DEXAMETHASONE

First draft prepared by
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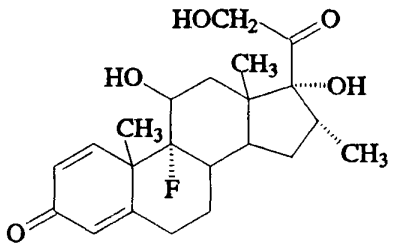
IDENTITY

Chemical name: (11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

Synonyms: Aeroseb-D, Anaflogistico, Calonat, Corson, Cortisumman, Decacortin, Decaderm, Decalix, Decaron, Decasone, Dectancyl, Dekacort, Deltafluorene, Dergramin, Deronil, Deseronil, Dexacortal, Dexa-Cortidel, Dexacortin, Dexa-cortisyl, Dexafarma, Dexa-Mamallet, Dexameth, Dexapos, Dexa-sine, Dexa-Scheron, Dexasone, Dexinolon, Dexralan, Dextelan, Dinormon, Dexiporal, Fluormone, Fortecortin, Gammacorten, Hexadrol, Isopto-Dex, Lokalison F, Loverine, Luxazone, Maxidex, Millicorten, Oradexon, Pet Derm III, Policort, Spoloven.

Structural formula:



Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.45

Important ester preparations:

Dexamethasone 21-acetate: Decadron-LA, Panasone

Dexamethasone 21-(3,3-dimethyl)-butyrate: Decadron TBA, Dexamethasone *tert*-butylacetate, Dexamedium

Dexamethasone 21-disodium phosphate: Dex, Baldex, Dalaron, Dexabene, Dexadreson, Dezone, Solu-Decadron, Tubinaire, Orgadron, Colvasone, Soldesam

Dexamethasone 21-disodiumphosphate + Dexamethasone 21-phenylpropionate: Dexafort

Dexamethasone 21-trimethylacetate: Opticortenol

Dexamethasone 21-isonicotinate: Auxiloson, Auxisone, Voren

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Dexamethasone

Appearance:

Melting point: 262-264°C

Optical rotation: $[a]_D^{25} = +77.5°$ (in dioxane)

Solubility:

Dioxane	24 g/L
Acetone	11 g/L
Chloroform	10 g/L
Methanol	96 g/L
Ethanol, 96%	8.3 g/L
Water	0.001 g/L

Indications:

cattle/sheep: acetonemia, orthopaedic disorders, inflammatory processes of the locomotor system, e.g. arthritis, tendovaginitis, bursitis and tendinitis, allergic skin diseases.

horses: orthopaedic disorders, inflammatory processes of the locomotor system, e.g. arthritis, tendovaginitis, bursitis and tendinitis, allergic disorders of the respiratory system, e.g. COPD. Allergic skin diseases.

pigs: orthopaedic disorders, inflammatory processes of the locomotor system, e.g. arthritis, oedema, *E. Coli* enterotoxaemia.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Dexamethasone is a potent synthetic analogue of hydrocortisone. It has been used for many years in human medicine for the treatment of a wide range of diseases. This wide range of therapeutic use reflects the broad spectrum of pharmacological actions of the corticosteroid hormones. The corticosteroids have effects on several important biochemical pathways and cellular transport mechanisms including, sodium transport in and out of the cell, glycogen synthesis and antiinflammatory responses.

Dexamethasone is also used in veterinary medicine for the treatment of a correspondingly broad spectrum of disorders in both companion and farm animals. Animal diseases in which dexamethasone is an effective treatment include, inflammation, acetonaemia, non-specific skin disease, shock and stress.

Dosages

Species: cattle, pigs, sheep and horses

Routes of administration: i.v., i.m., s.c., intra-articular

Dose range: 15-90 μg/kg
(20-100 μg/kg as ester preparations)

METABOLISM AND PHARMACOKINETICS

Dexamethasone and its ester derivatives have been widely used drugs for over 30 years and their pharmacokinetics have been studied in both animals and man.

Due to the large differences in the pharmacokinetic behaviour of the various esters only data on dexamethasone will be reviewed below. These differences originate from the different absorption rates following i.m. injection. However, esters are rapidly hydrolysed to dexamethasone by blood enzymes and metabolism studies conducted on esters are also valid for the metabolism of dexamethasone itself.

Radiometric studies with both tritiated dexamethasone and dexamethasone esters have been conducted.

