0.9 | 22.5 |
| Day 30 | 4.6 | 14.2 | 0.5 | 19.4 |

A further study on tissue concentrations of estradiol-17β, trenbolone-17β, and trenbolone-17α in heifers implanted with a combination of trenbolone acetate (180 mg) and estradiol-17β (18 mg) was conducted (Clemson University. undated). Certain methodological aspects of the study remain unclear. The author states, for example: "Standard curves for the $E_2$-B for muscle, fat, and liver tissues were constructed with tissue extracts from control heifers. The concentration of estradiol reported in these tissues is above the background level from control heifers. Standard curves for the kidney tissue were constructed using extraction solvent residues rather than tissue residues. Concentrations of estradiol reported in kidney tissues are total levels." Results appear in Table 43.

**Table 43. Estradiol in tissues of heifers implanted with estradiol-17ß/trenbolone acetate**

| Sample | Muscle | Fat | Liver | Kidney |
|---|---|---|---|---|
| | Concentration (ng/kg) | | | |
| control | 0.5 | 0.1 | 0.0 | 17.0 |
| day 15 | 3.1 | 23.9 | 4.1 | 35.9 |
| day 30 | 7.0 | 26.8 | 11.1 | 40.4 |

## CALCULATION OF THEORETICAL DAILY INTAKES

By multiplying the hormone concentrations [units/gram] found in the edible tissues with the arbitrary consumption figures established by JECFA, the theoretical daily intakes are obtained. The Theoretical Maximum Daily Intake (TMDI) is the sum of the individual daily intakes:

$$300\ c_{muscle} + 100\ c_{liver} + 50\ c_{kidney} + 50\ c_{fat} = \text{TMDI/500 g "meat"}$$

In this report the median concentration found is used for these calculations. The reasons are the following: It is obvious from the raw data that the concentrations of the hormones in the tissues are not normally distributed. A logarithmic transformation of the data is not always an appropriate solution to the problems, in particular, if analytical "zero's" are included in the results and/or if the number of results below the limit of quantification is significant. For the great majority of all these cases it is still possible to calculate a median even under these conditions. Table 44 gives an example of the calculations. The data used are the results of the study with Synovex S in steers.

**Table 44. Theoretical daily intake of estrone, estradiol and progesterone using median hormone levels found in and steers implanted with SYNOVEX-S and on JECFA tissue consumption figures.**

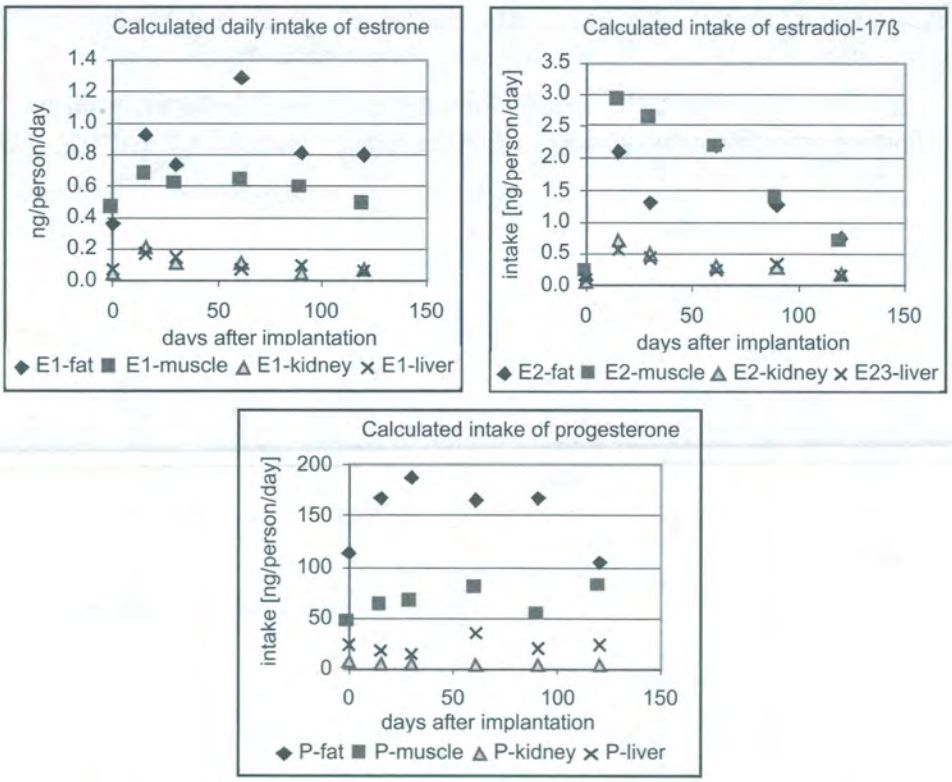
| Tissue | | n | Concentration (ng/kg) | | | Intake/day (ng) | | | Contribution to TMDI (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $E_1$ | $E_2$ | P | $E_1$ | $E_2$ | P | | | |
| Fat | Control | 21 | 7.37 | 1.91 | 2290 | 0.37 | 0.10 | 115 | 39 | 21 | 60 |
| | 15 | 8 | 18.6 | 42.2 | 3345 | 0.93 | 2.11 | 167 | 47 | 34 | 66 |
| | 30 | 8 | 14.7 | 26.0 | 3725 | 0.74 | 1.30 | 186 | 46 | 27 | 68 |
| | 61 | 8 | 25.7 | 43.9 | 3290 | 1.28 | 2.19 | 165 | 61 | 45 | 59 |
| | 90 | 7 | 16.3 | 25.6 | 3350 | 0.82 | 1.28 | 168 | 52 | 39 | 68 |
| | 120 | 7 | 16.1 | 14.8 | 2110 | 0.81 | 0.74 | 106 | 56 | 42 | 49 |
| Muscle | Control | 16 | 1.53 | 0.68 | 150 | 0.46 | 0.20 | 45 | 48 | 44 | 24 |
| | 15 | 8 | 2.24 | 9.71 | 210 | 0.67 | 2.91 | 63 | 34 | 46 | 25 |
| | 30 | 8 | 2.04 | 8.74 | 220 | 0.61 | 2.62 | 66 | 38 | 54 | 24 |
| | 61 | 8 | 2.13 | 7.23 | 260 | 0.64 | 2.17 | 78 | 30 | 44 | 28 |
| | 90 | 7 | 1.96 | 4.51 | 180 | 0.59 | 1.35 | 54 | 38 | 42 | 22 |
| | 120 | 7 | 1.63 | 2.23 | 270 | 0.49 | 0.67 | 81 | 34 | 38 | 38 |
| Kidney | Control | 18 | 1.02 | 1.53 | 135 | 0.05 | 0.08 | 6.8 | 5.4 | 17 | 3.5 |
| | 15 | 8 | 4.28 | 14 | 120 | 0.21 | 0.70 | 6.0 | 11 | 11 | 2.4 |
| | 30 | 8 | 2.18 | 9.68 | 120 | 0.11 | 0.48 | 6.0 | 6.8 | 10 | 2.2 |
| | 61 | 8 | 2.21 | 5.96 | 80 | 0.11 | 0.30 | 4.0 | 5.2 | 6.1 | 1.4 |
| | 90 | 7 | 1.08 | 5.38 | 90 | 0.05 | 0.27 | 4.5 | 3.5 | 8.3 | 1.8 |
| | 120 | 7 | 1.56 | 3.91 | 90 | 0.08 | 0.20 | 4.5 | 5.4 | 11 | 2.1 |
| Liver | Control | 18 | 0.73 | 0.87 | 240 | 0.07 | 0.09 | 24 | 7.7 | 19 | 13 |
| | 15 | 8 | 1.73 | 5.51 | 175 | 0.17 | 0.55 | 18 | 8.7 | 8.8 | 6.9 |
| | 30 | 8 | 1.48 | 4.24 | 145 | 0.15 | 0.42 | 15 | 9.2 | 8.8 | 5.3 |
| | 61 | 8 | 0.79 | 2.42 | 340 | 0.08 | 0.24 | 34 | 3.7 | 4.9 | 12 |
| | 90 | 7 | 1.06 | 3.37 | 195 | 0.11 | 0.34 | 20 | 6.8 | 10 | 7.9 |
| | 120 | 7 | 0.64 | 1.4 | 245 | 0.06 | 0.14 | 25 | 4.5 | 8.0 | 11 |

Table 45 summarises the Theoretical Maximum Daily Intake resulting from the consumption of 300g muscle, 100g liver and 50g of each, kidney and fat. The TMDI would be highest if the animals would be slaughtered 15 days after implantation (see also Figures 12 a-c where the controls are plotted on day 0).

**Table 45. Theoretical Maximum Daily Intake of estrone, estradiol, and progesterone resulting from the consumption of edible tissues of steers implanted with SYNOVEX-S**

| Days after implantation | TMDI [ng/day] | | |
|---|---|---|---|
| | Estrone | Estradiol | Progesterone |
| Control | 1.0 | 0.5 | 190 |
| 15 | 2.0 | 6.3 | 254 |
| 30 | 1.6 | 4.8 | 273 |
| 61 | 2.1 | 4.9 | 281 |
| 90 | 1.6 | 3.2 | 246 |
| 120 | 1.4 | 1.7 | 216 |

**Figure 12. Theoretical Maximum Daily Intake of estrone, estradiol, and progesterone resulting from the consumption of edible tissues of steers implanted with SYNOVEX-S**




Calculations of theoretical daily intake of estrone, estradiol and testosterone on the basis of median hormone levels found in control heifers and in heifers implanted with SYNOVEX-H and on JECFA consumption figures are presented in Table 46.

Table 47_summarises the Theoretical Maximum Daily Intake resulting from the consumption of 300g muscle, 100g liver and 50g of each, kidney and fat. The TMDI would be highest if the animals would be slaughtered 15 days after implantation.

Theoretical daily intakes were calculated in the same way on the data base available and described in previous paragraphs. Table 48 summarises the results obtained when the data from the pregnant heifer study were used. Depending on the duration of pregnancy at the time of implantation, treatment results in either a slight increase (estrone, late pregnancy; testosterone, early pregnancy;) or of a significant decrease (estradiol, late pregnancy) of the TMDI.

Table 49 summarises the theoretical daily intakes resulting from the consumption of edible tissues of pregnant heifers, both untreated animals and animals implanted with Synovex-H at various times of pregnancy. Table 50 summarises the theoretical daily intakes from the consumption of edible tissues obtained from calves implanted with SYNOVEX-implants.

**Table 46. Calculation of theoretical daily intake of estrone, estradiol and testosterone using median hormone levels in heifers implanted with SYNOVEX-H using JECFA consumption figures.**

| Tissue | Day after implantation | n | Concentration (ng/kg) | | | Intake/day (ng) | | | Contribution to TMDI (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $E_1$ | T | P | $E_1$ | T | P | | | |
| Fat | Control | 35 | 10 | 9.2 | 24 | 0.5 | 0.5 | 1.2 | 37 | 30 | 6.8 |
| | 30 | 20 | 36 | 73 | 290 | 1.8 | 3.7 | 15 | 47 | 25 | 21 |
| | 60 | 25 | 26 | 48 | 129 | 1.3 | 2.4 | 6.5 | 48 | 40 | 22 |
| | 89 | 10 | 29 | 54 | 132 | 1.5 | 2.7 | 6.6 | 45 | 42 | 18 |
| | 119 | 10 | 12 | 11 | 31 | 0.6 | 0.6 | 1.5 | 37 | 39 | 5.6 |
| Muscle | Control | 15 | 2.3 | 2.9 | 22 | 0.7 | 0.9 | 6.5 | 48 | 57 | 37 |
| | 30 | 20 | 5.3 | 30 | 101 | 1.6 | 9.1 | 30 | 41 | 63 | 44 |
| | 60 | 10 | 3.8 | 10 | 41 | 1.1 | 3.1 | 12 | 41 | 50 | 42 |
| | 89 | 10 | 4.8 | 10 | 52 | 1.4 | 3.1 | 16 | 44 | 48 | 42 |
| | 119 | 10 | 2.6 | 2.1 | 27 | 0.8 | 0.6 | 8.1 | 49 | 42 | 29 |
| Kidney | Control | 15 | 1.4 | 2.0 | 169 | 0.1 | 0.1 | 8.5 | 5.0 | 6.4 | 48 |
| | 30 | 10 | 5.4 | 23 | 431 | 0.3 | 1.1 | 22 | 6.9 | 7.8 | 31 |
| | 60 | 15 | 3.0 | 7.5 | 180 | 0.2 | 0.4 | 9.0 | 5.6 | 6.2 | 31 |
| | 89 | 10 | 4.0 | 7.7 | 256 | 0.2 | 0.4 | 13 | 6.1 | 6.0 | 35 |
| | 119 | 10 | 1.7 | 2.9 | 330 | 0.1 | 0.1 | 16 | 5.4 | 9.7 | 60 |
| Liver | Control | 10 | 1.4 | 1.0 | 13 | 0.1 | 0.1 | 1.3 | 9.8 | 6.5 | 7.6 |
| | 30 | 10 | 2.0 | 6.8 | 32 | 0.2 | 0.7 | 3.2 | 5.3 | 4.6 | 4.6 |
| | 60 | 10 | 1.4 | 2.2 | 16 | 0.1 | 0.2 | 1.6 | 5.3 | 3.7 | 5.6 |
| | 89 | 10 | 1.5 | 2.7 | 20 | 0.2 | 0.3 | 2.0 | 4.8 | 4.1 | 5.5 |
| | 119 | 10 | 1.3 | 1.4 | 15 | 0.1 | 0.1 | 1.5 | 8.6 | 9.3 | 5.4 |

**Table 47. Theoretical Maximum Daily Intake of estrone, estradiol, and testosterone from the consumption of edible tissues of heifers implanted with SYNOVEX-H**

| Days after implantation | TMDI (ng/person/day) | | |
|---|---|---|---|
| | Estrone | Estradiol | Testosterone |
| Control | 1.4 | 1.5 | 17 |
| 15 | 3.9 | 15 | 70 |
| 30 | 2.7 | 6.1 | 29 |
| 61 | 3.2 | 6.5 | 37 |
| 90 | 1.6 | 1.5 | 28 |

**Table 48.** **Theoretical Maximum Daily Intake of estrone, estradiol, and testosterone from the consumption of edible tissues of pregnant heifers implanted with SYNOVEX-H**

| Description of the treatment group | Duration of pregnancy at day of implantation | Days after implantation | TMDI (ng/person/day) | | |
|---|---|---|---|---|---|
| | | | Estrone | Estradiol-17β | Testosterone |
| Unsynchronised controls | 120 | | 93 | 16 | 203 |
| Synchronised controls | 120 | | 113 | 16 | 172 |
| Implanted | 120 | 61 | 34 | 15 | 233 |
| Synchronised controls | 180 | | 280 | 48 | 282 |
| Implanted | 180 | 61 | 107 | 24 | 237 |
| Synchronised controls | 240 | | 326 | 139 | 377 |
| Implanted | 240 | 61 | 377 | 49 | 326 |

**Table 49.** **Theoretical daily intakes from the edible tissues obtained from calves implanted with SYNOVEX-implants.**

| Treatment | Hormone | A: Studies with female calves/heifers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Muscle | | Fat | | Liver | | Kidney | | All tissues | |
| | | Treated | Control | Treated | Control | Treated | Control | Treated | Control | Treated | Control |
| | | Theoretical Intake (ng/person/day) | | | | | | | | TMDI (ng/person/day) | |
| A* | $E_1$ | 0.6 | 0.7 | 1.0 | 0.3 | 0.11 | 0.06 | 0.16 | 0.04 | 1.9 | 1.1 |
| | $E_2$ | 3.7 | 2.4 | 2.0 | 0.8 | 0.36 | 0.15 | 0.35 | 0.06 | 6.3 | 3.5 |
| | P | 134 | 0.7 | 337 | 0.1 | 9 | 7.27 | 24 | 14 | 504 | 22 |
| B* | $E_1$ | 1.2 | 0.5 | 1.4 | 0.4 | 0.20 | 0.16 | 0.16 | 0.08 | 3.0 | 1.2 |
| | $E_2$ | 1.7 | 1.4 | 1.4 | 0.5 | 0.36 | 0.18 | 0.29 | 0.10 | 3.7 | 2.2 |
| | P | 136 | 0.2 | 338 | 0.0 | 27 | 19 | 19 | 34 | 520 | 53 |
| C** | $E_1$ | 1.0 | 0.4 | 1.0 | 0.1 | 0.16 | 0.12 | 0.20 | 0.14 | 2.4 | 0.8 |
| | $E_2$ | 2.4 | 1.0 | 1.4 | 0.3 | 0.36 | 0.31 | 0.47 | 0.13 | 4.5 | 1.7 |
| | T | z | 0.6 | 5.9 | 0.2 | 3.2 | 1.9 | 11 | 11 | 30 | 13 |
| D** | $E_1$ | 1.3 | 0.9 | 1.5 | 0.3 | 0.29 | 0.18 | 0.29 | 0.12 | 3.4 | 1.4 |
| | $E_2$ | 6.9 | 2.5 | 3.8 | 0.8 | 1.3 | 0.75 | 0.94 | 0.34 | 13 | 4.4 |
| | T | 35 | 0.9 | 14 | 0.3 | 2.9 | 1.8 | 24 | 20 | 76 | 22 |
| E** | $E_1$ | 0.6 | 0.4 | 0.8 | 0.1 | 0.12 | 0.10 | 0.13 | 0.09 | 1.7 | 0.7 |
| | $E_2$ | 2.8 | 1.4 | 1.6 | 0.4 | 0.26 | 0.25 | 0.37 | 0.09 | 5.0 | 2.1 |
| | T | 16 | 0.7 | 9.7 | 0.1 | 2.4 | 1.8 | 14 | 20 | 42 | 22 |
| F** | $E_1$ | 1 | 1.0 | 1.4 | 0.7 | 0.2 | 0.2 | 0.14 | 0.04 | 2 | 2 |
| | $E_2$ | 1 | 2.4 | 2.2 | 0.2 | 0.5 | 0.2 | 0.42 | 0.10 | 4 | 3 |
| | T | 14 | 18 | 9.7 | 2.3 | 4.6 | 3.6 | 20 | 28 | 48 | 51 |

**Table 49 (continued). Theoretical daily intakes from the edible tissues obtained from calves implanted with SYNOVEX-implants.**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **A: Studies with castrated male calves/steers** | | | | | | | | | | |
| **A*** | $E_1$ | 0.6 | 0.2 | 0.8 | 0.3 | 0.2 | 0.2 | 0.2 | 0.0 | 1.9 | 0.8 |
| | $E_2$ | 3.5 | 0.3 | 1.8 | 0.2 | 0.3 | 0.1 | 0.3 | 0.1 | 5.9 | 0.7 |
| | P | 259 | 95 | 310 | 389 | 6.8 | 6.0 | 35.0 | 10.9 | 611 | 501 |
| **B*** | $E_1$ | 0.6 | 0.1 | 0.8 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 1.6 | 0.4 |
| | $E_2$ | 1.8 | 0.1 | 1.5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.0 | 3.7 | 0.5 |
| | P | 72 | 91 | 392 | 436 | 14 | 11 | 12 | 14 | 491 | 552 |
| **C**** | $E_1$ | 0.8 | 0.6 | 0.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 1.8 | 1.1 |
| | $E_2$ | 1.7 | 0.7 | 1.1 | 0.1 | 0.4 | 0.3 | 0.3 | 0.1 | 3.4 | 1.2 |
| | P | 111 | 152 | 595 | 489 | 16 | 10 | 17 | 18 | 739 | 669 |
| **D**** | $E_1$ | 1.4 | 0.2 | 1.9 | 0.1 | 0.1 | 0.1 | 0.2 | 0.0 | 3.7 | 0.5 |
| | $E_2$ | 6.5 | 0.4 | 2.8 | 0.1 | 0.4 | 0.3 | 0.9 | 0.1 | 11 | 0.9 |
| | P | 106 | 131 | 399 | 258 | 25 | 15 | 10 | 18 | 540 | 421 |
| **E**** | $E_1$ | 0.8 | 0.7 | 0.9 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 2.1 | 1.2 |
| | $E_2$ | 2.7 | 0.3 | 1.1 | 0.1 | 0.4 | 0.2 | 0.4 | 0.1 | 4.7 | 0.7 |
| | P | 99 | 163 | 285 | 345 | 21 | 13 | 10 | 14 | 414 | 536 |
| **C**** | $E_1$ | 0.9 | 0.3 | 0.8 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 2.2 | 0.8 |
| | $E_2$ | 1.7 | 0.3 | 0.9 | 0.2 | 0.4 | 0.4 | 0.4 | 0.1 | 3.4 | 1.0 |
| | P | 193 | 753 | 293 | 332 | 20 | 13 | 21 | 73 | 527 | 1170 |

**A** = implanted day 0, slaughtered day 61. **B** = implanted day 0, slaughtered day 119. **C** = implanted day 0, implanted day 118, slaughtered day 241. **D** = implanted day 0, implanted day 118, implanted day 240, slaughtered day 301. **E** = implanted day 0, implanted day 118, implanted day 240, slaughtered day 329. **F**= implanted day 0, implanted day 118, implanted day 240, slaughtered day 360.