Horses

Metabolism

Dumasia et. al. (1986) administered [1,2-^{3}H]-dexamethasone i.m. to castrated male crossbred horses (n = 2) at doses of 86 μg/kg bw (horse A) and 83 μg/kg bw (horse B). Urine was collected when voided up to 100 h post dosing. Radioactivity was determined in aliquots of the urine samples and urinary metabolites were separated by TLC and analysed by GC-MS. In total, 91.7% (horse A) and 117% (horse B) of the radioactivity was excreted in urine within 96 h (Table 1).

Table 1. Excretion of Radioactivity in the Urine of Two Horses after i.m. Administration of [^{3}H]-Dexamethasone (Dumasia, 1986).

	% Dose Excreted	
	Horse A	**Horse B**
24 h	40.2	56.0
24-96 h	51.5	61.0
Total	**91.7**	**117.0**
Composition of 24 h sample:		
Unconjugated	26.5	36.0
Conjugated	7.8	13.3
Unextractable	4.2	5.0
Total	**38.5**	**54.3**
Expressed as % of total in 24 h sample	95.8	97.0

The 24 h urine sample was investigated further in order to identify dexamethasone metabolites. In addition to dexamethasone the following 5 metabolites were identified, dexamethasone glucuronide, 6-hydroxydexamethasone, 17-oxodexamethasone, 11-dehydrodexamethasone and 20-dihydrodexamethasone. It is therefore clear that the predominant metabolic pathways in the horse involves hydroxylation at C6 with minor oxidation pathways at both C11 and C17 and reduction at C20.

The major urinary metabolite isolated after administration of dexamethasone to a horse is shown in Figure 1 (Skrabalak et. al., 1984). The ratio of this urinary metabolite to dexamethasone at time periods after administration is shown in Table 2. Most striking is the change which takes place between the third and fourth

hour following dosing. During this time the isolated dexamethasone metabolite assumes predominance and within the next two hours attains an average relative concentration of nearly 150 times that of the first hour. Thus a clear pattern is established 4 hours after dosing where the metabolite is the preferred marker residue for detection of dexamethasone administration. These data have been challenged on the grounds that the metabolite isolated is probably an artefact of the analytical work-up procedure (Bette 1993b).

Table 2. Relative Concentrations of Dexamethasone Metabolite to Dexamethasone in Urine (n=3)

Time Post-administration (h)	Relative Concentrations of Dexamethasone Metabolite to Dexamethasone ± SD
1	0.038 ± 0.013
2	0.182 ± 0.072
3	0.493 ± 0.281
4	1.982 ± 1.125
5	3.380 ± 1.935
6	5.619 ± 2.684

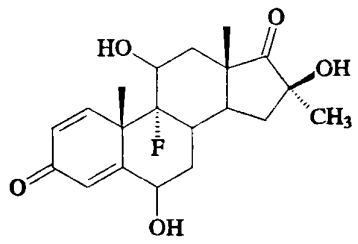


Figure 1. Major Urinary Metabolite Isolated after Administration of Dexamethasone to a Horse

Pharmacokinetics

Dexamethasone (3 mg per animal = 0.06 μg/kg) was administered i.v. to 6 female Welsh ponies (Intervet, 1990). Blood samples were taken at the following times 0, 5, 10, 20, 30 and 45 min, then 1, 1.5, 2, 3, 4, 6, 8, 11, 15, 24, 32 and 48 h after dosing. The disposition of dexamethasone in ponies fitted a three compartment open model, $t_{1/2}\alpha$ = 0.33 ± 0.13 h, $t_{1/2}\beta$ = 3.02 ± 0.71 h and $t_{1/2}\gamma$ = 13.28 ± 3.98 h. Clearance was 0.44 ± 0.039 L/h/kg.

Toutain et al. (1984) have also reported studies on the pharmacokinetics of dexamethasone in the horse. Six horses (saddle bred, 5 male, 1 female; aged 7 - 15 years) were administered i.m. or i.v. with dexamethasone at 50 μg/kg body weight (the same 6 horses were used for both i.m. and i.v. studies: a 10 day wash-out period was allowed between experiments). Blood samples were collected at 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 45, 60 and 75 min, then 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12 and 24 h. The mean times (± SD) were $t_{1/2}\beta$ = 53.3 ± 14.0 min, V_D = 0.96 ± 0.193 L/kg and the clearance was 12.8 cm^3/min/kg.

Rat

Metabolism and Pharmacokinetics

In the rat (Wistar, male; n=4), the majority of an oral dose (0.527 μg/kg body weight) of [1,2-^{3}H]-dexamethasone was excreted in the urine (31%) and the faeces (25%) within 4 days of dosing (English et al., 1975). Most of the radioactivity excreted in urine was eliminated within the first 24 h (Table 3).

The largest proportion (90%) of the radioactivity excreted in urine was in an unconjugated form. Dexamethasone accounted for 13.6%, 6-hydroxydexamethasone for 7.4% and 20-dihydrodexamethasone for 1.1% of the administered radioactive dose. The remaining fraction of the administered dose was not traced as metabolites.

Table 3. Excretion of Radioactivity in the Urine and Faeces of Rats Administered [1,2-^{3}H]-Dexamethasone Orally (English et al, 1975).

Time After Dosing (h)	Cumulative % Dose Excreted Mean ± SD, n = 4	
	Urine	Faeces
6	10.1 ± 2.0	1.7 ± 0.8
24	27.8 ± 1.8	17.0 ± 0.7
48	30.0 ± 1.5	23.4 ± 1.0
72	30.9 ± 1.4	24.2 ± 1.1
96	31.4 ± 1.3	24.8 ± 1.1

Four days after administration of radiolabelled dexamethasone to the rats, they were sacrificed and residual radioactivity in organs determined (Table 4). Very small amounts of radioactivity remained and the small proportion of residual radioactivity in the GI contents at this time is consistent with biliary excretion of dexamethasone in the rat.

Table 4. Residues of Radioactivity in Tissues 4 Days after Oral Administration of [1,2-^{3}H]-Dexamethasone (0.527 μg/kg) to Rats (English et al., 1975).

Tissue	% Dose Mean ± SD, n = 4
Liver	0.19 ± 0.008
Intestinal Tract	0.05 ± 0.003
GI Contents	0.26 ± 0.009
Adipose Tissue	278 ± 47*
Muscle	364 ± 23*

* expressed as DPM/g tissue.

In another study (Rice et al, 1974) [1,2-^{3}H]-dexamethasone was given i.p. to rats (Waster, male; n=4) at a dose of 114 μg/kg. Urine and faeces were collected at 6 h and 24 h, then at days 2, 3 and 4.

Excretion of radioactivity in urine and faeces demonstrated a cumulative excretion of 30.4 ± 1.6% (mean ± SD) of the administered dose in urine within 96 h, the corresponding value for faeces is 43.6 ± 8% (mean ± SD). The majority of the radioactivity was eliminated within the first 24 h. Further studies on the metabolites of dexamethasone in urine collected within 24 h after injection demonstrated that 32% of the radioactivity excreted was 6-hydroxydexamethasone.

It is important to ascertain the extent of tritium exchange when quantitative metabolic studies utilising tritium-labelled compounds are undertaken. Stewart et al. (1992) have determined tritium exchange with body water after intramuscular administration of tritiated dexamethasone to rats. They showed extensive tritium exchange in plasma (87%) and urine (37%) samples collected 4 days following i.m. administration of [1,2,4-^{3}H]-dexamethasone to rats (n = 7, Sprague-Dawley, male) at 9 μg/kg. The plasma radioactivity level peaked (3.68 μg equivalents/g) at 6 h post dosing, falling rapidly to 0.15 μg equivalents/g by 96 h with a $t_{1/2}$ of approximately 7 hours. It is concluded that tritiated dexamethasone is not suitable for metabolic studies due to the misleading excretory results which might occur due to isotope exchange.

Pigs

Metabolism

Studies in pigs utilising [1,2-^{3}H]-dexamethasone trimethylacetate (Horner, 1989) demonstrated extremely low levels of dexamethasone trimethylacetate in blood due to the rapid hydrolysis of the ester (at 4 h after dosing <1% of the plasma radioactivity was dexamethasone trimethylacetate) with concomitant release of dexamethasone.

About 5% of the administered radioactivity was found as an unidentified band on TLC which was tentatively identified as 6-hydroxydexamethasone. The author also speculated that the large amount of unextractable radioactivity in the plasma is due to polar metabolites rather than to protein bound parent compound and/or metabolites. The possibility of tritium exchange was not considered in the discussion of conclusions.