* = testosterone not determined ** = progesterone not determined

**Table 50. Theoretical daily intakes from the tissues of pregnant heifers (untreated animals and animals implanted with Synovex-H at various times of pregnancy)**

| Tissue | Description of the treatment groups | Days pregnant | Theoretical daily intake (ng/person/day) | | |
|---|---|---|---|---|---|
| | | | Estrone | Estradiol-17β | Testosterone |
| Fat | Unsynchronised controls | 120 | 41 | 2 | 21 |
| | Synchronised controls | 120 | 57 | 2 | 27 |
| | 61 Days implanted | 120 | 15 | 4 | 40 |
| | Synchronised controls | 180 | 137 | 3 | 37 |
| | 61 Days implanted | 180 | 65 | 7 | 51 |
| | Synchronised controls | 240 | 136 | 3 | 33 |
| | 61 Days implanted | 240 | 246 | 7 | 62 |
| Muscle | Unsynchronised controls | 120 | 46 | 3 | 74 |
| | Synchronised controls | 120 | 52 | 4 | 69 |
| | 61 Days implanted | 120 | 18 | 7 | 99 |
| | Synchronised controls | 180 | 127 | 7 | 94 |
| | 61 Days implanted | 180 | 36 | 7 | 93 |
| | Synchronised controls | 240 | 167 | 11 | 126 |
| | 61 Days implanted | 240 | 117 | 9 | 116 |
| Liver | Unsynchronised controls | 120 | 2 | 5 | 4 |
| | Synchronised controls | 120 | 1 | 5 | 5 |
| | 61 Days implanted | 120 | 1 | 2 | 4 |
| | Synchronised controls | 180 | 9 | 24 | 12 |
| | 61 Days implanted | 180 | 2 | 3 | 6 |
| | Synchronised controls | 240 | 16 | 109 | 27 |
| | 61 Days implanted | 240 | 2 | 15 | 9 |
| Kidney | Unsynchronised controls | 120 | 4 | 6 | 104 |
| | Synchronised controls | 120 | 3 | 5 | 71 |
| | 61 Days implanted | 120 | 1 | 2 | 90 |
| | Synchronised controls | 180 | 8 | 13 | 140 |
| | 61 Days implanted | 180 | 3 | 7 | 88 |
| | Synchronised controls | 240 | 7 | 15 | 191 |
| | 61 Days implanted | 240 | 12 | 18 | 139 |
| Theoretical Maximum Daily Intake (TMDI) | Unsynchronised controls | 120 | 93 | 16 | 203 |
| | Synchronised controls | 120 | 113 | 16 | 172 |
| | 61 Days implanted | 120 | 34 | 15 | 233 |
| | Synchronised controls | 180 | 280 | 48 | 282 |
| | 61 Days implanted | 180 | 107 | 24 | 237 |
| | Synchronised controls | 240 | 326 | 139 | 377 |
| | 61 Days implanted | 240 | 377 | 49 | 326 |

Table 51 calculates the theoretical daily intake resulting from the consumption of steers/heifers implanted with STEER-oid/HEIFER-oid. Tables 52- 56 summarise the intake calculations performed on the basis of the results obtained in studies with animals of both sexes and different ages/production classes, which had been treated with COMPUDOSE. Tables 63-66 summarise the intake calculations performed on the basis of the results obtained in studies with animals of both sexes and different ages/production classes, which had been treated with Finaplix, Revalor, Torelor and Revalor/Implix

**Table 51. Theoretical daily intakes of estradiol-17β and progesterone from the tissues of steers/heifers implanted with STEER-oid/HEIFER-oid.**

| Days after implantation | Daily intake (ng/person/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Steers | | | | | | | | |
| | Estradiol-17β | | | Progesterone | | | Testosterone | | |
| | Muscle | Fat | TMDI | Muscle | Fat | TMDI | Muscle | Fat | TMDI |
| Control | 13 | 8 | 21 | 186 | 113 | 299 | ND | ND | ND |
| 15 | 17 | 8 | 25 | 246 | 129 | 375 | ND | ND | ND |
| | Heifers | | | | | | | | |
| | Estradiol-17β | | | Progesterone | | | Testosterone | | |
| | Muscle | Fat | TMDI | Muscle | Fat | TMDI | Muscle | Fat | TMDI |
| Control | 11 | 5 | 16 | ND | ND | ND | 31 | 12 | 43 |
| 15 | 13 | 6 | 18 | ND | ND | ND | 34 | 14 | 48 |

ND = not determined

**Table 52. Theoretical Dietary Intakes from the tissues of steers implanted with COMPUDOSE implants**

| Tissue | Treatment group (Study APH216A) | Concentration (median) (ng/kg) | | Daily Intake (ng/person/day) | |
|---|---|---|---|---|---|
| | | Estrone | Estradiol-17β | Estrone | Estradiol-17β |
| Fat | Control | 10.9 | 6.3 | 0.5 | 0.3 |
| | Implanted | 39.5 | 20.9 | 2.0 | 1.0 |
| Kidney | Control | 9.1 | 9.4 | 0.5 | 0.5 |
| | Implanted | 24.2 | 29.9 | 1.2 | 1.5 |
| Liver | Control | 8.3 | 5.9 | 0.8 | 0.6 |
| | Implanted | 15.1 | 9.5 | 1.5 | 1.0 |
| Muscle | Control | 8.4 | 10.4 | 2.5 | 3.1 |
| | Implanted. | 9.1 | 7.4 | 2.7 | 2.2 |
| TMDI | Control | | | 4.4 | 4.5 |
| | Implanted. | | | 7.4 | 5.7 |

**Table 53. Theoretical Dietary Intakes from the tissues of heifers implanted with COMPUDOSE implants***

| Tissue | Treatment group | Concentration (mean) (ng/kg) | | Daily Intake (ng/person/day) | |
|---|---|---|---|---|---|
| | | Estrone | Estradiol-17β | Estrone | Estradiol-17β |
| Fat | Control | 5.3 | 5.6 | 0.3 | 0.3 |
| | Implanted | 9.9 | 9.3 | 0.5 | 0.5 |
| Kidney | Control | 6.5 | 8.5 | 0.3 | 0.4 |
| | Implanted | 10.2 | 26.3 | 0.5 | 1.3 |
| Liver | Control | 7.5 | 8.2 | 0.8 | 0.8 |
| | Implanted | 6.7 | 7.3 | 0.7 | 0.7 |
| Muscle | Control | 5.9 | 7.1 | 1.8 | 2.1 |
| | Implanted. | 6.4 | 5.8 | 1.9 | 1.7 |
| TMDI | Control | | | 3.1 | 3.7 |
| | Implanted. | | | 3.6 | 4.3 |

- = Calculations are based on average residue levels because individual animal data were not available and, therefore, median values could not be calculated.

**Table 54. Theoretical Dietary Intakes from the tissues of bull calves implanted with COMPUDOSE implants**

| Tissue | Treatment group | Concentration (median) (ng/kg) | | Daily Intake (ng/person/day) | |
|---|---|---|---|---|---|
| | | Estrone | Estradiol-17β | Estrone | Estradiol-17β |
| Fat | Control | 13 | 5 | 0.7 | 0.3 |
| | Implanted | 23 | 40 | 1.2 | 2.0 |
| Kidney | Control | 8 | 11 | 0.4 | 0.5 |
| | Implanted | 35 | 56 | 1.7 | 2.8 |
| Liver | Control | 9 | 14 | 0.9 | 1.4 |
| | Implanted | 23 | 31 | 2.3 | 3.1 |
| Muscle | Control | 7 | 5 | 2.1 | 1.5 |
| | Implanted. | 11 | 19 | 3.4 | 5.6 |
| TMDI | Control | | | 4 | 4 |
| | Implanted. | | | 9 | 14 |

**Table 55. Theoretical Dietary Intakes from the tissues of bulls implanted with COMPUDOSE implants**

| Tissue | Treatment group | Concentration (median) (ng/kg) | | Daily Intake (ng/person/day) | |
|---|---|---|---|---|---|
| | | Estrone | Estradiol-17β | Estrone | Estradiol-17β |
| Fat | Control | 13.6 | 9.0 | 0.7 | 0.5 |
| | Implanted | 33.6 | 19.1 | 1.7 | 1.0 |
| Kidney | Control | 8.5 | 9.7 | 0.4 | 0.5 |
| | Implanted | 13.6 | 20.9 | 0.7 | 1.0 |
| Liver | Control | 5.5 | 8.7 | 0.5 | 0.9 |
| | Implanted | 6.1 | 15.3 | 0.6 | 1.5 |
| Muscle | Control | 7.2 | 5.0 | 2.2 | 1.5 |
| | Implanted. | 6.8 | 8.0 | 2.0 | 2.4 |
| TMDI | Control | | | 3.8 | 3.3 |
| | Implanted. | | | 5.0 | 5.9 |

**Table 56. Theoretical Dietary Intakes from the tissues of Zebu steers implanted with COMPUDOSE implants**

| Tissue | Treatment group | Concentration (median) (ng/kg) | | Daily Intake (ng/person/day) | |
|---|---|---|---|---|---|
| | | Estrone | Estradiol-17β | Estrone | Estradiol-17β |
| Fat | Control | 5.3 | 5.0 | 0.3 | 0.3 |
| | Implanted | 7.7 | 6.7 | 0.4 | 0.3 |
| Kidney | Control | | | | |
| | Implanted | | | | |
| Liver | Control | 8.0 | 8.1 | 0.8 | 0.8 |
| | Implanted | 11 | 14 | 1.1 | 1.4 |
| Muscle | Control | 17 | 8.6 | 5.0 | 2.6 |
| | Implanted. | 10 | 5.5 | 2.9 | 1.6 |
| TMDI | Control | | | 6.1 | 3.6 |
| | Implanted. | | | 4.4 | 3.4 |

**Table 57. Theoretical daily intakes from the tissues of heifers treated with FINAPLIX**

| Day after implantation | Definition of the estrogen | Theoretical daily intake (ng/person/day) | | | | |
|---|---|---|---|---|---|---|
| | | Muscle | Liver | Kidney | Fat | TMDI |
| 15 | Free Estradiol-17β | 4.7 | 1.7 | 0.63 | 2.4 | 9.4 |
| | Conjugated Estradiol-17β | 3.3 | 8.9 | 1.3 | 0.70 | 14 |
| | Free Estrone | | 3.0 | | 1.7 | 4.6 |
| | Total Estrogens | 11 | 14 | 1.9 | 4.5 | 31 |
| 30 | Free Estradiol-17β | 6.8 | 2.7 | 0.58 | 2.0 | 12 |
| | Conjugated Estradiol-17β | 6.6 | 8.8 | 0.78 | 0.73 | 17 |
| | Free Estrone | | 2.5 | | 2.7 | 5.2 |
| | Total Estrogens | 13 | 13 | 1.6 | 5.6 | 33 |
| 60 | Free Estradiol-17β | 2.1 | 2.3 | 1.0 | 1.3 | 6.7 |
| | Conjugated Estradiol-17β | 0.90 | 4.0 | 0.88 | 0.65 | 6.4 |
| | Free Estrone | | 2.2 | | 1.7 | 3.9 |
| | Total Estrogens | 2.9 | 8.7 | 1.9 | 3.7 | 17 |
| 75 | Free Estradiol-17β | 2.9 | 1.9 | 1.5 | 0.40 | 6.6 |
| | Conjugated Estradiol-17β | 3.5 | 2.4 | 2.0 | | 7.8 |
| | Free Estrone | | 1.8 | | 1.9 | 3.7 |
| | Total Estrogens | 8.6 | 6.3 | 3.8 | 2.3 | 21 |

**Table 58. Theoretical daily intakes of estradiol-17β from the tissues of animals implanted with Revalor**

| Sex of animal | Sample | Theoretical Intakes (ng/person/day) | | | | |
|---|---|---|---|---|---|---|
| | | Muscle | Fat | Liver | Kidney | TMDI |
| Heifers | Control | 0.15 | 0.01 | 0.00 | 0.85 | 1.0 |
| | Day 15 | 0.93 | 1.2 | 0.41 | 1.8 | 4.3 |
| | Day 30 | 2.1 | 1.3 | 1.1 | 2.0 | 6.6 |
| Steers | Control | 0.63 | 0.33 | 0.00 | 1.1 | 2.0 |
| | Day 15 | 1.26 | 0.90 | 0.09 | 1.1 | 3.4 |
| | Day 30 | 1.38 | 0.71 | 0.05 | 0.97 | 3.1 |

**Table 59. Theoretical daily intakes from the tissues of steers implanted with TORELOR A: Effects of a single implantation**

| Days after the last implantation | Daily intake (ng/person/day) | | | | | | TMDI (ng/person/day) | |
|---|---|---|---|---|---|---|---|---|
| | Estrone | | Estradiol-17β | | | | Estrone | Estradiol-17β |
| | liver | fat | muscle | liver | kidney | fat | | |
| Control | 1.7 | 1.4 | 9.5 | 5.4 | 2.8 | 1.4 | 3.1 | 19.1 |
| 15 | 5.4 | 3.5 | 12 | 34 | 7.0 | 3.5 | 8.9 | 56 |
| 30 | 6.3 | 4.6 | 14 | 38 | 8.6 | 4.6 | 11 | 66 |
| 60 | 19 | 4.4 | 14 | 5 | 5.3 | 4.4 | 23 | 29 |
| 75 | 1.8 | 3.7 | 11 | 5.6 | 8.1 | 3.7 | 5.5 | 28 |
| B: Effect of a second implantation | | | | | | | | |
| 60 | 2.2 | 2.7 | 9.3 | 30 | 13 | 6.6 | 4.9 | 59 |
| 60+15 | 1.8 | 3.3 | 8.4 | 14 | 10 | 5.3 | 5.0 | 38 |
| 60+30 | 2.7 | 4.6 | 38 | 18 | 19 | 15 | 7.3 | 89 |
| 60+60 | 1.8 | 3.5 | 36 | 11 | 11 | 8.2 | 5.3 | 67 |

**Table 60. Theoretical intakes of hormones resulting from the consumption of tissues of animals treated with REVALOR/IMPLIX**

| | | | Estradiol-17α | Estradiol-17β | | | | | Progesterone | | | | | Testosterone | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group | Day | Liver | Muscle | Liver | Kidney | Fat | TMDI | Muscle | Liver | Kidney | Fat | TMDI | Muscle | Liver | Kidney | Fat | TMDI |
| Male Animals | Control | 30 | 57 | 2.2 | 3.2 | 0.65 | 0.59 | 6.6 | 270 | 75 | 203 | 80 | 628 | | | | | |
| | | 80 | 69 | 6.1 | | 0.46 | 1.2 | 7.7 | 123 | 186 | 51 | 133 | 493 | | | | | |
| | Revalor only | 65 | 111 | 28 | 14 | 7.7 | 4.8 | 55 | | | | | | | | | | |
| | | 80 | 132 | 35 | 16 | 8.9 | 3 | 63 | | | | | | | | | | |
| | | 100 | 67 | 34 | 11 | 8.6 | 2.5 | 55 | | | | | | | | | | |
| | Implix BM/ Revalor | 15 | 95 | 11 | 15 | 4.3 | 4.6 | 35 | 182 | 60 | 50 | 270 | 562 | | | | | |
| | | 30 | 68 | 14 | 7.6 | 6.3 | 1.9 | 30 | 179 | 92 | 140 | 326 | 737 | | | | | |
| | | 50 | 82 | 18 | 19 | 6.7 | 4.8 | 49 | 232 | 77 | 70 | 433 | 812 | | | | | |
| | | 65 | 130 | 34 | 18 | 10 | 8.0 | 70 | 80 | 2025 | 79 | 777 | 2961 | | | | | |
| | | 80 | 128 | 32 | 21 | 13 | 6.1 | 72 | 145 | 63 | 61 | 347 | 616 | | | | | |
| | | 100 | 233 | 52 | 23 | 13 | 10 | 97 | 150 | 44 | 73 | 400 | 667 | | | | | |
| Female Animals | Control | 30 | 60 | 1.7 | 3.2 | 0.85 | 0.76 | 6.5 | | | | | | 1.83 | 11 | 4.8 | 1.11 | 19 |
| | | 80 | 59 | 4.4 | 2.3 | 0.93 | 0.94 | 8.6 | | | | | | 123 | 186 | 51 | 133 | 493 |
| | Revalor only | 65 | 88 | 25 | 8.0 | 7.2 | 0.81 | 41 | | | | | | | | | | |
| | | 80 | 111 | 30 | 15 | 9.9 | 6.5 | 61 | | | | | | | | | | |
| | Implix BM/ Revalor | 15 | 179 | 32 | 51 | 12 | 3.8 | 99 | | | | | | 108 | 20 | 29 | 51 | 208 |
| | | 30 | 70 | 27 | 7.3 | 8.5 | 8.6 | 51 | | | | | | 74 | 6.6 | 28 | 63 | 172 |
| | | 50 | 70 | 27 | 9.5 | 7.3 | 6.0 | 49 | | | | | | 68 | 7.1 | 26 | 38 | 138 |
| | | 65 | 172 | 47 | 27 | 8.2 | 7.0 | 89 | | | | | | 56 | 7.9 | 35 | 29 | 129 |
| | | 80 | 156 | 47 | 15 | 23 | 6.6 | 91 | | | | | | 54 | 7.2 | 32 | 36 | 129 |

## APPRAISAL

The results of the residue studies were statistically evaluated. The distributions of the residues were described by a number of characteristics including the mean, standard deviation, geometric mean and median. The assumption of normal distribution of the hormone concentrations could not be defended. Taking into consideration that tissue concentrations were sometimes below the limits of detection (LOD) or the limits of quantification (LOQ) of the methods, the median was the most stable and convenient parameter to provide an estimate of a central tendency of the data without excluding any individual result or making specific assumptions on substitute values for results like "below the limit of quantification". Appropriate median values (see below) were therefore also used as the basis for the calculation of theoretical daily intakes.

The objective of the intake calculations was to obtain conservative estimates of the theoretically possible excess dietary intakes of preferential eaters of meat that could be attributed to the approved uses of the products reviewed. The calculations were, therefore, performed in the following stepwise manner:

For every given time point of every study the median hormone concentrations found in tissues of control animals and of treated animals were multiplied with the respective daily consumption figures for "meat" conventionally used by JECFA (300 g muscle, 100g liver, 50 g kidney, and 50 g fat per person per day). The median value of a residue or contaminant in food is the appropriate value to be used if lifetime dietary exposure was to be assessed. However, the procedure used here, is not directly comparable to the ones used in the cases of other substances for which the Committee had set MRLs and were the MRLs are used as an estimate of the concentrations of a given substance in a food commodity. The present approach was followed because no numerical MRLs were established by the Committee.

- The results obtained in this way for muscle, liver, kidney and fat were summed up to calculate a figure for the total intake for 500 g of "meat".

- If data for several time points after implantation were available, the time points with the highest values were used. By doing this it was taken into consideration that no withdrawal period had been established for the use of any approved product. If the highest values did not coincide in time for all hormones, the time point with the highest results for estrogen intakes was selected. This was done in order to ensure a very conservative approach to the intake estimates of these compounds for which safety margins are somewhat lower than for the other two compounds. The effects of this selection on the estimates obtained for the other hormones were negligible. For the purposes of this report these figures are referred to as "Theoretical Maximum Daily Intakes"

- In order to estimate the defined excess intakes, the TMDI calculated for the concurrent untreated control population was subtracted from the figure obtained using the corresponding data of implanted animals.

Table 61 summarizes the final results of all relevant intake calculations. It also provides information on excess intakes of estrogens, the most relevant group of residues.

For total estrogens the highest excess intakes calculated in this way were in the order of 30-50 ng/person/day (see Table 61 Synovex in heifer in conjunction with comment 1 to the table and Finaplex in heifers in conjunction with comment 5 to the table). This range of excess intakes is less than 2% of the ADI for estradiol-17β established by the Committee for a 60 kg person. For certain experimental studies carried out with experimental combinations the resulting excess intakes were more than twice as high (about 4% of the ADI) if compared with the approved uses.

For progesterone the highest excess intake of the parent compound (which represents the only relevant hormonally active residue) was below 500 ng/person/day for the approved uses of this hormone (see Table 61, Synovex in calves). This excess intake corresponds to approximately 0.003% of the ADI established by the Committee for a 60 kg person.

For testosterone the highest excess intake (see Table 61, Synovex in heifers) for the free hormone was approximately 60 ng/person/day for all approved uses. This intake is approximately 0.2 % of the ADI established by the Committee for a 60kg person. The not precisely known intakes of possible relevant metabolites could theoretically be of the same order of magnitude.

The Committee noted that the hormone concentrations found in individual populations of treated animals - despite the fact that they typically were statistically significantly higher than the corresponding values of the concurrent controls - were well within the physiological range of these substances in bovine animals. In addition, the calculated excess

intakes contributed only a small additional hormonal burden to the background dietary intakes resulting from the consumption of other normal foods of both animal and plant origin.

Taking into consideration that the available data on the identity and concentrations of residues of the approved veterinary drugs in animal tissues indicate a wide margin of safety for consumption of residues in food when the products are used according to good practice in the use of veterinary drug, the Committee concluded that there would be no need to specify numerical MRLs for the three hormones and recommended "MRLs not specified" in bovine tissues. It is, however, recommended to keep the total intake of estrogenic residues resulting from the use of any approved hormonal product below the above calculated excess intakes.