Cattle

Pharmacokinetics

Lactating cows (n=6) i.v. injected with 24.8 mg (i.e. 40 μg/kg) dexamethasone followed by radio immunoassay of plasma for dexamethasone up to 48 h post dosing, demonstrated a 3 compartment pharmacokinetic model with rapid distribution, slow distribution and elimination phases with $t_{1/2}\alpha$ (mean $\pm$ SD) = 5.8 $\pm$ 0.41 h, $t_{1/2}\beta$ = 1.6 $\pm$ 0.4 h and $t_{1/2}\gamma$ = 9.7 $\pm$ 1.9 h, respectively (Intervet, 1989). Dexamethasone had a total V_D= 2.7 $\pm$ 0.3 L/kg and a clearance of 0.196 $\pm$ 0.022 L/h/kg. The kinetics in milk were very similar to those in blood (Table 5) for the first 3 milkings (up to 32 h). After this time, the milk dexamethasone level was greater than the corresponding plasma level at the same time point. The $t_{1/2}$ for dexamethasone in milk was 9.6 $\pm$ 3.5 h which is very similar to the elimination $t_{1/2}$ from plasma.

Table 5. Concentrations of Dexamethasone in Milk and Plasma after i.v. Administration of 40 μg/kg bw of Dexamethasone to Milking Cows (Intervet, 1989).

Time after Treatment (h)	Dexamethasone Concentration (ng/cm^3) - Mean $\pm$ SD	
	Plasma	Milk
0	0.22 $\pm$ 0.16	0.01 $\pm$ 0.006
8	6.23 $\pm$ 0.34	3.96 $\pm$ 1.29
24	2.39 $\pm$ 0.71	0.82 $\pm$ 0.50
32	1.29 $\pm$ 0.31	0.58 $\pm$ 0.65
48	0.48 $\pm$ 0.18	1.02 $\pm$ 0.37

In another study (Toutain et al., 1982) dexamethasone was administered i.v or i.m. to cows (n=4) at a dose of 0.1 mg/kg. Blood samples were taken at 1, 2, 4, 8, 16, 30 min, then 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h

post dosing. The effect of dexamethasone on the ACTH response test and plasma dexamethasone concentrations were determined by HPLC.

The semi logarithmic plasma dexamethasone concentration time plot following i.v. dosing showed a biphasic elimination with $t_{1/2}\alpha$ = 8.06 min, $t_{1/2}\beta$ = 5.5 h. The V_D = 1.18 L/kg. Following i.m. administration, the peak plasma concentration was reached at about 4 h (T_{max} = 256 min) after dosing. Bioavailability was 67% with a C_{max} = 42.8 ng/cm^3, $t_{1/2}\alpha$ = 94 min and $t_{1/2}\beta$ = 5.7 h. The elimination parameters are very similar following both i.v. and i.m. administration of the drug.

Seutter (1975) reviewed the metabolic pathways of corticosteroids in humans. It appears that 6-hydroxydexamethasone is the major metabolite after oral administration. Its physiological properties are unknown. This is largely due to the fact, that this metabolite cannot be obtained in sufficient quantities.

HYDROLYSIS OF DEXAMETHASONE ESTERS IN VITRO AND IN VIVO

Dexamethasone esters are very efficiently and rapidly hydrolysed in both *in vitro* systems and *in vivo* by esterases. Because of their rapid hydrolysis it is very likely that relative tissue residue levels of dexamethasone in edible tissues resulting from the administration of different esters will not differ significantly between various ester preparations during the elimination phase.

The factor which governs the speed of residue accumulation is the absorption from the site of injection. Clearly this will vary between the different esters.

Hydrolysis of Dexamethasone Esters *in vitro*

Dexamethasone is often administered as an ester (e.g 21-isonicotinate, -disodiumphosphate, -trimethylacetate, -dimethylbutyrate, -phenylpropionate). Generation of glucocorticoid activity requires hydrolysis of the ester to release dexamethasone.

Studies on the kinetics of the hydrolysis reaction have demonstrated species differences in the hydrolysis rate for the 21-isonicotinate. Rabbit and rat serum enzymes very rapidly hydrolyse dexamethasone 21-isonicotinate (approximately 90% hydrolysis in 10 min equating to a $t_{1/2}$ of <3 min.) (Weisenberger, 1972). The hydrolysis speed of the isonicotinate ester in human serum is very much slower (hydrolysis $t_{1/2}$ = 90-100 min). This finding is particularly important when extrapolating animal studies to man, but also indicates that differences between other animal species might occur (Weisenberger, 1972).