**Table 61. Calculations of excess TMDI from bovine animals treated with estradiol-17β, progesterone and testosterone**

| Product | Animals | Comments | Description of the treatment of the animals | Theoretical Maximum Daily Intakes [nanograms/person/day] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $E_1$ | $E_2$-17α | $E_2$-17β | Excess $E_1$+$E_2$-β | P | T |
| Synovex-S ($E_2$-b+P) | Steers | 1 | **Control animals** | **1.0** | | **0.5** | | **190** | |
| | | | Animals slaughtered 15 days after implantation | 2.0 | | 6.3 | 6.8 | 254 | |
| Synovex H ($E_2$-b+T-p) | Heifers | 1 | **Control animals** | **1.4** | | **1.5** | | | **17** |
| | | | Animals slaughtered 15 days after implantation | 3.9 | | 15 | 16 | | 70 |
| Synovex-C ($E_2$-b+T) Synovex-H ($E_2$-b+T-p) | Calves a) female | 1 | **Control animals, slaughtered on day 61** | **1.1** | | **3.5** | | **22** | |
| | | | **Control animals, slaughtered on day 119** | **1.2** | | **2.2** | | **53** | |
| | | | **Control animals, slaughtered on day 240** | **0.8** | | **1.7** | | | **13** |
| | | | **Control animals, slaughtered on day 301** | **1.4** | | **4.4** | | | **22** |
| | | | **Control animals, slaughtered on day 329** | **0.7** | | **2.1** | | | **22** |
| | | | **Control animals, slaughtered on day 360** | **2.0** | | **3.0** | | | **51** |
| | | | implanted day 0; slaughtered on day119 | 3.0 | | 3.7 | 3.3 | 520 | |
| Synovex-C ($E_2$-b+T-p) Synovex S ($E_2$-b+P) | Calves b) castrated males | | **Control animals, slaughtered on day 61** | **0.8** | | **0.7** | | **501** | |
| | | | **Control animals, slaughtered on day 119** | **0.4** | | **0.5** | | **552** | |
| | | | **Control animals, slaughtered on day 240** | **1.1** | | **1.2** | | **669** | |
| | | | **Control animals, slaughtered on day 301** | **0.5** | | **0.9** | | **421** | |
| | | | **Control animals, slaughtered on day 329** | **1.2** | | **0.7** | | **536** | |
| | | | **Control animals, slaughtered on day 360** | **0.8** | | **1.0** | | **1170** | |
| | | | implanted on days 0, 118, 240; slaughtered on day 301 | 3.7 | | 11 | 13.3 | 540 | |
| Synovex H ($E_2$-b+T-p) | Pregnant heifers | 2 | **120 days pregnant , unsynchronized controls** | **93** | | **16** | | | **203** |
| | | | **120 days pregnant , synchronized controls** | **113** | | **16** | | | **172** |
| | | | 120 days pregnant , 61 days implanted | 34 | | 15 | -80 | | 233 |
| | | | **180 days pregnant , synchronized controls** | **280** | | **48** | | | **282** |
| | | | 180 days pregnant , 61 days implanted | 107 | | 24 | -197 | | 237 |
| | | | **240 days pregnant , synchronized controls** | **326** | | **139** | | | **377** |
| | | | 240 days pregnant , 61 days implanted | 377 | | 49 | -39 | | 326 |
| Steer-oid ($E_2$ + P) | Steers | 3 | **Control animals** | | | **21** | | **299** | |
| | | | Animals slaughtered 15 days after implantation | | | 25 | 4 | 375 | |
| Heifer-oid ($E_2$ +T-p) | Heifers | | **Control animals** | | | **16** | | | **43** |
| | | | Animals slaughtered 15 days after implantation | | | 18 | 2 | | 48 |
| Compudose ($E_2$) | Steers | 4 | **Control animals** | **4.4** | | **4.5** | | | |
| | | | Animals implanted 70-180 days | 7.4 | | 5.7 | 4.2 | | |

**Table 61. Calculations of excess TMDI from bovine animals treated with estradiol-17β, progesterone and testosterone**

| Product | Animals | Comments | Description of the treatment of the animals | Theoretical Maximum Daily Intakes [nanograms/person/day] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $E_1$ | $E_2$-17α | $E_2$-17β | Excess $E_1$+$E_2$-β | P | T |
| Synovex-S ($E_2$-b+P) | Steers | 1 | **Control animals** | **1.0** | | **0.5** | | **190** | |
| | | | Animals slaughtered 15 days after implantation | 2.0 | | 6.3 | 6.8 | 254 | |
| Synovex H ($E_2$-b+T-p) | Heifers | 1 | **Control animals** | **1.4** | | **1.5** | | | **17** |
| | | | Animals slaughtered 15 days after implantation | 3.9 | | 15 | 16 | | 70 |
| Synovex-C ($E_2$-b+T) Synovex-H ($E_2$-b+T-p) | Calves a) female | 1 | **Control animals, slaughtered on day 61** | **1.1** | | **3.5** | | **22** | |
| | | | **Control animals, slaughtered on day 119** | **1.2** | | **2.2** | | **53** | |
| | | | **Control animals, slaughtered on day 240** | **0.8** | | **1.7** | | | **13** |
| | | | **Control animals, slaughtered on day 301** | **1.4** | | **4.4** | | | **22** |
| | | | **Control animals, slaughtered on day 329** | **0.7** | | **2.1** | | | **22** |
| | | | **Control animals, slaughtered on day 360** | **2.0** | | **3.0** | | | **51** |
| | | | implanted day 0; slaughtered on day119 | 3.0 | | 3.7 | 3.3 | 520 | |
| Synovex-C ($E_2$-b+T-p) Synovex S ($E_2$-b+P) | Calves b) castrated males | | **Control animals, slaughtered on day 61** | **0.8** | | **0.7** | | **501** | |
| | | | **Control animals, slaughtered on day 119** | **0.4** | | **0.5** | | **552** | |
| | | | **Control animals, slaughtered on day 240** | **1.1** | | **1.2** | | **669** | |
| | | | **Control animals, slaughtered on day 301** | **0.5** | | **0.9** | | **421** | |
| | | | **Control animals, slaughtered on day 329** | **1.2** | | **0.7** | | **536** | |
| | | | **Control animals, slaughtered on day 360** | **0.8** | | **1.0** | | **1170** | |
| | | | implanted on days 0, 118, 240; slaughtered on day 301 | 3.7 | | 11 | 13.3 | 540 | |
| Synovex H ($E_2$-b+T-p) | Pregnant heifers | 2 | **120 days pregnant , unsynchronized controls** | **93** | | **16** | | | **203** |
| | | | **120 days pregnant , synchronized controls** | **113** | | **16** | | | **172** |
| | | | 120 days pregnant , 61 days implanted | 34 | | 15 | -80 | | 233 |
| | | | **180 days pregnant , synchronized controls** | **280** | | **48** | | | **282** |
| | | | 180 days pregnant , 61 days implanted | 107 | | 24 | -197 | | 237 |
| | | | **240 days pregnant , synchronized controls** | **326** | | **139** | | | **377** |
| | | | 240 days pregnant , 61 days implanted | 377 | | 49 | -39 | | 326 |
| Steer-oid ($E_2$ + P) | Steers | 3 | **Control animals** | | | **21** | | **299** | |
| | | | Animals slaughtered 15 days after implantation | | | 25 | 4 | 375 | |
| Heifer-oid ($E_2$ +T-p) | Heifers | | **Control animals** | | | **16** | | | **43** |
| | | | Animals slaughtered 15 days after implantation | | | 18 | 2 | | 48 |
| Compudose ($E_2$) | Steers | 4 | **Control animals** | **4.4** | | **4.5** | | | |
| | | | Animals implanted 70-180 days | 7.4 | | 5.7 | 4.2 | | |

**Table 62. Percent contributions of the TMDI in the four animal tissues**

| Substance implanted | Testosterone propionate | | Estradiol benzoate | | | | Progesterone | |
|---|---|---|---|---|---|---|---|---|
| Animals: | Heifers | | Heifers | | Steers | | Steers | |
| Fraction of total labeled residue | Free | Conjugated | Free | Conjugated | Free | Conjugated | Free | Conjugated |
| | Contribution (%) to the TMDI | | | | | | | |
| Muscle | 6.7 | 5.3 | 5.3 | 3.7 | 5.9 | 3.1 | 3.0 | 1 |
| Liver | 29.9 | 38.1 | 36.5 | 13.5 | 21.5 | 28.5 | 17.2 | 15.8 |
| Kidney | 3.8 | 4.2 | 9.9 | 18.1 | 9.9 | 18.1 | 26.5 | 22.5 |
| Fat | 11.0 | 1.0 | 12.2 | 0.8 | 11.6 | 1.4 | 13.2 | 0.8 |
| **Total** | **51.4** | **48.6** | **63.9** | **36.1** | **48.9** | **51.1** | **59.9** | **40.1** |

2 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. A correction of the data is not possible due to a lack of relevant information. However, in view of the well established significant reduction in the TMDI as a consequence of implantation of pregnant heifers such a correction is apparently unnecessary.

3 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle and fat. The given figures most likely greatly underestimate the "true" TMDI 's. No information was available to correct these estimates.

4 The method used includes the extraction and de-conjugation of conjugates. The estimated intake figures, therefore, represent total parent compound and can be used as they are given in the table.

5 The free and conjugated fractions of estradiol-17β were determined in all tissues. However, estrone (free fraction only) was only determined in liver and fat. The "true" TMDI 's for estrogens, therefore, could well be 50% higher than the values given in the table. Data on which a more precise estimate of a correction factor could be based were not available. The Torelor study represents research work and does not reflect an approved use.

6 From the method description it appears that conjugates are not included in the determination of the residues. In view of the effects of trenbolone/estradiol combinations on estrogen concentrations seen in other studies, it cannot be excluded that the data given in the table significantly underestimate the "true" TMDI for estrogens.

7 No description of the analytical method was given in the report. The values for estradiol-17α are based on concentrations found in liver only. The study represents research work and does not reflect an approved use.

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# NEOMYCIN

**First draft prepared by**

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**Addendum**
**to the Neomycin residue monographs prepared by the 43rd meeting and the 47th meeting of the Committee published in the**
**FAO Food and Nutrition Paper 41/7, Rome 1995 and 41/9, Rome 1997, respectively**

At the 43rd meeting of the Committee, a temporary ADI of 0 - 30 µg per kg of body weight was recommended for neomycin. The Committee evaluated both toxicological and microbiological data and concluded that the toxicological data provided the most appropriate end-point. A safety factor of 200 was used to compensate for the lack of genotoxicity data. Temporary MRLs of 5000 µg/kg for kidney and 500 µg/kg for muscle, liver, and fat, expressed as the parent drug, were recommended for cattle, sheep, goats, pigs, turkeys, ducks and chickens. The temporary MRLs recommended for chicken eggs and cow's milk were 500 µg/kg and 500 µg/L, respectively, also expressed as the parent drug.

After evaluating new genotoxicity data, the 47th meeting of the Committee recommended an ADI of 0 - 60 µg/kg BW using a safety factor of 100. Although no new residue depletion studies were submitted, the Committee concluded that the MRL for kidney should be doubled (to 10000 µg/kg) in order to permit practical withdrawal times to be established for all target animal species. The Committee had information indicating that adsorption of neomycin in calves that are just a few days old is higher than in older calves. The neomycin residue levels in the kidneys of these younger calves require approximately thirty days to deplete below 10,000 µg/kg.

## RESIDUES IN FOOD AND THEIR EVALUATION

The present Committee evaluated two new residue depletion studies. Previous studies assessed tissue residue depletion following oral dosing. The first of the two new studies compared tissue residues after oral dosing at 10 mg/kg, twice a day for 5 days and intramuscular dosing at 12 mg/kg once a day for 5 days. This study used only four animals per treatment group and one sacrifice time. Results provide a temporal comparison of the residue levels of neomycin in edible tissues resulting from an injectable and an oral route of administration. The second study assessed tissue residue depletion after intramuscular dosing at 12 mg/kg daily for 5 days. This study utilized 4 animals per slaughter point and met contemporary standards for a residue depletion study. The two studies also used a new HPLC method with fluorimetric detection that had a limit of quantification 5-fold lower than the microbiological method used in many of the earlier studies.

## TISSUE RESIDUE DEPLETION STUDIES

### Cattle

Two groups (2 male and 2 female each) of 3 week old, non-ruminating calves were dosed with neomycin sulfate. Calves in group 1 were given 12 mg neomycin/kg by intramuscular injection for 5 consecutive days (Birckel, 1999a). Those in group 2 were given neomycin,10 mg/kg, orally, twice a day for 5 days. Blood was collected at day 3 (pre-dose) and day 5 (post-dose). The animals were slaughtered 8 days after the first treatment, i.e., 4 days after the last treatment. Tissue samples (muscle, liver, kidney and fat) were collected at slaughter.

Concentrations of neomycin were determined in plasma and tissues by HPLC using fluorimetric detection. For plasma, the assay method was validated in terms of specificity, extraction recovery and linearity. The limit of quantification was 100 µg/l. In each tissue, the method was validated in terms of specificity, extraction recovery, linearity, precision and accuracy. A limit of quantification of 100 µg/kg was achieved for muscle, liver, kidney and fat.

Neomycin was not detected in plasma sampled at day 3, pretreatment. At day 5 (one day after the last treatment), neomycin was detected in plasma samples from all animals (range; 530 to 49 μg/L). Neomycin was detected in kidney, liver, fat and muscle from calves in Group 1 and in kidney and liver of those in Group 2. Residues of neomycin were highest in kidney, followed by liver, fat and muscle. Neomycin concentrations found in all tissues during this study are presented in Table 1. The residue concentrations in the edible tissues from calves in Group 2 are indicative of the relatively low absorption of orally administered neomycin. The injectable formulation was a four-way combination of neomycin (12 g/100 ml), procaine benzylpenicillin (21 g/100 ml), methylprednisolone (0.4 g/ 100 ml) and procaine hydrochloride (3 g/100 ml).

**Table 1. Concentration (μg/kg) of neomycin in tissues of individual calves administered 12 mg/kg BW of neomycin by injection (Group 1) and 10 mg/kg BW orally (Group 2).**

| Group | Day after the last treatment | Fat | Muscle | Liver | Kidney |
|---|---|---|---|---|---|
| 1 | 4 | 546 | 207 | 13100 | 145000 |
| | | 830 | 214 | 9880 | 106000 |
| | | 956 | 277 | 11800 | 109000 |
| | | 987 | 200 | 9020 | 132000 |
| 2 | 4 | <LOQ | <LOQ | 380 | 25100 |
| | | <LOQ | <LOQ | 141 | 7730 |
| | | <LOQ | <LOQ | 363 | 18700 |
| | | <LOQ | <LOQ | 361 | 17000 |

<LOQ = below the limit of quantification (100 μg/kg).

Tissue residue depletion of neomycin was assessed in 24 (12 males and 12 females) ruminating beef cattle, approximately 6 months old, following intramuscular injection of 12 mg/kg BW of neomycin for 5 consecutive days (Birckel, 1999b). The injection formulation was the same as that used in the previous study. Calves were randomly allocated to 6 groups of 4, two males and two females (Group 2 to 7). Two additional calves, 1 male and 1 female, (Group 1) were identified as untreated controls.

Blood was collected immediately prior to dosing and at day 5, post-dose. The calves (4 per slaughter time) were slaughtered 7, 14, 21, 30, 45 and 60 days after the last injection. Calves from group 1 (untreated controls) were slaughtered 7 days after the first injection. Tissues (muscle, liver, kidney, fat and the injection site of the last injection) were collected at each slaughter time.

Concentrations of neomycin were determined in plasma and tissues by HPLC with fluorimetric detection, as in the previous study. In this study, concentrations of benzylpenicillin and methylprednisolone were also determined in plasma and tissues by HPLC with UV detection. The limit of quantification (LOQ) of benzylpenicillin in tissues and plasma was 25 μg/L and the LOQ for methylprednisolone was 10 μg/L.

Benzylpenicillin was detected only in animals from Group 2, slaughtered 7 days after the fifth treatment. Residues were highest in injection site, followed by liver, kidney and fat. Benzylpenicillin was not detected in muscle. Methylprednisolone was detected only in the injection site in animals from Groups 2 and 3, slaughtered 7 and 14 days after the fifth treatment. Neomycin was detected in all tissues (kidney, liver, fat, muscle and injection site). Residues of neomycin were highest in kidney followed by liver, injection site, fat and muscle. Neomycin concentrations in the four tissues and the injection site are presented in Table 2. Mean residue concentrations are presented in Table 3.

**Table 2. Concentrations (µg/kg) of neomycin in tissues of individual calves administered 12 mg/kg of neomycin by intramuscular injection for 5 consecutive days.**

| Days after last treatment | Group | Fat | Muscle | Injection Site* | Liver | Kidney |
|---|---|---|---|---|---|---|
| | | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 7 | 1 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | | 323 | 109 | 791 | 4900 | 39300 |
| | 2 | 468 | 126 | 1920 | 8770 | 45200 |
| | | 615 | 169 | 2060 | 5540 | 71400 |
| | | 663 | 144 | 871 | 7290 | 40800 |
| | | 348 | <LOQ | 432 | 11700 | 25300 |
| 14 | 3 | 500 | <LOQ | 188 | 25400 | 29000 |
| | | 208 | <LOQ | 358 | 9710 | 22000 |
| | | 270 | <LOQ | 146 | 13400 | 19500 |
| | | 189 | <LOQ | 584 | 6650 | 21900 |
| 21 | 4 | 302 | <LOQ | 669 | 11700 | 16300 |
| | | 128 | <LOQ | 822 | 7880 | 26200 |
| | | 147 | <LOQ | 365 | 6990 | 18200 |
| | | <LOQ | <LOQ | 479 | 8180 | 17000 |
| 30 | 5 | 154 | <LOQ | 225 | 11000 | 10600 |
| | | <LOQ | <LOQ | 285 | 6360 | 13800 |
| | | <LOQ | <LOQ | 414 | 2330 | 14800 |
| | | <LOQ | <LOQ | 370 | 5050 | 7900 |
| 45 | 6 | 161 | - | 180 | 5390 | 9220 |
| | | <LOQ | - | 388 | 5740 | 13700 |
| | | <LOQ | - | 401 | 9360 | 8790 |
| | | <LOQ | - | 264 | 3190 | 5510 |
| 60 | 7 | <LOQ | - | <LOQ | 1760 | 3770 |
| | | <LOQ | - | <LOQ | 5840 | 4810 |
| | | <LOQ | - | 360 | 1140 | 3860 |

<LOQ = below the limit of quantification (100 µg/kg).

**Table 3. Mean concentrations (μg/kg) of neomycin residues treated by intramuscular injection for 5 consecutive days.**

| Days after the last treatment | Fat | Muscle | Injection Site | Liver | Kidney |
|---|---|---|---|---|---|
| Control | BLQ | BLQ | BLQ | BLQ | BLQ |
| 7 | 517 | 137 | 1410 | 6620 | 49200 |
| 14 | 332 | BLQ | 381 | 1510 | 24000 |
| 21 | 192 | BLQ | 610 | 8320 | 20700 |
| 30 | 114 | BLQ | 351 | 6960 | 14100 |
| 45 | 115 | BLQ | 335 | 6380 | 9920 |
| 60 | BLQ | BLQ | 206 | 2980 | 4500 |

BLQ: below the limit of quantification (100 μg/kg). Except for the control group, when an individual animal value was BLQ, 100 μg/kg was used in calculating the mean.

## METHODS OF ANALYSIS

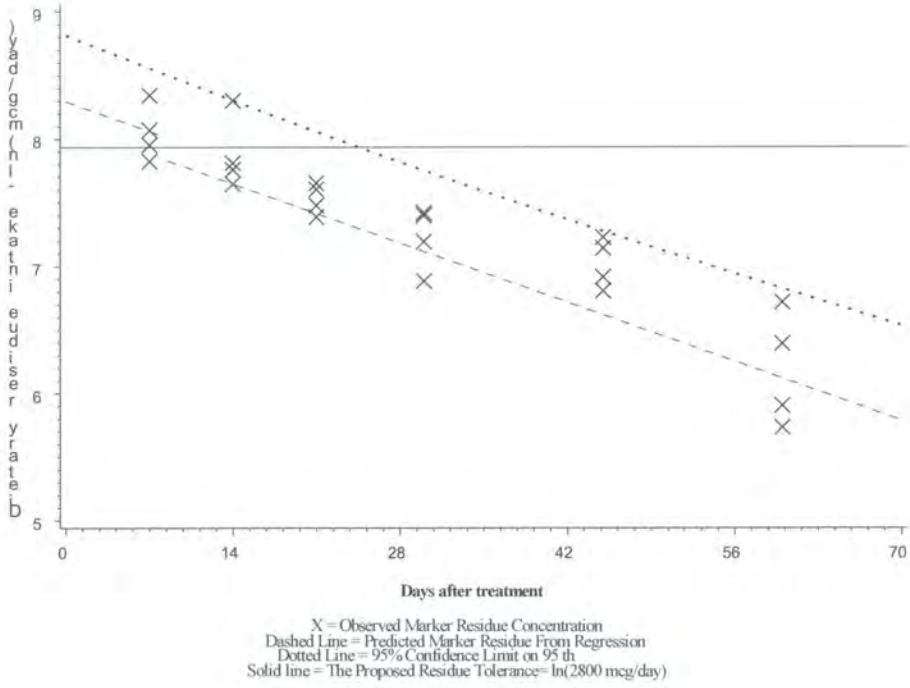
The HPLC method with fluorimetric detection used to determine the concentration of neomycin in tissues consists of four stages: homogenisation, precipitation of proteins with 5% trichloracetic acid, clean-up on ion-exchange column using CM-Sephadex and post-column derivatisation (Decolin and Nicolas, 1998). The absolute recoveries for muscle, liver, kidney and fat were 66%, 57%, 59% and 72%. The concentrations in Tables 1 and 2 were corrected for recoveries by the use of a standard curve prepared in the respective tissue matrix.

## APPRAISAL

An ADI of 0 – 60 μg/kg is equivalent to 3600 μg/day. The previously recommended MRLs for eggs and milk of 500 μg/kg and 500 μg/l would use up 800 μg of the acceptable daily intake, leaving up to 2800 μg to be distributed between the four edible tissues. Using the residue depletion data in Table 2, the dietary residue intake (DRI) can be calculated for each of the 24 animals by summing the individual tissues for that animal according to the market basket approach. Figure 1 demonstrates the relationship between the natural log of the dietary residue intake and the days after treatment. The intercept of the 95% Confidence Limit on the 95$^{th}$ Percentile (the 95/95 residue limit) and the natural log of 2800 μg is 30 days. A similar plot of the 95/99 residue limit intercepts the natural log of 2800 μg at 38 days. At a withdrawal period of 30 - 38 days, only the residue concentrations in kidney and liver contribute significantly to the daily residue intake. Therefore, the residue concentrations in liver and kidney at 30 - 38 days withdrawal can be used to estimate MRLs that would be necessary to permit the use of an injectable formulation of neomycin. The MRLs for liver and kidney also can be estimated to be 15,000 and 20,000 μg/kg, respectively, by using the mean concentrations ± three standard deviations for either the 30 or 45 day sacrifice times

SYMBOL

**Figure 1.** **Depletion of the dietary residue intake (DRI) of neomycin from cattle administered 12 mg neomycin/kg by intramuscular injection.**




## Maximum Residue Limits

The Committee recommends MRLs for sheep, goat, pig, turkey, duck and chicken of 0.5 mg/kg in muscle, liver, kidney, fat and eggs. Using the upper 99% confidence limits for cattle, the Committee recommends MRLs of 0.5 mg/kg in muscle, fat and cattle milk; for liver, 15 mg/kg and kidney, 20 mg/kg, espressed as equivalents of parent drug.

Using the MRL values for cattle and 0.5 mg/kg for eggs, the calculated theoretical maximum daily intake of neomycin residues is 3475 µg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.5 kg of milk (Annex 1, reference yy).

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# PHOXIM

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## INTRODUCTION

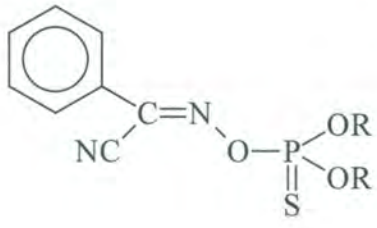
Phoxim has not previously been reviewed by the Committee, but was previously reviewed by the Joint Meeting on Pesticide Residues (JMPR), which established an ADI and recommended MRLs based on horticultural use. As the sponsor has indicated that it does not intend to continue to support horticultural use of phoxim, the JMPR has recommended that the previously established MRLs for such use should no longer be supported. The use of phoxim as a veterinary product has therefore been referred to the Committee, which received data for such use for number of food animal species, including cattle, pigs, sheep, goats and rabbits. Most of the studies provided were not conducted according to current GLP standards.

## IDENTITY

**Chemical name:** Diethyl O-(α-cyanobenzylideneamino)thiophosphate (IUPAC)
4-Ethoxy-7-phenyl-3, 5-dioxa-6-aza-4-phosphaoct-6-ene-8-nitrile 4-sulfide;
phenylglyoxylonitrile oxime O,O-diethyl phosphorothioate (CAS)

**Synonyms:** PHOXIM Baythion, Bay 5621, Bay 77488, Sebacil, Volaton, SRA 7502

**Structural formula:**




$R = CH_2CH_3$ phoxim
$R = CH_3$ phoxim methyl

**CAS number:** 14816-18-3

**Molecular formula:** $C_{12}H_{15}N_2O_3PS$

**Molecular weight:** 298.3

## OTHER INFORMATION ON IDENTITY AND PROPERTIES

**Pure active ingredient:** Phoxim

**Appearance:** Pale yellow oil.

**Boiling point:** 102°C ($bp_{0.01}$).

**Refractive Index:** 1.5405 ($n^{20}_{D}$).

**Solubility:** Freely soluble in alcohols, ketones, aromatic hydrocarbons and chlorinated aliphatic hydrocarbons; slightly soluble aliphatic hydrocarbons, vegetable oils and mineral oils; nearly insoluble in water.

**Optical rotation:** Optically inactive

**Ultraviolet maxima:** Not reported.

## RESIDUES IN FOOD AND THEIR EVALUATION

### Conditions of Use

General

Phoxim is an ectoparasiticide of the organophosphate group used for the control of Psoroptes-, Sarcoptes- and Chorioptes mites, biting and sucking lice, sheep keds, flies, ticks and fly maggots in wounds. Species for which registrations have been granted include cattle, pigs, sheep, goats and horses, but some countries have excluded the use of phoxim in lactating animals. Phoxim is sold as a 50% (w/v) formulation for dilutions as a wash, spray or dip and as a 7.5% (w/v) pour-on formulation.

Dosage

As a wash or spray treatment, phoxim is typically applied either as a single treatment or in two treatments at a 7-day interval. The formulated product (50 g phoxim/100 mL) is diluted, using 10 mL of per 10 L of water, and sufficient liquid is utilised to ensure that the total body of the animal has been moistened. The concentration of phoxim may be doubled for treatment of severe mange infestation. When used as a plunge dip, typically 1 L of the formulated product is diluted in 1000 L of water. A pour-on formulation (7.5 g phoxim/100 mL) is also available for treatment of pigs for mange or lice infestation and is applied at a recommended dose of 0.4 mL of formulated product per kg body weight.

## PHARMACOKINETICS AND METABOLISM

### Metabolism in Laboratory Animals

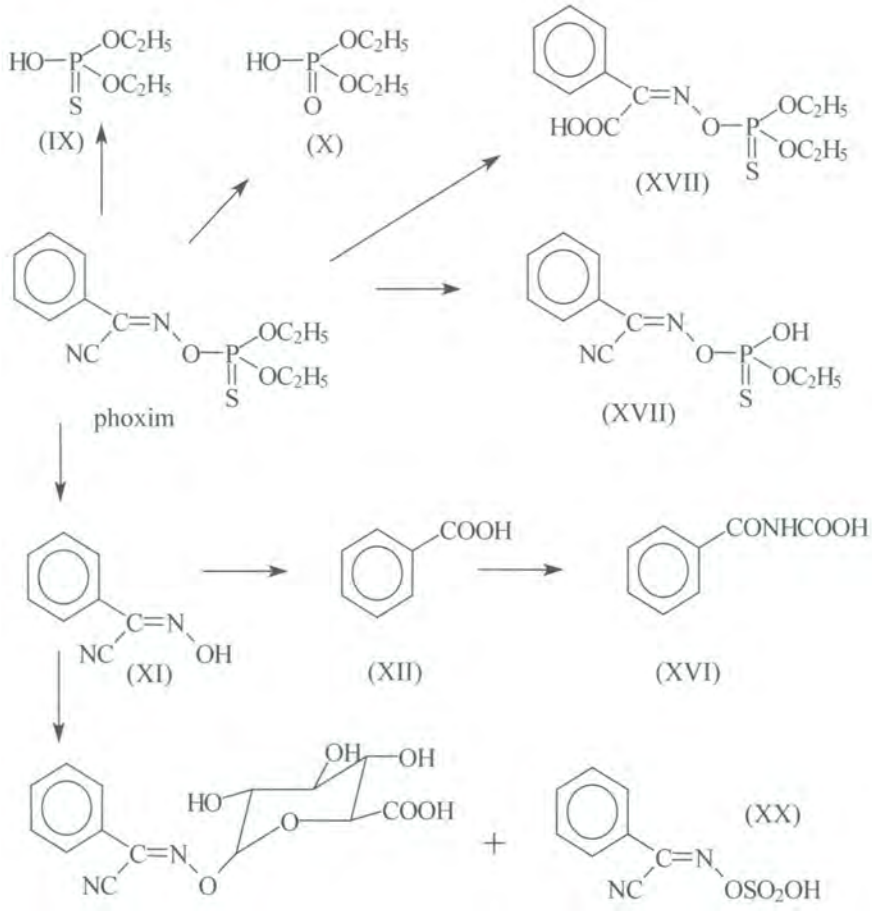
General

The proposed phoxim metabolic pathways are presented in Figure 1. Phoxim metabolism has been summarised in mice and rats for studies in which the compound was administered with two different radiolabels in its structure (Ecker, 1982). The two labels used were $^{14}C$, incorporated into the benzene ring, and side chain $^{32}P$-labelled phoxim. In a study with rats, 3 individual administration groups were used consisting of 5 rats each. A total of 93.6 ±5.1% of the $^{14}C$ labelled phoxim was excreted during the first 24 hours after oral administration of a single dose at 10 mg/kg body weight to male rats. The amount excreted in female rats after equal dose was slightly lower (85.6 ±7.5%). At 10 days post administration, the excretion was practically complete when over 90% of the excreted $^{14}C$ label was found in the urine, with the remainder in faeces. The total amount of $^{14}C$-labelled phoxim excreted (89.9 ±3.9%) during 10 days after a dose of 1 mg/kg BW was slightly lower compared to the higher dose. No data were available on the metabolite

profile in rat urine. The urinary metabolite profile in white mice was investigated 24 hours after administration of $^{32}$P-phoxim at 114 mg/kg BW and 30 hours after administration 955 mg/kg BW (Vinopal and Fukota, 1971). Practically all of the radioactivity excreted could be related to metabolites X, XVII, IX and XVIII (Figure 1) of which 85% was diethyl phosphoric acid (X) and O,O-diethyl thiophosphoric acid (IX), after the lower dose and 87% was diethyl phosphoric acid (X), "phoxim carbonic acid" and O,O-diethyl thiophosphoric acid (IX) after the higher dose.

In another study, containing both long-term and short-term exposures, [$^{14}$C, $^{3}$H]- doubly labelled phoxim methyl was given orally to rats at 1 mg/kg body weight (Puhl, 1976). The compound was labelled with $^{14}$C in the benzene ring and with $^{3}$H in the O-methyl group in the long-term study. Only $^{14}$C-labelled phoxim was used for the short-term (tissue) study. In the long-term study, 2 male and 2 female Sprague-Dawley albino rats were used, with an average weight of 245g. The animals were kept in metabolic cages, allowing collection of urine, faeces and expired air. Urine and faeces samples were collected at 4, 8, 12, and 24 hours and at subsequent 24 hourly intervals until the sacrifice of the rats. The air traps were sampled and changed at 24-hour intervals. In the short-term study 12 male and 12 female rats were used. Two rats of each sex were sacrificed at 1, 2, 4, 8, 12, and 24 h after administration of the compound. Urine, faeces, tissue, blood, and plasma samples were obtained at the time of sacrifice. Quantitative radiocarbon analysis was performed by use of liquid scintillation counting. Thin layer chromatography was used for separations of the metabolites and the bands identified by autoradiography. The bands were then subjected to liquid scintillation counting. The results showed that the majority of urinary metabolites were polar compounds based on the TLC characteristics and poor extractability into chloroform. Dimethylphosphate, dimethylphosphorothioate and an unknown third metabolite accounted for practically all excreted $^{3}$H radioactivity. Over 90% of the $^{14}$C-labelled metabolites were recovered in the urine. Metabolites designated as "B" and "E" appeared to be the major metabolites but these metabolites could not be identified. It was suggested that the metabolite "E" is not a pure compound but a mixture of which one component being a conjugate of phenylglyoxylnitrile oxime, one of the major metabolites found in liver.

**Figure 1. Metabolic pathways of phoxim**



**Metabolism in Food Animals**

Cattle

In a non-GLP study, a single lactating dairy cow was treated with ring-labelled $^{14}C$-phoxim methyl by oral administration of a capsule containing 0.2 mg/kg BW of phoxim (Puhl, 1976). The metabolism and excretion were studied after a single and after five consecutive daily oral administrations of the compound to the cow. Urine, milk, faeces and blood samples were collected after the single and multiple dose administrations. Phoxim methyl was rapidly absorbed and the radioactivity was excreted mainly in urine. The phoxim methyl was mainly converted to hippuric acid, indicating a complex metabolic pathway. The other minor metabolites were not identified. Amounts of radioactivity recovered in milk indicated rapid depletion of residues over several milkings, following the single treatment. The cow was sacrificed 5 hrs after the last administration and tissue samples were obtained. Combustion analysis of tissue samples indicated residues of phoxim methyl as follows: kidney, 0.62 mg/kg; liver, 0.38 mg/kg; muscle, 0.03 mg/kg; fat, 0.02 mg/kg.

Pigs

In the report by Klein and Weber (1988), biotransformation of $^{14}C$-phenyl-labelled phoxim was described in pigs in a GLP-study. Two animals were administered a single oral dose at 5 mg/kg. The animals were sacrificed at 24 and 72 h after administration. An additional untreated animal was sacrificed before the study in order to determine the background radioactivity of the tissues and to study the extraction procedures. The $^{14}C$-phenyl-labelled phoxim was administered to the 2 animals together with unlabelled drug in a ratio of 1.5:1. The animals received approximately 2 mCi in a dose of 5 mg $^{14}C$-labelled phoxim/kg BW.

A urine sample was collected at 8 h and urine and faecal samples were collected at 24, 48, and 72 h following administration of $^{14}C$-phenyl- phoxim. Venous blood samples were collected from one pig at 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 56, and 72 h and the other at 0.5, 1, 2, 3, 8, and 24 h after administration of the compound. Liver, kidney, muscle, loin, subcutaneous fat and blood were removed at sacrifice and stored frozen pending analysis.

As in laboratory animals and in the cow, phoxim methyl was rapidly absorbed in the pig and the majority of the radioactivity was excreted in urine. The faecal excretion appeared slightly higher at later time points but the minimal number of test animals do not allow any further conclusions concerning routes of excretion. The major metabolite in urine (almost 90%) was identified as cyanobenzaldoxime, which was excreted as its glucuronic acid conjugate. The fraction of total administered radioactivity found in the tissue was much below 1%. At 24 h, total phoxim residues in edible tissues were: liver, 0.60 mg/kg; kidney, 0.35 mg/kg; muscle, 0.05 mg/kg; fat, 1.32 mg/kg. At 72 hrs, total phoxim residues were: liver, 0.37 mg/kg; kidney, 0.12 mg/kg; muscle, 0.02 mg/kg; and fat, 0.68 mg/kg.

The pharmacokinetic analysis showed rapid absorption of the radioactivity. This is apparently consistent with rapid absorption of phoxim. However, the other pharmacokinetic parameters, such as total clearance, renal clearance and mean residence time (MRT) were obviously determined for the total radioactivity and not specifically for the parent compound and do not provide any information on the pharmacokinetics of phoxim methyl. The pharmacokinetic analysis presented may be representative for any combination of metabolites. Therefore, it is very difficult to conclude that the pharmacokinetic model of best fit would be a two-compartment model because the two phases observed may relate to two different compounds. Furthermore, determination of a parameter such as clearance requires knowledge of bioavailability, which was not determined in the present report.

## TISSUE RESIDUE DEPLETION STUDIES

**Radio-labelled Residue Depletion Studies**

No studies were reported in which target animals were treated using a radio-labelled phoxim formulation typical of those used in current veterinary applications.

**Other Residue Depletion Studies (with Unlabelled Drug)**

General Comment

The results reported in the studies discussed in this section were not corrected for analytical recovery.

Cattle

Four yearling cattle (250 to 370 kg BW) were sprayed with a formulation containing 1000 mg/L phoxim, with the treatment repeated at 8 days (Terblanche, 1978a). Insufficient data were provided in the report to calculate an approximate dose rate in mg/kg. The animals were slaughtered in pairs at 14 and 28 days following the second treatment and samples of liver, kidney, muscle and fat were collected for analysis. Samples were also collected from an untreated animal which served as a control. Analysis was by gas chromatography with a nitrogen-phosphorus detector, following extraction and clean-up of homogenised tissue. Analytical recoveries were approximately 92 - 104% and a limit of detection of 0.01 mg/kg. No details on the extraction, clean-up or conditions of chromatographic analysis were provided. Fat samples from the animals slaughtered at 14 days contained residues of 0.32 and 0.37 mg/kg, respectively, while residues in fat from both animals slaughtered at 28 days were 0.02 mg/kg. Residues in all other tissues were below the limit of detection. The study was not conducted to GLP.

Lactating Cattle

Three dairy cows each received two treatments 8 days apart with a spray formulation containing 1000 mg/L phoxim (Terblanche, 1978b). Milk samples were collected at 12 h and 1, 3, 7 and 14 days following the second treatment and were analysed by gas chromatography with a nitrogen-specific detector. Analytical recoveries from fortified controls were 90 - 95% at 200 µg/kg and the limit of detection was 2 µg/kg. Residues found were as follows: 12 h, 220-420 µg/kg; day 1, 120-200 µg/kg; day 3, 8-15 µg/kg; day 7, <2 µg/kg; day 14, <2 µg/kg.

In a subsequent study, three lactating cows were each sprayed twice at an 8 day interval with a dipwash formulation containing 500 mg/L phoxim (Terblanche, 1979a). Milk samples collected at 12 h, 1, 2 and 3 days following the second treatment were analysed as in the previous study. Residues found were as follows: 12 h, 80-210 µg/kg; day 1, 22-55 µg/kg; day 2, 6-20 µg/kg; day 3, 4-10 µg/kg.

A third study was reported in which 5 cows each received a single treatment with a spray containing 500 mg/L phoxim (FAO, 1985). Milk samples were collected at morning and evening milkings for 13 days following treatment. Subsequently, the same cows received two treatments with the same formulation at an interval of 6 days, after which milk samples were collected for 5 days following the second treatment. Following the initial treatment, residues reported in milk samples were as follows: 0.5 day, 40-80 µg/L; day 1, 10-20 µg/L; day 1.5, 8-10 µg/L; day 2, not detected to 2 µg/L; subsequent milkings, not detected. Residues in samples collected following the two treatments separated by 6 days were as follows: 0.5 day, 2-4 µg/L; day 1, 2-16 µg/L; day 1.5, 2-5 µg/L; day 2, 1-2 µg/L; day 2.5, 1-5 µg/L; day 3, not detected at 1 µg/L; subsequent samples, not detected. No explanation was provided for the discrepancy in residue levels observed between the single treatment and two treatment experiments.

None of the above studies were conducted to current GLP standards.

Pig

Twenty-two pigs (average weight 40 kg) were treated in a GLP study with phoxim as a pour-on formulation at a dose corresponding to 30 mg/kg BW, with a second treatment 14 days following the initial application (Greife *et al*, 1991). Groups of 4 animals were slaughtered at 7, 14, 21, 28 and 35 days following the second treatment. Samples of muscle (semimembranosus), belly fat, liver and kidney were collected, as well as muscle, skin and fat from the application area (M. longissimus dorsi). After extraction and partitioning using acetonitrile and hexane, extracts were cleaned up on silica gel and analysed by liquid chromatography with UV detection. Additional details on the methodology are provided in the discussion in the Methods Section (see Heukamp, 1991). No residues of phoxim were detected in muscle from the application area (from days 7-35) or from other portions of the carcass (from days 7-21), nor were residues found in liver or kidney samples at day 7. Samples of these tissues for the remaining slaughter dates were not tested. Residues were found in skin and fat samples and these are summarised in Table 1.

**Table 1. Phoxim residues found in skin and fat from pigs administered two treatments of phoxim at a 14-day interval at an estimated dose rate of 30 mg/kg BW.**

| Withdrawal time (days after 2nd application of phoxim) | Phoxim Residues (mg/kg) | | |
|---|---|---|---|
| 7 | 0.492 ± 0.083 | 0.511 ± 0.136 | 0.346 ± 0.030 |
| 14 | 0.218 ± 0.072 | 0.222 ± 0.095 | 0.107 ± 0.028 |
| 21 | 0.135 ± 0.095 | 0.108 ± 0.093 | 0.049 ± 0.034 |
| 28 | 0.046 ± 0.023* | 0.034 ± 0.008 | 0.023 ± 0.011 |
| 35 | 0.023 ± 0.008 | n.d. - 0.033 | n.d. - 0.012 |

* Mean calculated for only 3 animals; a result of 0.441 mg/kg in fat from one animal was rejected as an outlier. Other samples from this animal were consistent with results for other animals and were not excluded. n.d. = not detected

Nine pigs received a pour-on application of phoxim applied along the dorsal midline at a dose of 30 mg/kg BW, repeated after 14 days. The pigs were slaughtered in groups of 3 animals at 7, 14 and 28 days following the second application (Gyr, 1985). An untreated pig was used as a control. No detectable residues were present in any liver, kidney or muscle samples at the detection limit of 0.01 mg/kg. In fat samples, residues were as follows: day 7, 0.3-1.1 mg/kg; day 14, 0.05-0.10 mg/kg; day 28, 0.06-0.09 mg/kg. The study was not conducted to current GLP standards.

In an earlier study, 4 pigs were sprayed with a solution containing 1000 mg/L of phoxim, with a repeat application after 8 days (Terblanche, 1978c). The pigs were slaughtered in pairs at 14 and 28 days after the second application. Liver, kidney, muscle and fat were collected for analysis from the 4 treated pigs and from an untreated control. Following extraction and clean-up, samples extracts were analysed by gas chromatography using a nitrogen-phosphorus detector. Recoveries were 78-82% for phoxim from fortified liver, kidney and muscle and 100% for fat, with a limit of detection of 0.01 mg/kg for phoxim in all tissues. No residues were detectable in any liver, kidney or muscle samples. In pigs slaughtered 14 days following the second treatment, phoxim residues in fat were 0.04 and 0.05 mg/kg, respectively, while residues in fat from the two pigs slaughtered 28 days after the second treatment were <0.01 mg/kg and 0.13 mg/kg, respectively.

Subsequently, an experiment was conducted in which 6 pigs received a spray application of 500 mg/L phoxim, repeated after 1 week (SABS, 1980). Pigs were slaughtered in groups of 3 animals at 7 and 14 days following the second application, with fat collected for analysis. Following Soxhlet extraction and clean-up, samples were analysed by gas chromatography with a nitrogen-phosphorus detector. No phoxim residues were found in excess of the limit of detection of 0.05 mg/kg. Neither of these two studies was conducted according to current GLP standards.