Dexamethasone trimethylacetate is rapidly hydrolysed by serum from cows and horses (Houghton, 1989), as is dexamethasone dimethylbutyrate by cattle plasma (Coert et al., 1988).

Hydrolysis of Dexamethasone Esters *in vivo*

In all of the pharmacokinetics studies reviewed dexamethasone *per se* was measured by specific immunoassays or by HPLC following administration of long acting ester preparations. The presence of free dexamethasone is important evidence that hydrolysis also takes place *in vivo*. The pharmacokinetic data obtained in cows by Toutain and co-workers (1982) further corroborate this finding. They reported a complete and rapid hydrolysis of the isonicotinic acid ester in cattle following i.v. injection. In their report they also compare the absorption kinetics of free dexamethasone and dexamethasone-21-isonicotinate after i.m. administration. Since both C_{max} and t_{max} are similar for dexamethasone *per se* and for the ester, it is therefore likely that a rapid and complete hydrolysis also occurs following i.m. administration.

Dexamethasone trimethylacetate is very rapidly hydrolysed on entry into cells or the circulatory system. Opticortenol - the marketed product containing dexamethasone trimethylacetate - was administered i.m. to dairy cows (Holstein Friesian; n=4) as two separate injections (this corresponds to a dose of 20 mg dexamethasone per injection which in turn corresponds to an approximate dose of 40 μg/kg bw). At 4 h after dosing only 1.2% of the total plasma radioactivity was in the form of the ester (Ciba Geigy, 1987).

Studies in pigs utilising [1,2-^{3}H]-dexamethasone trimethylacetate demonstrated extremely low levels of dexamethasone trimethylacetate in blood due to the rapid hydrolysis of the ester (at 4 h after dosing $<1\%$ of the plasma radioactivity was from dexamethasone trimethylacetate) with concomitant release of dexamethasone. This is consistent with *in vitro* findings for the horse and cow (Houghton, 1989).

[^{3}H]-Dexamethasone trimethylacetate was administered i.m. to two horses (Horner, 1991). The dose was 50 μg/kg and samples were taken at -1.5, -0.5, 0, 1, 2, 4, 8, 12, 24, 36, 48, 72, 86, 120, 168, 240, 312, 384 and 504 h post dosing. Urine samples were also collected using 24 hours collection. Dexamethasone trimethyl acetate was very rapidly hydrolysed in blood *in vivo*. At 4 h after dosing only 1.2% of the total plasma radioactivity was in the form of the ester.

These reports show that all of the dexamethasone esters studied are likely to be rapidly hydrolysed to liberate dexamethasone in blood. Therefore the ratio of residue concentrations in different tissues is likely to be constant during the elimination phase, irrespective of the ester preparation administered.

Conclusion

Ester preparations are rapidly and efficiently hydrolysed to dexamethasone.

In addition to the parent compound, dexamethasone glucuronide and 6β-hydroxydexamethasone are the major urinary metabolites in rats, pigs, horses. C6-hydroxylation results in a massive decrease in the steroid activity of corticosteroids.

Due to extensive tritium exchange *in vivo*, total residue studies in food animals with ^{3}H-dexamethasone are prone to errors. Consequently it appears justified to evaluate the tissue depletion of the parent compound, dexamethasone, as the marker residue.

TISSUE RESIDUE DEPLETION STUDIES

General

Tissue residue depletion studies of dexamethasone based on the use of tritium labelled compounds are suspect because of recent findings of Stewart et al. (1992) that tritium exchange of [1,2,4-^{3}H]-dexamethasone with body water after intramuscular administration to rats was extensive 4 days after dosing.

These findings, coupled with the lack of a secure analytical method to analyse dexamethasone at the low levels required to assess tissue depletion over an extended period, resulted in the lack of reliable animal tissue depletion data for this drug. Although this may appear surprising in such a well established and widely used drug, it was only recently that analytical methodology has been able to deliver reliable results at the relatively low dosage levels commonly employed.

Therefore only two separate recent studies are considered and the major data presented here are preliminary results supplied by Boehringer Ingelheim Vetmedica GmbH International, Ingelheim, Germany (P. Bette and H. Hummelt, 1993) and Intervet International B. V., Boxmeer, The Netherlands (Intervet) (A. Coert, 1993).