Sheep

In a non-GLP study, nine sheep were sprayed twice at an interval of 8 days with a solution containing 1000 mg/kg phoxim, with groups of 3 sheep each slaughtered at 7, 14 and 21 days following the second treatment (Terblanche, 1978d). Liver, kidney, muscle and fat samples from these sheep, plus from an untreated control sheep, were analyzed following extraction and clean-up by gas chromatography using a nitrogen-phosphorus detector. The method had a claimed limit of detection of 0.01 mg/kg and recoveries using fortified tissues were 92% for 1 mg/kg phoxim in fat and, at a fortification level of 0.50 mg/kg, were 88%, 100% and 88% in muscle, liver and kidney, respectively. Residues found in tissues from the treated animals are summarised in Table 2.

**Table 2. Residues in edible tissues of sheep following two spray treatments 8 days apart with a solution containing 1000 mg/L phoxim (3 animals per treatment group).**

| Withdrawal period (days) | Phoxim residues (mg/kg) | | | |
|---|---|---|---|---|
| | fat | muscle | Kidney | liver |
| 7 | 2.40 - 2.75 | 0.03 - 0.07 | <0.01 - 0.07 | <0.01 |
| 14 | 0.74 - 1.45 | <0.01 - 0.03 | <0.01 - 0.03 | <0.01 |
| 21 | 0.11 - 0.62 | 0.02 - 0.04 | <0.01 | <0.01 |

Nine sheep were divided into groups of 3 each, with two groups (groups 1 and 2) receiving two spray treatments at an interval of 8 days with 500 mg/L phoxim , while group 3 received phoxim at 1000 mg/L in two spray treatments also 8 days apart (Terblanche, 1979b). Groups 1 and 2 were slaughtered at 14 and 21 days, respectively, after the second treatment, while group 3 was also slaughtered at 21 days after the second treatment. Samples of liver, kidney, muscle and fat were analyzed for phoxim residues, following extraction and clean-up, by gas chromatography using a nitrogen-phosphorus detector (SABS, 1979). The method had a claimed limit of detection of 0.01 mg/kg, with analytical recoveries from fortified tissues reported as follows: fat, 105%; muscle, 88%; liver, 65%; kidney, 75%. No residues were detected in tissues from a sheep used as a control, or in muscle, kidney or liver from the treated sheep. Residues in fat samples from sheep in the three treatment groups were as follows: Group 1, 0.17-0.66 mg/kg; Group 2, 0.03-0.17 mg/kg; Group 3, 0.20-0.52 mg/kg. The study was not conducted to current GLP standards.

In another non-GLP study, 18 sheep were divided into groups of 6 animals which received a plunge-dip treatment with phoxim for 1 minute in a tank containing, respectively, 1000, 2000 or 3000 mg/L phoxim solution (Hopkins *et al.*, 1980). Within each group, 3 sheep were treated 21 days prior to slaughter and 3 sheep were treated 45 days prior to slaughter. One untreated sheep was slaughtered as a source of control tissue. No residues of phoxim were found in liver, kidney or muscle samples from any of the treated animals using a gas chromatographic method of analysis with flame photometric detection in the phosphorus mode (limit of detection, 0.05 mg/kg). In fat samples, residues ranged from 1.6 - 2.8 mg/kg in sheep slaughtered 30 days after treatment at 3000 mg/L and from <0.05-1.0 mg/kg at 45 days post-treatment. With dip treatment at 2000 mg/L, residues in fat at 30 days were 1.2-1.8 mg/kg and at 45 days were <0.05 - 0.6 mg/kg. In the 1000 mg/kg treatment group, no residues were detectable in fat at 45 days, while at 30 days residues were <0.05 - 0.7 mg/kg.

Twelve sheep were divided into two equal groups which received, respectively, 500 mg/L phoxim as a plunge dip (single treatment) or 1000 mg/L phoxim as a spray solution, repeated after 7 days (Schröder, 1988). The animals were slaughtered in groups of 3, at 28 and 35 days after the plunge dip or the second spray treatment. Only 2 sheep were included in the day 28 group for the spray treatment due to the death of one animal from causes not related to the treatment during the study. Analysis of fat samples for phoxim and its oxygen analogue by a gas chromatographic method with a limit of detection of 0.05 mg/kg for both phoxim and the oxygen analogue revealed no traces of the analogue in any samples. For the plunge dip treatment samples, residues declined from <0.05 - 0.18 mg/kg at day 28 to <0.05 mg/kg at day 35. For samples from animals that received the spray treatment, residues were <0.05 and 0.05 mg/kg at day 28 and <0.05 to 0.10 mg/kg at day 35. This study also did not meet current GLP standards.

One study was also conducted to assess the effect of phoxim treatment of lactating sheep (Hapke, 1980). Following treatment of 6 lactating sheep in a plunge dip containing 500 mg/L phoxim, milk was collected for the next 3 days and tested for residues using a liquid chromatographic (HPLC) method with a claimed limit of detection of 0.03 mg/L. No phoxim residues were detected in any of the samples.

Goats

Nine goats were sprayed twice, at an interval of 8 days, with a solution containing 1000 mg/L phoxim (Terblanche, 1978e). The goats were divided into groups of 3 animals each, which were slaughtered at 7, 14 and 21 days, respectively, following the second treatment. Liver, kidney, muscle and fat samples from the nine goats and an untreated control animal were tested for phoxim residues by a gas chromatographic method using a nitrogen-phosphorus detector (limit of detection, 0.01 mg/kg). Analytical recoveries from fat at 1 mg/kg were 79%, while recoveries from liver, kidney and muscle at 0.5 mg/kg were 102, 90 and 98%, respectively. In the day 7 samples, liver and kidney from one goat and kidney from a second goat contained 0.01 mg/kg phoxim. All other liver and kidney samples for this and the other sampling dates contained <0.01 mg/kg phoxim. In muscle, phoxim residues were: day 7, 0.01 - 0.05 mg/kg; day 14, 0.01 - 0. 2 mg/kg; day 21, <0.01 - 0.03 mg/kg. In fat, phoxim residues were: day 7, 0.53 - 0.85 mg/kg; day 14, 0.16 - 0.46 mg/kg; day 21, 0.08 - 0.10 mg/kg. This study was not conducted to current GLP standards.

Rabbits

Sixteen rabbits were treated on 7 consecutive days with a lotion containing 0.1% phoxim using a topical application in the ears, corresponding to 1 mg phoxim per day (Marcotrigiano, 1991). Animals were sacrificed, in pairs at 1, 3, 5, 7, 10, 15, 21 and 28 days following the final treatment, with samples of liver, kidney, muscle and fat collected for analysis. Samples were also collected from two other rabbits, which did not receive the phoxim treatment. Analysis was conducted by liquid chromatography (HPLC) with UV detection, with a claimed limit of detection of 0.005 mg/kg for phoxim and mean recoveries of 76%. Maximum residues were found in kidney at day 1 (0.13 mg/kg), while residues peaked in other tissues at day 3 (fat, 0.13 mg/kg; liver, 0.08 mg/kg; muscle, 0.01 mg/kg). By day 7, residues were at or approaching the detection limit in all tissues. The experiment was repeated using a spray formulation, at an estimated

dosage of 1 mg/rabbit/day. Highest residues were found in day 1 samples (fat, 0.14 mg/kg; liver, 0.11 mg/kg; kidney, 0.10 mg/kg; muscle, not quantifiable). Residues depleted rapidly and were not detectable in any tissues at day 7. The study did not meet GLP requirements.

## METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Two distinct analytical approaches have been developed for the determination of phoxim residues, with earlier studies using gas chromatography and more recent studies using liquid chromatography as the preferred analytical technique. In the earliest method reported by the sponsor (Blass and Reigner, 1978), homogenised liver, kidney, muscle or fat tissue was extracted with hexane, partitioned with acetonitrile to remove lipids, then cleaned up on a column packed with basic alumina. Phoxim residues were eluted with benzene and analysed by gas chromatography using a nitrogen-phosphorus (alkali flame) detector. It was noted that the gas chromatography column packed with 2% SE-30 on Chromosorb GAW, DMCS, required conditioning by heating and by frequent injection of the test substance. A limit of detection of 0.04 mg/kg was claimed for the method, with analytical recoveries in the range 75-86%. The report does not specify the species from which tissues were tested.

A subsequent report described a method used in analytical work conducted by the South African Bureau of Standards (SABS, 1979). This method was applicable to muscle, liver, kidney and fat from sheep and goat. The procedure was essentially the same as that described above, with hexane extraction, partitioning with acetonitrile and clean-up on alumina. Analysis was by gas chromatography with nitrogen-phosphorus detector, using an equivalent column packing material to that described above. The claimed limit of detection was 0.05 mg/kg for all tissues. This method was apparently also applied in studies involving cattle, milk from cattle and pigs (Terblanche, 1978a-c, 1979a).

A variation on the previous methodology was used in a depletion study of phoxim in sheep. The use of a more selective flame photometric detector enabled the analysis of samples with less clean-up than required when an alkali flame detector was selected, as in the above reports (Hopkins *et al*, 1980). In this study, tissue samples were extracted with hexane, after the addition of anhydrous sodium sulphate to absorb water. The hexane extract was concentrated, partitioned with acetonitrile, with the final extract injected into the GC without further clean-up. The limit of detection achieved was 0.05 mg/kg, with recoveries ranging from 73 - 99% at 1 mg/kg.

The first reported use of liquid chromatography (HPLC) was in a study on phoxim residues in pig tissues (Gyr, 1985). Samples of liver, kidney, muscle and fat were extracted and partitioned between acetonitrile and hexane, following which extracts were analysed by liquid chromatography (HPLC) with UV detection at 285 nm. Unlike HPLC methods described subsequently, no additional clean-up using solid phase extraction was reported. Recoveries were 82-90% for all tissues at 0.1 mg/kg and from 85-90% at 1 mg/kg fortification levels, with a claimed limit of detection of 0.01 mg/kg.

A method using liquid chromatography (HPLC) with UV detection for the analysis of phoxim in swine tissues is similar to the methods reported using gas chromatography, in that partitioning between hexane and acetonitrile is followed by clean-up on alumina (Maasfeld, 1990). Liver, kidney and muscle were initially extracted with acetonitrile, then partitioned with hexane, while fat samples were homogenized with hexane and partitioned with acetonitrile. In either case, the acetonitrile phase was concentrated and cleaned up on an alumina column, after which phoxim was eluted with dichloromethane, taken to dryness, then taken up in acetonitrile and water for HPLC analysis. The limit of determination of the method was 0.01 mg/kg, with detection limits of 0.002-0.003 mg/kg for tissues. Analytical recoveries determined at 0.01 and 0.05 mg/kg fortification levels were 67-68% for fat, 72% for kidney, 52-55% for liver and 71-75% for muscle. For 5 replicate determinations at each fortification level in each tissue, relative standard deviation was <10%.

A similar approach for the analysis of swine tissues has also been reported in which, following extraction and partitioning as described above, the extract was cleaned up on a silica gel packing instead of alumina (Heukamp, 1991). In this procedure, elution of phoxim residues from the silica gel was performed with hexane/dichloromethane (50/50, v/v), after which the extract was dried and reconstituted in acetonitrile/water for analysis. the limit of determination was again 0.01 mg/kg for all tissues, with limits of detection reported from 0.002-0.003 mg/kg for the various tissues. Analytical recoveries were improved for kidney (82-108%) and fat (77-86%), but less so for muscle (52-89%) and liver (56-69%). A revised edition of the method is also available in a more recent report (Krebber and Heukamp, 1995).

## APPRAISAL

A number of factors require consideration by the Committee in recommending the MRLs for veterinary use of phoxim. The Committee established an ADI of 0 - 4 µg/kg BW per day, equivalent to 240 µg per day for a 60 kg person and, because phoxim is not currently planned to be used for crop protection; the whole ADI is available for veterinary usage.

The total residue represented by the marker residue can not be determined from available studies, which also did not permit a complete assessment of the toxicity of the metabolites. As no other residues were detected or identified in the depletion studies, the marker residue is parent phoxim. Depletion studies demonstrate that residues in treated animals, other than rabbits, are at very low concentrations immediately following treatment and subsequently in all tissues except fat. MRLs recommended for muscle, liver and kidney are based on 2x the LOQ of available analytical methods (0.01 mg/kg). Based on depletion studies with unlabelled compound, the target tissue is fat in all species.

MRLs for fat are based on the GLP study in pigs, using an estimate based on the mean plus 3 standard deviations, and are extended to other species based on the similar results obtained in available depletion studies. MRLs for sheep could be extended to goats, based on the similar results observed in the available studies in the two species. The Joint Meeting on Pesticide Residues (JMPR) had previously recommended an MRL of 0.01 mg/kg for phoxim in milk from cattle.

Suitable analytical methodology is available for determining phoxim at the recommended MRLs for edible tissues.

On the basis of the maximum observed residues in cattle, pigs, sheep and goats, and in milk from cattle treated with phoxim by the recommended topical routes of administration, the Committee recommended temporary MRLs for edible tissues of cattle, pigs, sheep and goats of 20 µg/kg in muscle, 20 µg/kg in liver, 20 µg/kg in kidney, 400 µg/kg in fat and 10 µg/kg in cattle whole milk, expressed as parent compound. The MRLs as recommended would result in a daily maximum intake of 44µg of phoxim (Table 3).

**Table 3. Theoretical Maximum Daily Intake (TMDI) of Phoxim Residues**

| Food Item | MRL (µg/kg) | Food Basket (g) | TMDI (µg ) |
|---|---|---|---|
| Muscle | 20 | 300 | 6 |
| Liver | 20 | 100 | 2 |
| Kidney | 20 | 50 | 1 |
| Fat | 400 | 50 | 20 |
| Milk | 10 | 1500 | 15 |
| **Total:** | | | **44** |

The Committee required the following data for evaluation in 2002:

1. Radiolabel studies to relate the marker residue to the total residue in ruminants and pigs following topical application of the formulated product.

2. GLP residue studies using the current recommended treatments in sheep and cattle.

3. A study to confirm distribution of the marker residue in rabbits.

4. Validation of available methodology for phoxim residues in tissues of sheep, cattle, goats, rabbits and in milk from cattle.

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# PORCINE SOMATROPINS

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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 $190^0$C.

**Denaturation:** $52^0$C (Somagrepor); $56^0$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 50$^{th}$ Meeting of JECFA concluded that IGF-I, resulting from use of bST, was orally inactive (WHO TRS 888, 1999). The 50$^{th}$ 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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# THIAMPHENICOL

**First draft prepared by**
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**ADDENDUM**
**to the thiamphenicol residue monograph**
**prepared by the 47th meeting of the Committee and published in**
**FAO Food and Nutrition Paper 41/9, Rome 1996.**

## INTRODUCTION

The Committee has previously considered the antimicrobial agent thiamphenocol at the forty-seventh meeting. At that meeting the Committee recommended temporary MRLs of 40 µg/kg for poultry and cattle muscle, liver, kidney and fat, expessed as the parent drug. MRLs were not recommended for eggs because of unacceptably high thiamphenicol residues. No MRLs were proposed for cattle milk or pigs because no data were supplied on total residues in milk and the data supplied for pigs were considered insufficient to allow a recommendation for MRLs.

In reaching this decision, based on the data package supllied by the sponsor, the 47th Committee considered the following factors:

- A temporary ADI of 0-6 µg/kg BW was recommended based on a toxicological endpoint, that corresponds to 300 µg per day for a 60 kg person.
- There was a dearth of data to determine the percentage of marker residue to total residue in the edible tissues of target species.
- The limits of quantification and detection of available analytical methods were 0.02 mg/kg and 0.01 mg/kg, respectively.
- There was a a lack of depletion studies in target animals extending to periods beyond the withdrwal times at maximum recommended dosage.

The Committee requested that the following additional information be required for evaluation in 1999:

- Detailed reports of the carcinogenicity study in rats on which the summary report was available at the 47th meeting and the range-finding study used to establish dose levels in that study.
- Residue depletion studies with radiolabeled and unlabeled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs.

The evidence on which the Committee made its determination has been reported previously. (FAO, 1997). This monograph reviews data submitted by the sponsor that addresses the second of the Committee's requests for new information. In addition, new material supplied by the sponsor on pharmacokinetics and residue depletion in fish and sheep is also included. However, no radiolabeled studies were reported, as requested by the Committee.

## IDENTITY

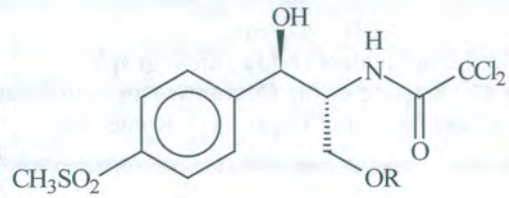
**Chemical name:** D-d-threo-2-dichloroacetamido-1-(4-methylsulfonylphenyl)-1,3-propanediol (IUPAC) [R-(R*.R*)]-2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-(methylsulfonyl) phenyl]ethyl]acetamide (CAS)

**Molecular formula:** $C_{12}H_{15}Cl_2NO_3S$

**Molecular weight:** 356.23

**Chemical structure:**

**OTHER INFORM**




R = H
Thiamphenicol

R = $COCH_2NH_2$ HCl
Thiamphenicol glycinate hydrochloride

**ATION ON IDENTITY AND PROPERTIES**

| | |
|---|---|
| **Appearance:** | White crystalline powder |
| **Melting point:** | 164-166$^0$C |
| **Optical rotation:** | $[\alpha]_D^{25°}$ = +12.9$^0$ (ethanol) |
| **UV spectrum (max):** | 224, 266, 274 nm (ε, 13,700,800,700) |

**RESIDUES IN FOOD AND THEIR EVALUATION**

**CONDITIONS OF USE**

General

Thiamphenicol is used for the treatment of certain bacterial diseases in cattle, pigs, poultry and fish. The product is usually used as an oral preparation, but is not suitable for the treatment of ruminating cattle.

Dosage

Thiamphenicol is used for oral administration and thiamphenicol glycine hydrochloride is utilised in formulations for parenteral use.

**METABOLISM AND PHARMACOKINETIC STUDIES**

Pigs

In a study which complied with GLP (Redgrave *et al.*, 1991), sixteen pigs weighing 15-22 kg and about 7 weeks old were fed thiamphenicol in the diet twice a day for five consecutive days. Each dose was approximately 30 mg/kg BW and three untreated animals acted as controls. Blood samples were taken immediately prior to dosing and at periods during the next 10 days. Results, which appear in Table 1, show that maximum mean plasma levels of 1.28 mg/L were attained 8 h after the first administration. After the withdrawal of treatment, plasma concentrations declined to close to or below, the LOD of the assay method after five days.

In another non-GLP study in pigs (Fornasini, 1992), thiamphenicol was orally administered twice daily to 15 pigs of mean weight 30 kg, randomly divided into three groups of five, and dosed at 10, 15 and 20 mg/kg BW for five consecutive days. The plasma values for thiamphenicol and thiamphenicol glucuronide were determined by GC using electron capture detection. The results of this study, previously summarised (FAO, 1997), are important in that they showed that glucuronide formation is an important route of elimination of thiamphenicol in pigs. Indeed, the mean plasma glucuronide concentrations at all time points after the last dose were higher than the concentrations of free

thiamphenicol. However, whereas plasma concentrations of thiamphenicol were dose related, those of thiamphenicol glucuronide were not.

**Table 1. Mean plasma concentrations of thiamphenicol in pigs fed a diet containing 900mg/kg of thiamphenicol (approximately 30 mg/kg BW) twice daily for five consecutive days**

| Study day (withdrawal day) | Time (h) | Thiamphenicol (mg/L) | | Study day (withdrawal day) | Time (h) | Thiamphenicol (mg/L) | |
|---|---|---|---|---|---|---|---|
| | | Mean (n = 6) | Range | | | Mean (n = 6) | Range |
| 1 | 0 | ND | - | 6 (1) | 8 | 0.05 | 0.03 – 0.11 |
| | 2 | 1.25 | 0.93 – 1.67 | | 12 | 0.04 | 0.02 – 0.06 |
| | 4 | 1.25 | 0.81 – 1.85 | | 16 | 0.02 | ND – 0.04 |
| | 6 | 0.85 | 0.57 – 1.47 | | 20 | <0.02 | ND – 0.02 |
| | 8 | 1.28 | 0.85 – 1.65 | 7 (2) | 24 | 0.02 | 0.02 – 0.03 |
| | 16 | 0.80 | 0.57 – 1.51 | | 12 | <0.02 | ND – 0.03 |
| 2 | 24 | 0.24 | 0.15 – 0.58 | 8 (3) | 24 | ~0.01 | ND – 0.03 |
| 3 | 24 | 0.34 | 0.14 – 1.12 | | 12 | ~0.01 | ND – 0.02 |
| 4 | 24 | 0.31 | 0.17 – 0.88 | 9 (4) | 24 | ND | ND |
| 5 | 24 | 0.39 | 0.14 – 1.14 | | 12 | ~0.01 | ND – 0.04 |
| 6 (1) | 24 | 0.22 | 0.12 – 0.48 | 10 (5) | 24 | ND | ND |
| | 4 | 0.08 | 0.05 – 0.17 | | | | |

In a more recent study, in conformance with GLP, four castrated male pigs weighing 100-120 kg were concurrently treated orally with 30 mg/kg BW and IV with 10 mg/kg BW thiamphenicol (Villa and Brightwell, 1997a,b). Blood samples were collected in the first 24 h and urine was collected to 48 h post dosing. All samples were measured by a validated HPLC method with a LOQ of 21 μg/L in plasma and 210 μg/L in urine. Results are shown in Table 2. After oral administration, the $t_{1/2}$ was 3.88 h and 4.57 h and AUC was 23.96 mg x h/L and 91.04 mg x h/L for unconjugated and total thiamphenicol, respectively. After IV administration, the $t_{1/2}$ was 3.43 h and 4.64 h and AUC was 12.64 mg x h/L and 23.29 mg x h/L for unconjugated and total thiamphenicol, respectively. In urine, highest concentrations of total thiamphenicol occurred 4 h after oral dosing and ranged between 610-723 mg/L while, after IV dosing, maximum urine concentrations also occurred after 4 h with a range of 363-1136 mg/L.

**Table 2. Mean plasma concentrations of thiamphenicol in pigs administered a single oral dose of 30 mg/kg BW or a single IV dose of 10 mg/kg BW**

| Time (h) | Thiamphenicol (TAP) conc. (mg/L) after oral dosing | | | Time (h) | Thiamphenicol conc. (mg/L) after IV dosing | | |
|---|---|---|---|---|---|---|---|
| | Free TAP (SD) | Glucuronide (SD) | Total TAP (SD) | | Free TAP (SD) | Glucuronide (SD) | Total TAP (SD) |
| 0.25 | 1.72 (0.43) | 4.73 (2.67) | 6.45 (3.09) | 5 (min) | 36.46(10.60) | 12.17 (9.28) | 48.64 (2.51) |
| 0.5 | 2.88 (0.47) | 6.31(1.64) | 9.20 (1.98) | 10 (min) | 20.31(7.10) | 14.57 (2.40) | 34.88 (6.23) |
| 1 | 3.08 (0.94) | 7.11(1.12) | 10.20 (2.01) | 20 (min) | 11.96 (2.53) | 8.57 (3.87) | 20.53 (1.77) |
| 2 | 2.80 (0.61) | 6.27 (2.30) | 9.08 (2.23) | 45 (min) | 3.23 (1.38) | 4.60 (2.44) | 7.84 (1.03) |
| 3 | 2.15 (0.60) | 4.84 (0.80) | 7.00 (1.17) | 1 | 2.20 (0.59) | 1.70 (1.28) | 3.90 (1.03) |
| 4 | 2.06 (1.530 | 7.52 (1.14) | 9.59 (1.36) | 2 | 0.85 (0.37) | 0.75 (0.58) | 1.61(0.55) |
| 6 | 1.53 (0.43) | 4.33 (1.06) | 5.87 (1.45) | 3 | 0.28 (0.10) | 0.38 (0.09) | 0.66 (0.06) |
| 8 | 0.83 (0.24) | 2.82 (1.21) | 3.66 (1.35) | 4 | 1.51(1.04) | 0.39 (0.36) | 0.27 (0.06) |
| 12 | 0.35 (0.10) | 1.16 (0.65) | 1.51 (0.75) | 8 | 0.05 (0.03) | 0.10 (0.06) | 0.16 (0.06) |
| 24 | 0.35 (0.11) | 0.85 (0.39) | 1.21 (0.48) | 12 | 0.02 (0.005) | 0.07 (0.04) | 0.10 (0.04) |
| | | | | 24 | 0.005(0.002) | 0.02 (0.02) | 0.03 (0.02) |

Fish

Pharmacokinetic studies of the use of thiamphenicol in yellowtail have been reviewed (Eisai Co Ltd, 1997a). Thiamphenicol was administered as a single dose, mixed in feed, to fish weighing 190g and reared at 28°C, at 100 mg/kg BW. Blood was collected, using 7 fish for each time-point, at 3, 6, 12, 24 and 48h. Peak thiamphenicol concentrations, as measured by a colorimetric method, occurred at 3-6 h with a $C_{max}$ 9.4-12.1 mg/L. No drug could be detected in blood after 48 h but high levels were detected in bile at 24 h, supporting the hypothesis that bialy excretion is the major elimination pathway in fish. No metabolites were identified.

The bioavailability of thiamphenicol in seabass (*Dicentrarchus labrax*), reared at 15 ± 2°C after a single dose and also after dosing for five days has been studied. Thiamphenicol was administered in the feed at 15 and 30 mg/kg/day, the fish being force-fed for single dose studies and in the feed for multiple dose studies. Adsorption of the drug from medicated feed was slow and incomplete in comparison with the forced feeding study ($AUC_{0-72h}$ = 39.34 mg h/L compared to 197.39 mg h/L at the higher dose). Thiamphenicol concentrations in blood declined rapidly and were below the LOQ of the analytical method (50 µg/L) 7 days after the last treatment.

## RESIDUE DEPLETION STUDIES

Pigs

In a recent GLP-compliant residue depletion study in pigs, 25 crossbreds weighing from 25-30 kg each were randomly divided into 5 equal groups (Villa and Brightwell, 1997a). Four groups were fed a thiamphenicol-containing diet such that each pig consumed 30 mg/kg/day BW of drug for 5 days while the last group (controls) received standard non-medicated diet. Groups were treated at different times so that all animals could be sacrificed together after withdrawal of drug, which occurred at 5,10,16 and 18 days for the respective groups. All tissues were analysed for the marker residue, thiamphenicol, by a validated HPLC method with an LOQ and LOD of 21 µg/kg and 5 µg/kg, respectively (Villa and Brightwell, 1997b). The results are shown in Table 3. Thiamphenicol was not present in muscle, except for two animals at day 10. In liver, mean residues of thiamphenicol were 27.35 µg/kg at day 18 while, at the same time point, kidney and skin values were 39.14 and 94.6 µg/kg, respectively. It should be noted that %CV were very large at several time points, particularly for kidney.

In a second GLP-compliant residue depletion study, 32 crossbred pigs weighing from 45-65.2 kg each were divided into 8 groups, each containing two males and two females (Luperi and Villa 1998a). Seven groups were dosed IM with 30 mg/kg/day BW thiamphenicol for 5 days while the last group acted as a control. Groups were sacrificed at 8 hours, and 1,4,8, 15, 21, and 28 days after the last administration of drug, respectively. Samples of muscle, muscle injection site, liver, kidney, visceral fat and skin/fat were taken from each animal while lung samples were also taken from animals in the 8 hour and one day groups. All tissues were analysed for thiamphenicol, by the same validated HPLC method used in the previous study (Villa and Brightwell, 1997b). The results are shown in Table 4. Thiamphenicol concentrations decreased to below 50 µg/kg in all tissues investigated 8 days after the last administration. After 28 days, thiamphenicol residues could only be detected in injection sit muscle at levels above the LOQ.

In neither of the two studies, summarised in Tables 3 and 4, were investigations undertaken to determine if thiamphenicol glucuronide was a major metabolite in any tissue and, if so, how much was present in that tissue. This was despite the fact that pharmacokinetic studies (*vide supra*) had demonstrated that the glucuronide conjugate of thiamphenicol represented the dominant proportion of thiamphenicol in plasma and a significant metabolite in the urine of pigs after oral dosing. The glucuronide conjugate could represent a significant proportion of total metabolites in any tissue. The 47$^{th}$ meeting of the JEFCA Committee had considered this possibility in requesting the sponsor to provide new residue depletion studies with radiolabelled and unlabeled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs. The studies discussed above (Villa and Brightwell, 1997a; Luperi and Villa 1998a) neither unambiguously identifies the thiamphenicol as the correct marker residue in all pig tissues nor gives any information of the ratios of thiamphenicol to the total residues in pig tissue at any time point.

**Table 3. Mean tissue concentrations (µg/kg) of thiamphenicol in the tissues of pigs following oral administration of 30 mg /kg /day BW (n=5) for 5 consecutive days.**

| Days after withdrawal | | Thiamphenicol concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|
| | | Muscle | Liver | Kidney | Fat | Skin |
| 5 | Mean (%CV) | ND | 56.3 (50.4) | 534.6 (157.6) | <LOQ | 228.8 (57.4) |
| | Range | ND | 29.2-96.2 | 18.7-1975 | LOD-<LOQ | 101.6-403.0 |
| 10 | Mean (%CV) | 10. 6 (137.5)* | 38. 5(40.74) | 127.7 (64.4)* | 11.3 (64.3)* | 120.2 (34.8) |
| | Range | ND-28.3 | 21.7-62.5 | 15.2-236.6 | LOD-23.9 | 57.3-167.0 |
| 16 | Mean (%CV) | ND | 22.5 (57.1)* | 83.5 (78.7)* | LOD-LOQ | 153.1 (38.9) |
| | Range | ND | ND-30.9 | <LOQ-173.2 | <LOQ-<LOQ | 93.0-230.3 |
| 18 | Mean (%CV) | ND | 27.4 (11.4) | 39.1 (159.2)* | ~LOD | 94.6 (24.8) |
| | Range | ND | 24.9-32.6 | ~LOD -150.3 | ND-<LOQ | 67.4-129.9 |

* estimated value only because some individual values were below the quantification range of the analytical method.
ND = not detected; LOD = 5 µg/kg; LOQ = 21 µg/kg

**Table 4. Mean tissue concentrations (µg/kg) of thiamphenicol in the tissues of pigs following IM administration of 30 mg /kg /day BW (n=4) for 5 consecutive days.**

| Days after withdrawal | | Thiamphenicol concentration (µg/kg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Muscle | Liver | Kidney | Visceral Fat | Skin/Fat | Lung |
| 8 (hours) | Mean (%CV) | 1756 (8.2)# | 642 (28.1) | 6675 (20.3)# | 552.9(44.0) | 991.1(26.3)# | 2240 (28)# |
| | Range | 1570-1910 | 459-806 | 5240-8510 | 265-857 | 647-1240 | 1430-2820 |
| 1 | Mean (%CV) | 389.5 (49.5) | 111.2 (127.9) | 1648 (69.9)# | 84.1 (20.5) | 203.7 (60.0) | 529.1 (38.8) |
| | Range | 151.4-585.0 | 14.3-322.4 | 401.1-3090 | 61.6-103.5 | 120.2-384.8 | 326.4-814.8 |
| 4 | Mean (%CV) | 10.8 (58.1)* | LOD | 55.0 (66.2)* | 22.6 (76.9)* | 32.3 (85.2)* | - |
| | Range | <LOQ-20.0 | <LOD-<LOQ | <LOQ-104.9 | ND-41.8 | <LOQ-72.9 | - |
| 8 | Mean (%CV) | LOD-LOQ | <LOD | 24.0 (78.0)* | <LOQ | LOD-LOQ | - |
| | Range | ~LOD-<LOQ | <LOD-LOD | ~LOD-47.18 | ND-22.3 | <LOQ-32.9 | - |
| 15 | Mean (%CV) | ~LOD-LOQ | ND | 16.5 (56.8)* | ND | <LOD | - |
| | Range | ~LOD-LOQ | ND | <LOQ-28.2 | ND | ND-~LOD | - |
| 21 | Mean (%CV) | ~LOD | ND | 14.2 (92.7)* | ND | ND | - |
| | Range | <LOD-LOD | ND | ~LOD-30.0 | ND | ND | - |
| 28 | Mean (%CV) | ND | ND | ND | ND | ND | - |
| | Range | ND | ND | ND | ND | ND | - |

* estimated value only because some individual values were below the quantification range of the analytical method
\# estimated value only because some individual values were above the validation range of the analytical method
ND = not detected ; LOD = 5 µg/kg; LOQ = 21 µg/kg

Sheep

A GLP-compliant residue depletion study in sheep has recently been reported (Luperi and Villa 1998a). Sixteen crossbred sheep, weighing from 55.0-73.7 kg for males and 38.6-56.0 kg for females, were divided into 4 groups, each containing two males and two females. Each group was dosed IM with 30 mg/kg/day BW thiamphenicol for 5 consecutive days. Groups were sacrificed at 4, 8, 12, and 16 days after the last administration of drug, respectively. Samples of muscle, muscle injection site, liver, kidney, and abdominal fat, taken from each animal, were analysed for thiamphenicol, by a validated HPLC method with an LOQ and LOD of 21 µg/kg and 2.4-5.2 µg/kg, respectively (Villa and Brightwell, 1997b). The results are shown in Table 5. Thiamphenicol was not present in muscle or liver, and in kidney and fat values were 43.2 and 342.5 µg/kg, respectively at day 4. No residues could be quantified in any tissue at all later time points

This study did not attempt to detect or quantify the presence of thiamphenicol glucuronide. There is no data that establishes that thiamphenicol is the correct marker residue for sheep tissues, nor is there data that enables the determination of the relationship between unconjugated thiamphenicol and total residues in any tissue.

**Table 5. Mean tissue concentrations (µg/kg) of thiamphenicol in the tissues of sheep following IM administration of 30 mg /kg /day BW (n=4) for 5 consecutive days.**

| Days after withdrawal | | Thiamphenicol concentration (µg/kg) | | | |
|---|---|---|---|---|---|
| | | Muscle | Liver | Kidney | Fat |
| 4 | Mean (%CV) | <LOD | ND | 40.2 (129.6) | 342.5 (112.8) |
| | Range | ND-LOD | ND | ND-116.9 | 79.1-905.8 |
| 8 | Mean (%CV) | <LOD | ND | ND | ~LOD |
| | Range | <LOD | ND | ND | ND-<LOQ |
| 12 | Mean (%CV) | ND | ND | ND | ~LOD |
| | Range | ND | ND | ND | ND-<LOQ |
| 16 | Mean (%CV) | ND | ND | ND | ND |
| | Range | ND | ND | ND | ND |

* estimated value only because some individual values were below the quantification range of the analytical method. ND = not detected; LOD = 5 µg/kg; LOQ = 21 µg/kg

Fish

Thiamphenicol was administered, mixed in feed, to yellowtail fish weighing 210g and reared at 23.0-27.5°C at doses of 45 (recommended dose) and 90 mg/kg/day BW for 14 days in a non-GLP compliant study (Eisai Co Ltd, 1997b). Muscle, liver, kidney and skin with fat were collected on day 10 of administration and at 0, 3, 7, 10, 14 21 and 28 days after the finish of treatment. Residue levels of free thiamphenicol were highest in liver, followed by kidney liver and skin, but were very low in muscle. Residues fell below the detection limit of the GC analytical method used (20 µg/kg) 3 days after cessation of treatment.

In a recent study (Della Rocca *et al.*, 1997a), which was not performed to GLP requirements, thiamphenicol was fed to seabass (*Dicentrarchus labrax*), weighing 140-150g and reared at 18-28°C at 40 mg/kg/day BW for 5 days. This is both the recommended dosing rate and time of administration. Muscle, liver, skin and vertebrae were collected from groups of 10 fish on days 2 and 4 of administration and at 1, 2, 3, 5, 7 and 10 days after the finish of treatment and thiamphenicol concentrations determined by HPLC. Results of the study are summarised in Table 6. Residue levels of free thiamphenicol were highest in liver, followed by muscle and skin. Residues fell below the detection limit of the HPLC analytical method used (20 - 100 µg/kg) 5 days after cessation of treatment.

**Table 6. Mean tissue concentrations (µg/kg) of thiamphenicol in the tissues of seabass (*Dicentrarchus labrax*) following oral administration of 40 mg /kg /day BW (n=10) for 5 consecutive days.**

| Day of sampling | Thiamphenicol concentration (µg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Muscle | | Liver | | Skin | | Vertebrae | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 2 During dosing | 1270 | 920 | 6360 | 3450 | 650 | 360 | 440 | 190 |
| 4 During dosing | 3010 | 1180 | 6450 | 6020 | 1140 | 420 | 780 | 290 |
| 1 After dosing | 1830 | 760 | 6380 | 3520 | 890 | 420 | 160 | 50 |
| 2 After dosing | 60 | 30 | 1500 | 810 | 90 | 50 | 120 | 70 |
| 3 After dosing | 28 | 30 | 410 | 230 | <50 | | 90 | 30 |
| 5 After dosing | <20 | | <100 | | <50 | | <50 | |

LOQ = 20 µg/kg in muscle, 50 µg/kg in skin and vertebrae and 100 µg/kg in liver

In a second related study (Della Rocca *et al.*, 1997b), which was not performed to GLP requirements, thiamphenicol was fed to seabream (*Spartus aurata*), weighing 100-120g and reared at 18-28°C at 40 mg/kg/day BW for 5 days. This is both the recommended dosing rate and time of administration. Muscle, liver, skin and vertebrae were collected from groups of 10 fish on days 2 and 4 of administration and at 1, 2, 3 and 5 days after the cessation of treatment and thiamphenicol concentrations determined by HPLC. Results of the study are summarised in Table 7. Residue levels of free thiamphenicol were highest in liver, followed by muscle and skin. Residues fell below the detection limit of the HPLC analytical method used (20 - 100 µg/kg) 5 days after cessation of treatment.

**Table 7. Mean tissue concentrations (µg/kg) of thiamphenicol in the tissues of seabream (*Spartus aurata*) following oral administration of 40 mg /kg /day BW (n=10) for 5 consecutive days.**

| Day of sampling | Thiamphenicol concentration (µg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Muscle | | Liver | | Skin | | Vertebrae | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 2 During dosing | 1160 | 1070 | 8500 | 5400 | 2640 | 1730 | 220 | 190 |
| 4 During dosing | 1970 | 910 | 6900 | 3410 | 2520 | 2540 | 530 | 340 |
| 1 After dosing | 1470 | 810 | 3890 | 1740 | 700 | 370 | 380 | 230 |
| 2 After dosing | 240 | 140 | 380 | 100 | 180 | 60 | 120 | 70 |
| 3 After dosing | 30 | - | <100 | | <50 | | 80 | 20 |
| 5 After dosing | <20 | | <100 | | <50 | | <50 | |

LOQ = 20 µg/kg in muscle, 50 µg/kg in skin and vertebrae and 100 µg/kg in liver

## METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A number of methods for the analysis of thiamphenicol residues have been reported which were not previously discussed in the monograph from the 47th meeting of the Committee (FAO, 1997). These are discussed below.

An HPLC method for the detection and quantification of residues of free thiamphenicol in the plasma, urine and tissues of pigs has been validated in accordance with GLP regulations (Villa and Brightwell, 1997b). Tissue is homogenised, extracted with ethyl acetate and the ethyl acetate extract washed with aqueous sodium chloride. The ethyl acetate is removed in a stream of nitrogen, the residue dissolved in water and extracted with hexane and the final aqueous layer injected onto a reverse phase HPLC column. Thiamphenicol is determined by elution with methanol-water and detection by UV at 224 nm. For kidney and urine samples, a florisil clean-up stage is inserted after evaporation of the ethyl acetate. Also, the elution solvent is modified when dealing with these samples. The method has been validated for a LOQ of 21 µg/kg and a LOD of 5 µg/kg in all matrices except urine, with a CV below 5% for all matrices except liver where the CV was 8%. However, quantitative results have been quoted using this method that are well below the stated LOQ, and even below the stated LOD (Villa and Brightwell, 1997a). The accuracy and precision of the method at these values was not stated although both quantification values and coefficients of variation were provided to two decimal places. For urine, the LOQ was 210 µg/L and a LOD of 25 µg/L.

The method has also been used to determine the sum of thiamphenicol and thiamphenicol glucuronide in plasma and urine samples by the introduction of a gluronidase deconjugation step at the beginning of the procedure. This work was not extended to the determination of thiamphenicol glucuronide in tissue samples.

This same HPLC procedure has also been validated for determination of thiamphenicol in sheep plasma, urine and tissues (Villa and Brightwell, 1998).

A HPLC procedure for the determination of thiamphenicol in fish tissues has not been validated in accordance with GLP. The LOQs obtained for different tissues were 20 μg/kg in muscle, 50 μg/kg in skin and vertebrae and 100 μg/kg in liver (Anfossi, 1998)

## APPRAISAL

At the 47th meeting The Committee requested that residue depletion studies with radiolabelled and unlabelled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs. The data that was submitted by the sponsor only partially addressed the Committee's requests for new information. The sponsor also provided unsolicited information on pharmacokinetics and residue depletion in fish and sheep. However, no radiolabelled studies were reported as requested by the Committee.

The 47th meeting of the JEFCA Committee requested the sponsor to provide new residue depletion studies with radiolabelled and unlabelled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs. The data provided neither unambiguously identifies thiamphenicol as the correct marker residue in all pig tissues nor provides any information of the ratios of thiamphenicol to the total residues in pig tissue at any time point. This was despite the fact that pharmacokinetic studies have demonstrated that the thiamphenicol glucuronide conjugate represented the dominant proportion of thiamphenicol in plasma and a significant metabolite in the urine of pigs after oral dosing.

Furthermore, no residue study undertaken has attempted either to detect or quantify the presence of thiamphenicol glucuronide as a portion of the total residues. The major thiamphenicol metabolites in tissues of any food animal or fish species are unknown and the occurrence of conjugated metabolites of thiamphenicol in edible tissues has not been investigated. The possibility of extensive metabolism of thiamphenicol in liver tissues cannot be disregarded. However, there is no data that allow the determination of the ratio of marker residue to total residues in any species. It is recognised that thiamphenicol glucuronide was not microbiologically active but could, on human ingestion, be converted to the microbiologically active parent drug.

Due to the lack of information on tissue metabolites, the Committee recommends that the marker residue for thiamphenicol should be the sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol. This marker residue will apply to temporary MRLs until further work has been conducted to establish the metabolite distribution in edible tissue.

Because the sponsor did not supply the data requested by the 47th Committee to support the use of thiamphenicol in cattle and chickens, the temporary MRLs previously recommended for these species are withdrawn.

The Committee requests the sponsor to provide for consideration by the Committee in 2002:

1. A satisfactory depletion study in pigs to determine the relationship between free thiamphenicol, thiamphenicol conjugates and total residues in all tissues.
2. A validated analytical method for use in all animal tissues, which incorporates an enzymatic hydrolysis step that will allow the determination of thiamphenicol and thiamphenicol conjugates as as free thiamphenicol.