Cattle

Two steers weighing 227 and 454 kg, respectively, received i.v. doses of 5 and 20 mg per day of dexamethasone, for a total of seven days and were slaughtered 24 hr after the last dose was administered. The resulting liver and hindquarter muscle tissues were analysed by HPLC with UV detection. The average dexamethasone residue levels in liver and standard deviations from low and high dosed animals were 29.2 $\pm$ 2.6 and 69.5 $\pm$ 3.1 μg/kg, respectively. No dexamethasone was detected in muscle tissue above 4 μg/kg, the limit of detection of the analytical method for this matrix (McLauchlin and Henion, 1990).

An aqueous solution of dexamethasone disodium phosphate (Dexadreson, 6 mg per 100 kg bw) was administered

to 12 Holstein, Dutch Friesian and MRY or pure bred MYR animals aged between 5.5 and 9 months (2 males and 2 females per group, weighing between 135 and 250 kg) by intramuscular injection into the neck. Blood was sampled 15 min post dosing and muscle, liver, kidney, fat and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 6.

Table 6. Dexamethasone Concentrations (μg/kg) in Tissues of Heifers and Bull Calves after i.m. Administration of a 60 μg/kg dose of Dexamethasone Disodium Phosphate (Dexadreson)(A. Coert, 1993)

Tissue	Day 1	Day 2	Day 4
Muscle	3.25	0.72	<0.5**
Liver	127.0	15.7	2.59
Kidney	76.4	12.6	0.87
Fat	1.2	<0.5**	<0.5**
Injection Site*	7.35	3.74	2.99

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*Total amount dexamethasone esters found (μg) per injection site

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver.

An aqueous suspension of crystalline dexamethasone phenylpropionate in an aqueous solution of dexamethasone disodium phosphate (Dexafort, equivalent to 6 mg of dexamethasone per 100 kg bw) was administered to 16 Holstein X Dutch Friesian or pure bred MYR animals aged between 5 and 9 months (2 males and 2 females per group, weighing between 160 and 240 kg) by intramuscular injection into the neck. Blood was sampled 30 min post dosing and muscle, liver, kidney, fat and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 7.

Table 7. Dexamethasone Concentrations (μg/kg) in Organs of Heifers and Bull Calves after i.m. Administration of a 60 μg/kg Dexamethasone Dose as an Aqueous Suspension of Crystalline Dexamethasone Phenylpropionate in a Solution of Dexamethasone Disodium Phosphate (Dexafort)(A. Coert, 1993)

Tissue	Day 8	Day 16	Day 32	Day 48
Muscle	<0.5**	<0.5**	nd	nd
Liver	16.2	3.9	na	na
Kidney	12.6	1.2	na	na
Fat	<0.5**	<0.5**	nd	nd
Injection Site*	114.1	19.2	na	na

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*Total amount dexamethasone esters found (μg) per injection site

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver

nd = not determined, na = not available

An aqueous suspension of crystalline dexamethasone dimethylbutyrate (Dexamedium, equivalent to 1.7 mg of dexamethasone per 100 kg bw) was administered to 16 Holstein X Dutch Friesian or pure bred MYR animals aged between 5.5 and 9 months (2 males and 2 females per group, weighing between 160 and 220 kg) by intramuscular injection into the neck. Blood was sampled 24 h post dosing and muscle, liver, kidney, fat and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 8.

Table 8. Dexamethasone Concentrations (μg/kg) in Organs of Heifers and Bull Calves after i.m. Administration of a 17 μg/kg Dexamethasone Dose as an Aqueous Suspension of Crystalline Dexamethasone Dimethylbutyrate (Dexamedium)(A. Coert, 1993)

Tissue	Day 6	Day 12	Day 24	Day 36
Muscle	<0.5**	<0.5**	nd	nd
Liver	7.89	5.09	na	na
Kidney	6.31	2.67	na	na
Fat	<0.5**	<0.5**	nd	nd
Injection Site*	114.2	32.2	na	na

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*Total amount dexamethasone esters found (μg) per injection site

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver

nd = not determined, na = not available

An aqueous suspension of crystalline dexamethasone 21-isonicotinate (Voren®), equivalent to 2 mg of dexamethasone per 100 kg bw) was administered to 16 cross bred calves about 7 months old (2 males and 2 females per group, weighing between 170 and 230 kg) by intramuscular injection into the neck. Blood was sampled 3 h post dosing and muscle, liver, kidney, fat and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 9.

Table 9. **Dexamethasone Concentrations (μg/kg) in Organs of Ruminating Calves after i.m. Administration of a Single 20 μg/kg Dexamethasone Dose as an Aqueous Suspension of Crystalline Dexamethasone 21-Isonicotinate (Voren suspension) (P. Bette and H. Hummelt, 1993)**

Tissue	Day 4	Day 8	Day 16	Day 28
Muscle	<0.6 (2)	<0.5**	<0.5**	nd
Liver	9.53 (4)	4.22 (4)	1.97 (2)	na
Kidney	5.58 (4)	2.94 (4)	0.88 (3)	na
Fat	<0.5**	<0.5**	<0.5**	nd
Injection Site*	144.87 (4)	57.09 (4)	65.44 (4)	2.62 (3)

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*Total amount dexamethasone esters found (μg) per injection site

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver

nd = not determined, na = not available

An aqueous solution of crystalline dexamethasone disodium phosphate (equivalent to 6 mg dexamethasone per 100 kg bw) was administered to 8 cross bred Holstein X Dutch Friesian and Holstein X MRY dairy cows from 3-7 years old, weighing between 450 and 600 kg (2 low milk yielders and 2 high milk yielders per group), by intramuscular injection into the neck. Blood was sampled 15 min post dosing and milk once daily Plasma and milk samples were analysed by HPLC-MS. Results are shown in Table 10.

Table 10. **Dexamethasone Concentrations (ng/ml) in Milk of Cows after i.m. Administration of a Single 60 μg/kg Dexamethasone Dose as an Aqueous Solution of Dexamethasone Disodium Phosphate (Dexadreson)(A. Coert, 1993)**

Time Post Dose (Milkings)	Mean (ng/ml)	No of Samples above LOQ*
0 (Control)	<0.25	0
1	6.91	8
2	1.7	8
3	1.13	8
4	0.31	8
5	0.29	1
6	<0.25	0
7	<0.25	0
8	<0.25	1

*Limit of quantitation (LOQ) for the analytical method of 0.25 μg/kg for milk. Geometric mean, values below the LOQ have been included in the calculation as the LOQ; the resulting

average residue concentrations reported as "< values".

An aqueous suspension of crystalline dexamethasone 21-isonicotinate (Voren®), equivalent to 2 mg dexamethasone per 100 kg bw was administered to 8 cross bred Holstein X Dutch Friesian dairy cows about 8 years old, weighing between 570 and 780 kg (4 low milk yielders at late stage of lactation, 4 high milk yielders at early stage of lactation), by intramuscular injection into the neck. Blood was sampled 3 h post dosing and milk twice daily, morning and afternoon. Plasma and milk samples were analysed by HPLC-MS. Results are shown in Table 11.

Table 11. Dexamethasone Concentrations (ng/ml) in Milk of Cows after i.m. Administration of a Single 20 μg/kg Dexamethasone Dose as an Aqueous Suspension of Crystalline Dexamethasone 21-Isonicotinate (Voren suspension)(P. Bette and H. Hummelt, 1993)

Time Post Dose (hours)	Mean (ng/ml)	No of Samples above LOQ*
0	<0.25	0
1	<0.45	5
8	0.39	8
24	<0.45	7
32	<0.32	5
48	<0.26	2
56	<0.25	0
72	<0.25	0
80	<0.25	0
96	<0.25	0
104	<0.25	0

*Limit of quantitation (LOQ) for the analytical method of 0.25 μg/kg for milk. Geometric mean, values below the LOQ have been included in the calculation as the LOQ; the resulting average residue concentrations reported as "< values".

Pigs

An aqueous suspension of crystalline dexamethasone 21-isonicotinate (Voren®), equivalent to 10 mg of dexamethasone per 100 kg bw) was administered to 16 large white pigs about 3.5 months old (2 males and 2 females per group, weighing about 40 kg) by intramuscular injection into the neck. Blood was sampled 3 h post dosing and muscle, liver, kidney, fat, skin and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 12.