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ANNEX 1

## SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

This attached table summarises the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 47th (1996), 48th (1997), 50th (1998) and 52nd (1999) meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. Please see reports of those meetings, published in WHO Technical Report Series (TRS).

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (T).
- Where an MRL is temporary, it is so indicated by "T".
- Several compounds have been evaluated more than once. The data given is for the most recent evaluation.

| Substance | ADI (µg/kg bw) | ADI Status | JECFA | MRL (µg/kg) | Tissue | Species | Marker residue and other remarks |
|---|---|---|---|---|---|---|---|
| Abamectin | 0-1 (1995 JMPR) | Full | 47 (1996) | 100<br>50 | Liver, fat<br>Kidney | Cattle | Avermectin $B_{1a}$ |
| Albendazole | 0-50 | Full | 34 (1989) | 100<br>5000 | Muscle, fat, milk<br>Liver, kidney | Cattle, sheep | MRLs analysed as 2-amino-benzimidazole and expressed as parent drug equivalents, see WHO TRS 788 |
| Azaperone | 0-6 | Full | 50 (1998) | 60<br>100 | Muscle, fat<br>Liver, kidney | Pigs | Sum of azaperone and azaperol |
| Benzylpenicillin | 30 µg/person/day | Full | 36 (1990) | 50<br>4 | Muscle, liver, kidney<br>Milk | All species | Parent drug |
| Bovine Somatotropins | Not specified | Full | 50 (1998) | Not specified | Muscle, liver, kidney, fat, milk | Cattle | |
| Carazolol | 0-0.1 | Full | 43 (1994) | 5<br>25 | Muscle, fat/skin<br>Liver, kidney | Pigs | Parent drug. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effect of carazolol |
| Carbadox | Limited acceptance | Full | 36 (1990) | 30<br>5 | Liver<br>Muscle | Pigs | Quinoxaline-2-carboxylic acid |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ceftiofur | 0-50 | Full | 45 (1995)<br>48 (1997) | <br>1000<br>2000<br>6000<br>2000<br>100 µg/l | <br>Muscle<br>Liver<br>Kidney<br>Fat<br>Milk | <br>Cattle, pigs<br><br><br><br>Cattle | <br>Desfuroylceftiofur |
| Chloramphenicol | No ADI | | 42 (1994) | No MRL | | | |
| Chlorpromazine | No ADI | | 38 (1991) | No MRL | | | |
| Chlortetracycline, oxytetracycline, tetracycline | 0-30 (Group ADI) | Full | 50 (1998) | 200<br><br>600<br>1200<br>400<br>100 µg/l<br>100 T<br>100 | Muscle<br><br>Liver<br>Kidney<br>Eggs<br>Milk<br>Muscle<br>Muscle | Cattle, pigs, sheep, poultry<br><br><br><br>Poultry<br>Cattle, sheep<br>Fish<br>Giant prawn | Parent drugs, singly or in combination<br><br><br><br><br><br>Oxytetracycline only<br>Oxytetracycline only |
| Clenbuterol | 0-0.004 | Full | 47 (1996) | 0.2<br>0.6<br>0.05 µg/l | Muscle, fat<br>Liver, kidney<br>Milk | Cattle, horses<br><br>Cattle | Parent drug |
| Closantel | 0-30 | Full | 36 (1990)<br>40 (1992) | <br>1000<br>3000<br>1500<br>5000<br>2000 | <br>Muscle, liver<br>Kidney, fat<br>Muscle, liver<br>Kidney<br>Fat | <br>Cattle<br><br>Sheep | <br>Parent drug |
| Cyfluthrin | 0-20 | Full | 48 (1997) | 20<br>200<br>40 µg/l | Muscle, liver, kidney<br>Fat<br>Milk | Cattle | Parent drug |
| Cypermethrin | 0-50 | Full | 47 (1996) | 200 T<br><br>1000 T<br>100 T<br>50 µg/l T | Muscle, liver, kidney<br><br>Fat<br>Eggs<br>Milk | Cattle, sheep, chickens<br><br>Chickens<br>Cattle | Parent drug |
| α-Cypermethrin | 0-20 | Full | 47 (1996) | 100 T<br><br>500 T<br>50 T<br>25 µg/l T | Muscle, liver, kidney<br><br>Fat<br>Eggs<br>Milk | Cattle, sheep, chickens<br><br>Chickens<br>Cattle | |

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|---|---|---|---|---|---|---|---|
| Danofloxacin | 0-20 | Full | 48 (1997) | 200<br>400<br>100<br><br>100<br>50<br>200<br>100 | Muscle<br>Liver, kidney<br>Fat<br><br>Muscle<br>Liver<br>Kidney<br>Fat | Cattle, chickens<br><br><br><br>Pigs | Parent drug<br><br>For chickens fat/skin in normal proportion |
| Deltamethrin | 0– 0 (JMPR 1982) | Full | 52 (1999) | 30 (guidance)<br><br>50<br>500<br>30<br>30 | Muscle<br><br>Liver, kidney<br>Fat<br>Milk<br>Egg | Cattle, sheep, chicken, salmon<br>Cattle, sheep, chicken<br>Cattle, sheep, chicken<br>Cattle<br>Chicken | Parent drug |
| Dexamethasone | 0-0.015 | Full | 42 (1994)<br>48 (1997)<br>50 (1998) | <br>No MRL<br>No MRL | | | <br>Temporary MRLs were not extended<br>Regulatory method not available |
| Diclazuril | 0-30 | Full | 50 (1998) | 500<br>3000<br>2000<br>1000 | Muscle<br>Liver<br>Kidney<br>Fat | Sheep, rabbits, poultry | Parent drug<br><br><br>Poultry skin/fat |
| Dihydrostrepto-mycin, streptomycin | 0-50 (Group ADI) | Full | 48 (1997)<br>52 (1999) | <br>600<br>1000<br>200 µg/kg T | <br>Muscle, liver, fat<br>Kidney<br>Milk | <br>Cattle, pigs, sheep, chickens<br>Cattle | Sum of dihydrostreptomycin and streptomycin |
| Demetridazole | No ADI | | 34 (1989) | No MRL | | | |
| Diminazene | 0-100 | Full | 42 (1994) | 500<br>12000<br>6000<br>150 µg/l | Muscle<br>Liver<br>Kidney<br>Milk | Cattle | Parent drug |
| Doramectin | 0-0.5 | Full | 45 (1995)<br>52 (1999) | 10<br>5<br>100<br>30<br>150 | Muscle<br>Muscle<br>Liver<br>Kidney<br>Fat | Cattle<br>Pigs<br>Cattle, Pigs | Parent drug. The Committee noted the high concentration of residues at the injection site over a 35-day period after subcutaneous or intramuscular administration of the drug at the recommended dose. |
| Enrofloxacin | 0-2 | Full | 48 (1997) | No MRL | | | |

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|---|---|---|---|---|---|---|---|
| Eprinomectin | 0-10 | Full | 50 (1998) | 100<br>2000<br>300<br>250<br>20 µg./l | Muscle<br>Liver<br>Kidney<br>Fat<br>Milk | Cattle | Eprinomectin $B_{1a}$ |
| Estradiol-17β | 0-0.05 | Full | 52 (1999) | Not specified | Muscle, liver, kidney, fat | Cattle | |
| Febantel, fenbendazole, oxfendazole | 0-7 (Group ADI) | Full | 50 (1998) | 100<br><br>500<br>100 µg/l | Muscle, kidney, fat<br><br>Liver<br>Milk | Cattle, sheep, pigs, horses, goats<br>Cattle, sheep | Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents |
| Fenbendazole (see febantel) | | | | | | | |
| Fluazuron | 0-40 | Full | 48 (1997) | 200<br>500<br>7000 | Muscle<br>Liver, kidney<br>Fat | Cattle | Parent drug |
| Flubendazole | 0-12 | Full | 40 (1992) | 10<br>200<br>500<br>400 | Muscle, liver<br>Muscle<br>Liver<br>Eggs | Pigs<br>Poultry | Parent drug |
| Flumequine | 0-30 | Full | 48 (1997) | 500<br>1000<br>3000<br>500 T<br>1000 T<br>3000 T<br>500 T | Muscle<br>Liver, fat<br>Kidney<br>Muscle<br>Liver, fat<br>Kidney<br>Muscle/skin | Cattle<br><br><br>Pigs, sheep, chickens<br><br><br>Trout | Parent drug<br><br><br><br><br><br>Muscle/skin in normal proportion |
| Furazolidone | No ADI | | 40 (1992) | No MRL | | | |
| Gentamicin | 0-20 | Full | 50 (1998) | 100T<br>2000<br>5000<br>200 µg/l | Muscle, fat<br>Liver<br>Kidney<br>Milk | Cattle, pigs<br><br><br>Cattle | Parent drug |
| Imidocarb | 0-10 | Full | 50 (1998) | 300 T<br>2000 T<br>1500 T<br>50 T<br>50 µg/l T | Muscle<br>Liver<br>Kidney<br>Fat<br>Milk | Cattle | Parent drug |
| Ipronidazole | No ADI | | 34 (1989) | No MRL | | | |

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|---|---|---|---|---|---|---|---|
| Isometamidium | 0-100 | Full | 40 (1992) | 100<br>500<br>1000 | Muscle, fat, milk<br>Liver<br>Kidney | Cattle | Parent drug |
| Ivermectin | 0-1 | Full | 40 (1992) | 100<br>40<br>15<br>20 | Liver<br>Fat<br>Liver<br>Fat | Cattle<br><br>Pigs, sheep | Ivermectin $H_2B_{1a}$ |
| Levamisole | 0-6 | Full | 42 (1994) | 10<br><br>100 | Muscle. Kidney, fat<br><br>Liver | Cattle, sheep, pigs, poultry | Parent drug |
| Metronidazole | No ADI | | 34 (1989) | No MRL | | | |
| Moxidectin | 0-2 | Full | 45 (1995)<br><br><br>50 (1998)<br><br><br><br>47 (1996)<br>48 (1997) | 100<br>50<br>500<br>20<br>100<br>50<br>500<br>50<br>20 | Liver<br>Kidney<br>Fat<br>Muscle<br>Liver<br>Kidney<br>Fat<br>Muscle<br>Muscle | Cattle, sheep<br><br><br>Deer<br><br><br><br>Sheep<br>Cattle | Parent drug. The Committee noted the very high concentration and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing. |
| Neomycin | 0-60 | Full | 47 (1996)<br><br><br>52 (1999)<br><br><br>52 (1999)<br><br><br>

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|---|---|---|---|---|---|---|---|
| Olaquindox | Limited acceptance | T | 42 (1994) | No MRL (see remarks) | Muscle | Pigs | MQCA. The Committee recommended no MRLs but noted that 4 µg/kg of MQCA (T) is consistent with Good Veterinary Practice |
| Oxfebdazole (see febantel) | | | | | | | |
| Oxolinic acid | No ADI | | 43 (1994) | No MRL | | | |
| Oxytetracycline (see chlortetracycline) | | | | | | | |
| Phoxim | 0-4 | Full | 52 (1999) | 50 T<br>400 T<br>10 T | Muscle, liver, kidney<br>Fat<br>Milk | Cattle, pigs sheep, goat<br>Cattle, pigs, sheep, goat<br>Cattle | Parent drug |
| Porcine somatotropins | Not specified | | 52 (1999) | Not specified | Muscle, liver, kidney, fat | Pigs | |
| Procaine benzylpenicillin | Less than 30 µg of penicillin per person per day | Full | 50 (1998) | 50<br>4 | Muscle, liver, kidney<br>Milk | Cattle, pigs, chickens<br>Cattle | Benzylpenicillin |
| Progesterone | 0-30 | Full | 52 (1999) | Not specified | Muscle, liver, kidney, fat | Cattle | |
| Propionyl-promazine | No ADI | | 38 (1991) | No MRL | | | |
| Ractopamine | No ADI | | 40 (1992) | No MRL | | | |
| Ronidazole | Withdrawn | | 42 (1994) | No MRL | | | |
| Sarafloxacin | 0-0.3 | Full | 50 (1998) | 10<br>80<br>20 | Muscle<br>Liver, kidney<br>Fat | Chicken, turkey<br>Chicken, turkey<br>Chicken, turkey | Parent drug |
| Spectinomycin | 0-40 | Full | 42 (1994)<br>50 (1998) | 500<br>2000<br>5000<br>2000<br>2000<br>200 µg/l | Muscle<br>Liver<br>Kidney<br>Fat<br>Eggs<br>Milk | Cattle, pigs, sheep, chickens<br>Chickens<br>Cattle | Parent drug |

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|---|---|---|---|---|---|---|---|
| Spiramycin | 0-50 | Full | 43 (1994)<br>47 (1996)<br><br><br><br><br><br><br><br>48 (1997) | <br>200<br>200<br>600<br>600<br>300<br>300<br>800<br>300<br>300<br>200 µg/l | <br>Muscle<br>Muscle<br>Liver<br>Liver<br>Kidney<br>Kidney<br>Kidney<br>Fat<br>Fat<br>Milk | <br>Cattle, chickens<br>Pigs<br>Cattle, chickens<br>Pigs<br>Cattle<br>Pigs<br>Chickens<br>Cattle, chickens<br>Pigs<br>Cattle | For cattle and chickens MRLs are expressed as the sum of spiramycin and neospiramycin<br><br>For pigs MRLs expressed as spiramycin equivalents (antimicrobially active residues) |
| Streptomycin (see dihydrostrepto-mycin | | | | | | | |
| Sulfadimidine | 0-50 | Full | 42 (1994) | 100<br><br>25 µg/l | Muscle, liver, kidney, fat<br><br>Milk | Cattle, sheep, pigs, poultry<br>Cattle | Parent drug |
| Sulphthiazole | No ADI | | 34 (1989) | No MRL | | | |
| Testosterone | 0-2 | Full | 52 (1952) | Not specified | Muscle, liver, kidney, fat | Cattle | |
| Tetracycline (see Chlortetracycline) | | | | | | | |
| Thiamphenicol | 0-5 | Full | 52(1999) | 50 T<br>100 T<br>500 T<br>50 T | Muscle, fat<br>Liver<br>Kidney<br>Muscle | Pigs<br>Pigs<br>Pigs<br>Fish | Thiamphenicol and thiamphenicol conjugates measured as thiamphenicol |
| Thiabendazole | 0-100 | Full | 48 (1997)<br>40 (1992) | <br>100<br><br>100 µg/l | <br>Muscle, liver, kidney, fat<br><br>Milk | <br>Cattle, pigs, goats, sheep<br>Cattle, goats | Sum of thiabendazole and 5-hydroxythiabendazole |
| Tilmicosin | 0-40 | Full | 47 (1996) | 100<br><br>1000<br>1500<br>300<br>1000<br>50 µg/l T | Muscle, fat<br><br>Liver<br>Liver<br>Kidney<br>Kidney<br>Milk | Cattle, pigs, sheep<br>Cattle, sheep<br>Pigs<br>Cattle, sheep<br>Pigs<br>Sheep | Parent drug |

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|---|---|---|---|---|---|---|---|
| Trenbolone acetate | 0-0.02 | Full | 34 (1989) | 2<br>10 | Muscle<br>Liver | Cattle | β-Trenbolone for muscle<br>α-Trenbolone for liver |
| Triclabendazole | 0-3 | Full | 40 (1992) | 200<br>300<br>100<br>100 | Muscle<br>Liver, kidney<br>Fat<br>Muscle, liver kidney, fat | Cattle<br><br><br>Sheep | 5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one |
| Tylosin | No ADI | | 38 (1991) | No MRL | | | |
| Xylazine | No ADI | | 47 (1996) | No MRL | | | |
| Zeranol | 0-0.5 | Full | 32 (1987) | 2<br>10 | Muscle<br>Liver | Cattle | Parent drug |

**ANNEX 2**

# SUMMARY OF 52nd JECFA RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

***ß-Adrenoceptor-blocking agent***

**Carazolol**

Acceptable daily intake: 0 – 0.1 µg/kg bw (established at the forty-third meeting of the Committee (WHO TRS 855, 1995))
Acute RfD: 0 – 0.1 µg/kg bw
Residue definition: Carazolol

***Recommended maximum residue limits (MRLs)[1]***

| Species | Muscle (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) |
|---|---|---|---|---|
| Pigs | 5 | 25 | 25 | 5[2] |

[1]Recommended at the forty-third meeting of the Committee (WHO TRS 855, 1995). Residues of carazolol at the injection site two hours after treatment may result in an intake that exceeds the acute RfD. Therefore, unless appropriate measures can be taken to ensure that residues at the injection site do not exceed the acute RfD, the use of carazolol during the transport of animals to slaughter is inconsistent with safe use of the drug.
[2]Fat/skin

***Anthelminthic agent***

**Doramectin**

Acceptable daily intake: 0 – 0.5 µg/kg bw (established at the forty-fifth meeting of the Committee (WHO TRS 864, 1996))
Residue definition: Doramectin

***Recommended maximum residue limits (MRLs)[1]***

| Species | Muscle (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) |
|---|---|---|---|---|
| Cattle[2] | 10 | 100 | 30 | 150 |
| Pigs | 5 | 100 | 30 | 150 |

[1]The Committee noted the high concentrations of residues at the injections sites.
[2]Recommended at the forty-fifth meeting of the Committee (WHO TRS 864, 1996)

*Antimicrobial agents*

**Dihydrostreptomycin/Streptomycin**

Acceptable intake: 0 – 50 µg/kg bw (established at the forty-eighth meeting of the Committee (WHO TRS 879, 1998))

Residue definition: Sum of the concentrations of dihydrostreptomycin and streptomycin

***Recommended maximum residue limits (MRLs)***

| Species | Muscle[1] (µg/kg) | Liver[1] (µg/kg) | Kidney[1] (µg/kg) | Fat[1] (µg/kg) | Milk (µg/kg) |
|---|---|---|---|---|---|
| Cattle | 600 | 600 | 1000 | 600 | 200[2] |
| Pigs | 600 | 600 | 1000 | 600 | |
| Sheep | 600 | 600 | 1000 | 600 | |
| Chickens | 600 | 600 | 1000 | 600 | |

[1]The Committee was aware of more sensitive analytical methods for dihydrostreptomycin and streptomycin in edible tissue and requested that additional analysis methods be made available to the Committee for evaluation in 2001.
[2]Temporary. The following information is required for evaluation in 2001:
1. A validated analytical method that will quantitate both compounds in milk at a low level.

**Neomycin**

Acceptable daily intake: 0 – 60 µg/kg bw (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1998))

Residue definition: Neomycin

***Recommended maximum residue limits (MRLs)***

| Species | Muscle (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) | Milk (µg/kg) | Eggs (µg/kg) |
|---|---|---|---|---|---|---|
| Cattle | 500[1] | 15 000 | 20 000 | 500[1] | 500[1] | |
| Pigs | 500[1] | 500[1] | 10 000[2] | 500[1] | | |
| Sheep | 500[1] | 500[1] | 10 000[2] | 500[1] | | |
| Goats | 500[1] | 500[1] | 10 000[2] | 500[1] | | |
| Turkeys | 500[1] | 500[1] | 10 000[2] | 500[1] | | |
| Ducks | 500[1] | 500[1] | 10 000[2] | 500[1] | | |
| Chickens | 500[1] | 500[1] | 10 000[2] | 500[1] | | 500[1] |

[1]Recommended at the forty-third meeting of the Committee (WHO TRS 855, 1995)
[2]Recommended at the forty-seventh meeting of the Committee (WHO TRS 876, 1998)

**Thiamphenicol**

Acceptable daily intake: 0 – 5 µg/kg bw

Residue definition: Sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol

***Recommended maximum residue limits (MRLs)***

| Species | Muscle (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) |
|---|---|---|---|---|
| Cattle[1] | withdrawn | Withdrawn | Withdrawn | withdrawn |
| Pigs[2] | 50 | 100 | 500 | 50 |
| Chickens[1] | withdrawn | withdrawn | withdrawn | withdrawn |
| Fish[2] | 50 | | | |

[1]The previous temporary MRLs for cattle and chickens were withdrawn as the data required by the forty-seventh meeting of the Committee (WHO TRS 876, 1998) were not provided.
[2]Temporary. The following information is required for evaluation in 2002:

1. A radiolabel depletion study in pigs to determine the relationship between free thiamphenicol, thiamphenicol conjugates and total residues in all tissues.
2. A validated analytical method for use in all animal tissues, which incorporates an enzymatic hydrolysis step allowing the determination of the sum of thiamphenicol and thiamphenicol conjugates as free thiamphenicol.

***Insecticides***

**Deltamethrin**

Acceptable daily intake: 0 – 10 µg/kg bw (established by the1982 Joint FAO/WHO Meeting on Pesticide Residues (FAO Plant Production and Protection Paper 46, 1983))
Residue definition: Deltamethrin

***Recommended maximum residue limits (MRLs)***

| Species | Muscle[1] (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) | Milk[1] (µg/kg) | Egg[1] (µg/kg) |
|---|---|---|---|---|---|---|
| Cattle | 30 | 50 | 50 | 500 | 30 | |
| Sheep | 30 | 50 | 50 | 500 | | |
| Chickens | 30 | 50 | 50 | 500 | | 30 |
| Salmon | 30 | | | | | |

[1]No residues were detected. MRLs are for guidance only and are based on two times the limit of quantification of the analytical method.

**Phoxim**

Acceptable daily intake: 0 – 4 µg/kg bw
Residue definition: Phoxim

***Recommended maximum residue limits (MRLs)***[1]

| Species | Muscle (µg/kg) | Liver (µg/kg) | Kidney (µg/kg) | Fat (µg/kg) | Milk (µg/kg) |
|---|---|---|---|---|---|
| Cattle | 50 | 50 | 50 | 400 | 10 |
| Pigs | 50 | 50 | 50 | 400 | |
| Sheep | 50 | 50 | 50 | 400 | |
| Goats | 50 | 50 | 50 | 400 | |

[1]Temporary. The following information is required for evaluation in 2002:

1. Radiolabel studies to relate the marker residue to the total residue in ruminants and pigs following topical application of the formulated product
2. Residue studies in accordance with Good Laboratory Practice using the current recommended treatments in cattle and sheep
3. Validation of available analytical methods for phoxim residues in tissues of cattle, sheep, goats and in milk from cattle

*Production aids*

**Estradiol-17ß, progesterone, and testosterone**

Acceptable daily intakes

| | |
|---|---|
| Estradiol-17ß: | 0 – 0.05 µg/kg bw |
| Progesterone: | 0 – 30 µg/kg bw |
| Testosterone: | 0 – 2 µg/kg bw |

Maximum residue limits: MRLs "not specified"[1] in cattle muscle, liver, kidney, and fat

[1]MRL "not specified" means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

**Porcine Somatotropin**

Acceptable daily intake: ADI "not specified"[1] (applies to Grolene®, Reporcin®, and Somagrepor®)
Maximum residue limit: MRLs "not specified"[2] in pig muscle, liver, kidney, and fat (applies to Grolene®, Reporcin®, and Somagrepor®)

[1]ADI "not specified" means that available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that use of the veterinary drug does not represent a hazard to human health and that there is no need to specify a numerical ADI.

[2]See definition of MRL "not specified" under estradiol-17ß, progesterone, and testosterone.

*Tranquilizing agent*

**Azaperone**

New information on the method of analysis in tissues of pigs was reviewed. Insufficient characterization on specificity, accuracy, and reproducibility of the method was provided. The Committee recommended that the method be improved and after further development it be forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

**FAO TECHNICAL PAPERS**

FAO FOOD AND NUTRITION PAPERS

| | |
|---|---|
| 1/1 | Review of food consumption surveys 1977 – Vol. 1. Europe, North America, Oceania, 1977 (E) |
| 1/2 | Review of food consumption surveys 1977 – Vol. 2. Africa, Latin America, Near East, Far East, 1979 (E) |
| 2 | Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E F S) |
| 3 | Report of a joint FAO/WHO expert consultation on dietary fats and oils in human nutrition, 1977 (E F S) |
| 4 | JECFA specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers, 1978 (E) |
| 5 | JECFA – guide to specifications, 1978 (E F) |
| 5 Rev. 1 | JECFA – guide to specifications, 1983 (E F) |
| 5 Rev. 2 | JECFA – guide to specifications, 1991 (E) |
| 6 | The feeding of workers in developing countries, 1976 (E S) |
| 7 | JECFA specifications for identity and purity of food colours, enzyme preparations and other food additives, 1978 (E F) |
| 8 | Women in food production, food handling and nutrition, 1979 (E F S) |
| 9 | Arsenic and tin in foods: reviews of commonly used methods of analysis, 1979 (E) |
| 10 | Prevention of mycotoxins, 1979 (E F S) |
| 11 | The economic value of breast-feeding, 1979 (E F) |
| 12 | JECFA specifications for identity and purity of food colours, flavouring agents and other food additives, 1979 (E F) |
| 13 | Perspective on mycotoxins, 1979 (E F S) |
| *14* | *Manuals of food quality control:* |
| 14/1 | Food control laboratory, 1979 (Ar E) |
| 14/1 Rev.1 | The food control laboratory, 1986 (E) |
| 14/2 | Additives, contaminants, techniques, 1980 (E) |
| 14/3 | Commodities, 1979 (E) |
| 14/4 | Microbiological analysis, 1979 (E F S) |
| 14/5 | Food inspection, 1981 (Ar E) (Rev. 1984, E S) |
| 14/6 | Food for export, 1979 (E S) |
| 14/6 Rev.1 | Food for export, 1990 (E S) |
| 14/7 | Food analysis: general techniques, additives, contaminants and composition, 1986 (C E) |
| 14/8 | Food analysis: quality, adulteration and tests of identity, 1986 (E) |
| 14/9 | Introduction to food sampling, 1988 (Ar C E F S) |
| 14/10 | Training in mycotoxins analysis, 1990 (E S) |
| 14/11 | Management of food control programmes, 1991 (E) |
| 14/12 | Quality assurance in the food control microbiological laboratory, 1992 (E F S) |
| 14/13 | Pesticide residue analysis in the food control laboratory, 1993 (E F) |
| 14/14 | Quality assurance in the food control chemical laboratory, 1993 (E) |
| 14/15 | Imported food inspection, 1993 (E F) |
| 14/16 | Radionuclides in food, 1994 (E) |
| 14/17 | Unacceptable visible can defects – a pictorial manual, 1998 (E F S) |
| 15 | Carbohydrates in human nutrition, 1980 (E F S) |
| 16 | Analysis of food consumption survey data for developing countries, 1980 (E F S) |
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| 18 | Bibliography of food consumption surveys, 1981 (E) |
| 18 Rev. 1 | Bibliography of food consumption surveys, 1984 (E) |
| 18 Rev. 2 | Bibliography of food consumption surveys, 1987 (E) |
| 18 Rev. 3 | Bibliography of food consumption surveys, 1990 (E) |
| 19 | JECFA specifications for identity and purity of carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents and other food additives, 1981 (E F) |
| 20 | Legumes in human nutrition, 1982 (E F S) |
| 21 | Mycotoxin surveillance – a guideline, 1982 (E) |
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| 27 | Review of food consumption surveys 1981, 1983 (E) |
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| 29 | Post-harvest losses in quality of food grains, 1983 (E F) |
| 30 | FAO/WHO food additives data system, 1984 (E) |
| 30 Rev. 1 | FAO/WHO food additives data system, 1985 (E) |
| 31/1 | JECFA specifications for identity and purity of food colours, 1984 (E F) |
| 31/2 | JECFA specifications for identity and purity of food additives, 1984 (E F) |
| 32 | Residues of veterinary drugs in foods, 1985 (E/F/S) |
| 33 | Nutritional implications of food aid: an annotated bibliography, 1985 (E) |
| 34 | JECFA specifications for identity and purity of certain food additives, 1986 (E F) |
| 35 | Review of food consumption surveys 1985, 1986 (E) |
| 36 | Guidelines for can manufacturers and food canners, 1986 (E) |
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| 38 | JECFA specifications for identity and purity of certain food additives, 1988 (E) |
| 39 | Quality control in fruit and vegetable processing, 1988 (E F S) |
| 40 | Directory of food and nutrition institutions in the Near East, 1987 (E) |
| 41 | Residues of some veterinary drugs in animals and foods, 1988 (E) |
| 41/2 | Residues of some veterinary drugs in animals and foods. Thirty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1990 (E) |
| 41/3 | Residues of some veterinary drugs in animals and foods. Thirty-sixth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E) |
| 41/4 | Residues of some veterinary drugs in animals and foods. Thirty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E) |

41/5 Residues of some veterinary drugs in animals and foods. Fortieth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1993 (E)
41/6 Residues of some veterinary drugs in animals and foods. Forty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/7 Residues of some veterinary drugs in animals and foods. Forty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/8 Residues of some veterinary drugs in animals and foods. Forty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1996 (E)
41/9 Residues of some veterinary drugs in animals and foods. Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1997 (E)
41/10 Residues of some veterinary drugs in animals and foods. Forty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1998 (E)
41/11 Residues of some veterinary drugs in animals and foods. Fiftieth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1999 (E)
41/12 Residues of some veterinary drugs in animals and foods. Fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2000 (E)
42 Traditional food plants, 1988 (E)
42/1 Edible plants of Uganda. The value of wild and cultivated plants as food, 1989 (E)
43 Guidelines for agricultural training curricula in Arab countries, 1988 (Ar)
44 Review of food consumption surveys 1988, 1988 (E)
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47/1 Utilization of tropical foods: cereals, 1989 (E F S)
47/2 Utilization of tropical foods: roots and tubers, 1989 (E F S)
47/3 Utilization of tropical foods: trees, 1989 (E F S)
47/4 Utilization of tropical foods: tropical beans, 1989 (E F S)
47/5 Utilization of tropical foods: tropical oil seeds, 1989 (E F S)
47/6 Utilization of tropical foods: sugars, spices and stimulants, 1989 (E F S)
47/7 Utilization of tropical foods: fruits and leaves, 1990 (E F S)
47/8 Utilization of tropical foods: animal products, 1990 (E F S)
48 Number not assigned
49 JECFA specifications for identity and purity of certain food additives, 1990 (E)
50 Traditional foods in the Near East, 1991 (E)
51 Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation, 1991 (E F)
52/1 Compendium of food additive specifications – Vol. 1, 1993 (E)
52/2 Compendium of food additive specifications – Vol. 2, 1993 (E)
52 Add. 1 Compendium of food additive specifications – Addendum 1, 1992 (E)
52 Add. 2 Compendium of food additive specifications – Addendum 2, 1993 (E)
52 Add. 3 Compendium of food additive specifications – Addendum 3, 1995 (E)
52 Add. 4 Compendium of food additive specifications – Addendum 4, 1996 (E)
52 Add. 5 Compendium of food additive specifications – Addendum 5, 1997 (E)
52 Add. 6 Compendium of food additive specifications – Addendum 6, 1998 (E)
52 Add. 7 Compendium of food additive specifications – Addendum 7, 1999 (E)
53 Meat and meat products in human nutrition in developing countries, 1992 (E)
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55 Sampling plans for aflatoxin analysis in peanuts and corn, 1993 (E)
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## CORRIGENDA

FAO Food and Nutrition Paper No. 41/12 – 2000
*Residues of some veterinary drugs in animals and foods*

Page 85 Replace Table 61 with the following Table 61.
Page 120 Replace with the following page 120.

TC/M/X4259E/Corr.1/9.00/2000
ISSN 0254-4725
ISBN 92-5-104401-5

**Table 61. Calculations of excess TMDI from bovine animals treated with estradiol 17-β, progesterone and testosterone**

| Product | Animals | Comments | Description of the treatment of the animals | Theoretical Maximum Daily Intakes [nanograms/person/day] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $E_1$ | $E_2$-17α | $E_2$-17β | Excess $E_1$+$E_2$-β | P | T |
| Synovex-S ($E_2$-b+P) | Steers | 1 | **Control animals** | **1.0** | | **0.5** | | **190** | |
| | | | Animals slaughtered 15 days after implantation | 2.0 | | 6.3 | 6.8 | 254 | |
| Synovex H ($E_2$-b+T-p) | Heifers | 1 | **Control animals** | **1.4** | | **1.5** | | | **17** |
| | | | Animals slaughtered 15 days after implantation | 3.9 | | 15 | 16 | | 70 |
| Synovex-C ($E_2$-b+P) | Calves a) female | 1 | **Control animals, slaughtered on day 61** | **1.1** | | **3.5** | | **22** | |
| | | | **Control animals, slaughtered on day 119** | **1.2** | | **2.2** | | **53** | |
| | | | **Control animals, slaughtered on day 240** | **0.8** | | **1.7** | | | **13** |
| | | | **Control animals, slaughtered on day 301** | **1.4** | | **4.4** | | | **22** |
| | | | **Control animals, slaughtered on day 329** | **0.7** | | **2.1** | | | **22** |
| | | | **Control animals, slaughtered on day 360** | **2.0** | | **3.0** | | | **51** |
| | | | implanted day 0; slaughtered on day119 | 3.0 | | 3.7 | 3.3 | 520 | |
| Synovex-C ($E_2$-b+P) Synovex S ($E_2$-b+P) | Calves b) castrated males | | **Control animals, slaughtered on day 61** | **0.8** | | **0.7** | | **501** | |
| | | | **Control animals, slaughtered on day 119** | **0.4** | | **0.5** | | **552** | |
| | | | **Control animals, slaughtered on day 240** | **1.1** | | **1.2** | | **669** | |
| | | | **Control animals, slaughtered on day 301** | **0.5** | | **0.9** | | **421** | |
| | | | **Control animals, slaughtered on day 329** | **1.2** | | **0.7** | | **536** | |
| | | | **Control animals, slaughtered on day 360** | **0.8** | | **1.0** | | **1170** | |
| | | | implanted on days 0, 118, 240; slaughtered on day 301 | 3.7 | | 11 | 13.3 | 540 | |
| Synovex H ($E_2$-b+T-p) | Pregnant heifers | 2 | **120 days pregnant , unsynchronized controls** | **93** | | **16** | | | **203** |
| | | | **120 days pregnant , synchronized controls** | **113** | | **16** | | | **172** |
| | | | 120 days pregnant , 61 days implanted | 34 | | 15 | -80 | | 233 |
| | | | **180 days pregnant , synchronized controls** | **280** | | **48** | | | **282** |
| | | | 180 days pregnant , 61 days implanted | 107 | | 24 | -197 | | 237 |
| | | | **240 days pregnant , synchronized controls** | **326** | | **139** | | | **377** |
| | | | 240 days pregnant , 61 days implanted | 377 | | 49 | -39 | | 326 |
| Steer-oid ($E_2$ –b+ P) | Steers | 3 | **Control animals** | | | **21** | | **299** | |
| | | | Animals slaughtered 15 days after implantation | | | 25 | 4 | 375 | |
| Heifer-oid ($E_2$ –b +T-p) | Heifers | | **Control animals** | | | **16** | | | **43** |
| | | | Animals slaughtered 15 days after implantation | | | 18 | 2 | | 48 |
| Compudose ($E_2$) | Steers | 4 | **Control animals** | **4.4** | | **4.5** | | | |
| | | | Animals implanted 70-180 days | 7.4 | | 5.7 | 4.2 | | |

| Product | Animals | Comments | Description of the treatment of the animals | Theoretical Maximum Daily Intakes [nanograms/person/day] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $E_1$ | $E_2$-17α | $E_2$-17β | excess $E_1$+$E_2$-β | P | T |
| Compudose ($E_2$) | Heifers | 4 | **Control animals** | **3.1** | | **3.7** | | | |
| | | | Animals implanted 84 days | **3.6** | | **4.3** | | | |
| Compudose ($E_2$) | Bull calves | 4 | **Control animals** | **4.0** | | 4.0 | | | |
| | | | Animals implanted | 9.0 | | 14 | 15 | | |
| | Bulls | 4 | **Control animals** | **3.8** | | **3.3** | | | |
| | | | Animals implanted | 5.0 | | 5.9 | 3.8 | | |
| | Zebu Steers | 4 | **Control animals** | **6.1** | | **3.6** | | | |
| | | | Animals implanted | 4.4 | | 3.4 | -1.9 | | |
| FINAPLIX (Tb-ac) | Heifers | 5 | implanted animals, slaughtered on day 15 | 4.6 | | 23 | 27.6 | | |
| | | | implanted animals, slaughtered on day 30 | 5.2 | | 29 | 34.2 | | |
| | | | implanted animals, slaughtered on day 60 | 3.9 | | 13 | 16.9 | | |
| | | | implanted animals, slaughtered on day 75 | 3.7 | | 14 | 17.7 | | |
| TORELOR (Tb-ac+$E_2$) | Steers | 5 | **Control animals** | **3.1** | | **19** | | | |
| | | | Animals implanted on day 0; slaughtered on day 30 | 11 | | 66 | 54.9 | | |
| | | | Animals implanted on days 0, 60; slaughtered on day 90 | 7.3 | | 89 | 74.2 | | |
| Revalor (Tb-ac +$E_2$) | Heifers | 6 | **Control animals** | | | **1.0** | | | |
| | | | Animals implanted 30 days | | | 6.6 | 5.6 | | |
| | Steers | | **Control animals** | | | **2.0** | | | |
| | | | Animals implanted 15 days | | | 3.4 | 1.4 | | |
| Revalor (Tb-ac +$E_2$) Implix BM ($E_2$+P) | Calves | 7 | **Male control animals, slaughtered on day 30** | | **57** | **6.6** | | **628** | |
| | | | **Male control animals, slaughtered on day 80** | | **69** | **7.7** | | **493** | |
| | | | Revalor implanted, slaughtered on day 80 | | 132 | 63 | 55.3 | | |
| | | | Revalor/Implix implanted, slaughtered on day 100 | | 233 | 97 | 89.3 | 667 | |
| Revalor (Tb-ac +$E_2$) Implix BF ($E_2$+T) | | | **Female control animals, slaughtered on day 30** | | **60** | **6.5** | | | **19** |
| | | | **Female control animals, slaughtered on day 80** | | **59** | **8.6** | | | **493** |
| | | | Revalor implanted, slaughtered on day 80 | | 111 | 61 | 52.4 | | |
| | | | Revalor/Implix implanted, slaughtered on day 15 | | 179 | 99 | 90.4 | | 208 |

1 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. To obtain an estimate of the degree of under-estimation of the "true" TMDI, information from a study with implants containing $^{14}C$-labeled hormones/hormone esters in the same proportions as in the commercial products can be used. Based on total radioactivity found in tissues of animals slaughtered 15 days after implantation and on the fractions of total residues identified as conjugates the individual contributions in percent of the TMDI of the free vs. conjugated fractions present in the four standard edible tissues have been calculated in Table 62. From these data it appears justifiable to multiply the estimates of TMDI 's for consumption of tissues from steers/heifers implanted with Synovex S / Synovex H with a factor of two. This correction is probably not relevant in the case of progesterone, where the tentatively identified conjugated metabolites have no significant gestagenic properties.

2 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. A correction of the data is not possible due to a lack of relevant information. However, in view of the well established significant reduction in the TMDI as a consequence of implantation of pregnant heifers such a correction is apparently unnecessary.

3 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle and fat. The given figures most likely greatly underestimate the "true" TMDI 's. No information was available to correct these estimates.

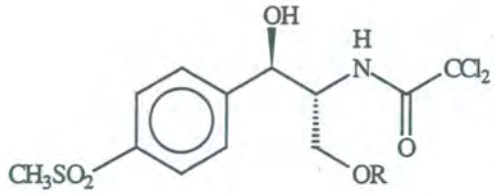
4 The method used includes the extraction and de-conjugation of conjugates. The estimated intake figures, therefore, represent total parent compound and can be used as they are given in the table.

5 The free and conjugated fractions of estradiol-17β were determined in all tissues. However, estrone (free fraction only) was only determined in liver and fat. The "true" TMDI 's for estrogens, therefore, could well be 50% higher than the values given in the table. Data on which a more precise estimate of a correction factor could be based were not available. The Torelor study represents research work and does not reflect an approved use.

6 From the method description it appears that conjugates are not included in the determination of the residues. In view of the effects of trenbolone/estradiol combinations on estrogen concentrations seen in other studies, it cannot be excluded that the data given in the table significantly underestimate the "true" TMDI for estrogens.

7 No description of the analytical method was given in the report. The values for estradiol-17α are based on concentrations found in liver only. The study represents research work and does not reflect an approved use.

**Chemical structure:**




R = H
Thiamphenicol

R = $COCH_2NH_2$ HCl
Thiamphenicol glycinate hydrochloride

## OTHER INFORMATION ON IDENTITY AND PROPERTIES

**Appearance:** White crystalline powder

**Melting point:** 164-166$^0$C

**Optical rotation:** $[\alpha]_D^{25°} = +12.9^0$ (ethanol)

**UV spectrum (max):** 224, 266, 274 nm (ε, 13,700,800,700)

## RESIDUES IN FOOD AND THEIR EVALUATION

### CONDITIONS OF USE

General

Thiamphenicol is used for the treatment of certain bacterial diseases in cattle, pigs, poultry and fish. The product is usually used as an oral preparation, but is not suitable for the treatment of ruminating cattle.

Dosage

Thiamphenicol is used for oral administration and thiamphenicol glycine hydrochloride is utilised in formulations for parenteral use.

## METABOLISM AND PHARMACOKINETIC STUDIES

Pigs

In a study which complied with GLP (Redgrave *et al.*, 1991), sixteen pigs weighing 15-22 kg and about 7 weeks old were fed thiamphenicol in the diet twice a day for five consecutive days. Each dose was approximately 30 mg/kg BW and three untreated animals acted as controls. Blood samples were taken immediately prior to dosing and at periods during the next 10 days. Results, which appear in Table 1, show that maximum mean plasma levels of 1.28 mg/L were attained 8 h after the first administration. After the withdrawal of treatment, plasma concentrations declined to close to or below, the LOD of the assay method after five days.

In another non-GLP study in pigs (Fornasini, 1992), thiamphenicol was orally administered twice daily to 15 pigs of mean weight 30 kg, randomly divided into three groups of five, and dosed at 10, 15 and 20 mg/kg BW for five consecutive days. The plasma values for thiamphenicol and thiamphenicol glucuronide were determined by GC using electron capture detection. The results of this study, previously summarised (FAO, 1997), are important in that they showed that glucuronide formation is an important route of elimination of thiamphenicol in pigs. Indeed, the mean plasma glucuronide concentrations at all time points after the last dose were higher than the concentrations of free

This document is one of three publications prepared by the fifty-second session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome, in February 1999, and dedicated exclusively to the evaluation of veterinary drug residues in food. The report of the meeting will be published in the WHO Technical Report Series, and the toxicological monographs as No. 43 in the WHO Food Additives Series. Residue monographs in this document provide information on chemical identity, properties, use, pharmacokinetics, metabolism, tissue residue depletion of and analytical methods for the substances indicated on the cover. This publication is meant for regulatory authorities, veterinary drug researchers and any other concerned persons who wish to gain information on and insights into the needs and problems involved in establishing maximum limits for veterinary drug residues in food.

ISBN 92-5-104401-5 ISSN 0254-4725

9 789251 044018

M-84 X4259E/1/1.00/2050