Table 12. Dexamethasone Concentrations (μg/kg) in Tissues of Pigs after i.m. Administration of a Single 100 μg/kg Dexamethasone Dose as an Aqueous Suspension of Crystalline Dexamethasone 21-Isonicotinate (Voren suspension)(P. Bette and H. Hummelt, 1993)

Tissue	Day 4	Day 8	Day 16	Day 28
Muscle	<0.5**	<0.5**	<0.5**	nd
Liver	<2.5**	<2.5**	<2.5**	na
Kidney	<0.5**	<0.5**	<0.5**	na
Fat	<0.5**	<0.5**	<0.5**	nd
Injection Site*	247.1 (4)	119.5 (4)	7.14 (4)	0.47 (2)

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*Total amount dexamethasone esters found (μg) per injection site

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver

nd = not determined, na = not available

An aqueous solution of dexamethasone disodium phosphate (Dexadreson, 6 mg per 100 kg bw) was administered to 16 cross-bred Yorkshire white pigs aged about 4 months (2 castrated males and 2 females per group, weighing about 70 kg) by intramuscular injection into the neck. Blood was sampled 15 min post dosing and muscle, liver, kidney, fat and injection site were sampled upon slaughter. Plasma and tissue samples were analysed by HPLC-MS. Results are shown in Table 13.

Table 13. Dexamethasone Concentrations (μg/kg) in Tissues of Pigs after i.m. Administration of a 60 μg/kg Dose of Dexamethasone Disodium Phosphate (Dexadreson)(A. Coert, 1993)

Tissue	Day 1	Day 2	Day 4
Muscle	<0.5**	<0.5**	<0.5**
Liver	<2.5**	<2.5**	<2.5**
Kidney	<0.5**	<0.5**	<0.5**
Fat	<0.5**	<0.5**	<0.5**
Injection Site*	BLQ	BLQ	BLQ

Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the number of samples above the LOQ)

*No dexamethasone ester found per injection site. All values were below the limit of quantitation (BLQ)

**All values below the limit of quantitation for the analytical method of 0.5 μg/kg for muscle, kidney and fat, and 2.5 μg/kg for liver

The results obtained so far suggested

- that no dexamethasone residues can be detected in fat at any time point,
- that the marker residue quickly depletes from milk and muscle, and
- that liver is the tissue with the slowest depletion rate. Thus, the liver appears to be the target tissue.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Because of the wide spread use of dexamethasone both in human and veterinary treatment , there are a plethora of methodologies which have been used to determine dexamethasone and related corticosteroids in a wide variety of matrices. Many of these are of limited value for the residue analysis in food commodities. Methods either measure the concentration of free dexamethasone in a matrix or determine the combined free and conjugated dexamethasone content after enzymic hydrolysis of samples with β-glucuronidase.

Although only three discrete methods are fully validated for a range of animal tissues, a number of methods in the literature appear to offer the potential sensitivity and ruggedness to be adaptable to a wide range of biological matrices. These potentially useful methodologies are therefore included in the review analytical procedures for dexamethasone.

Radioimmunoassays

Radioimmunoassays have been developed which give picogram sensitivity but are matrix dependent. It has been surggested that routine use for surveillance purposes is not practicable because standardised antibody batches are difficult to obtain routinely in sufficient quantities (Bette 1993a). However, 1 ml of antisera is enough to perform $5x10^5$ assays and should routine monitoring of dexamethasone ever prove necessary, a primary screen based on immunoassay would offer the desired sensitivity providing it was supported by a confirmatory chemically based method.

Biological Tests

Biological tests e.g. based on the chicken growth depression are not specific.

High Performance Liquid Chromatography (HPLC)

HPLC systems based on UV detection are not considered sensitive enough to measure dexamethasone in the concentration ranges that are required by the MRL (Bette, 1993a). Nevertheless, an HPLC procedure based on a normal phase chromatographic separation with UV detection has been successfully developed to monitor dexamethasone in cattle. Sample clean-up involved selective partition in a three phase extraction system. Dexamethasone could be determined in muscle tissue above 4 μg/kg and in liver above 10 μg/kg, the limit of quantitation of the analytical method for these matrixes (McLauchlin and Henion, 1990). The method used by the Food Safety and Inspection Service of the USDA for any necessary monitoring of dexamethasone is based on the McLauchlin and Henion publication (Ellis, 1991). These procedures appear satisfactory for the monitoring of dexamethasone used in excess of therapeutic dosages.

A similar determination utilising solid phase sample clean-up and reverse-phase HPLC separation of dexamethasone has a stated detection level of 10-100 μg/kg in muscle, kidney, liver and fat. Methylprednisolone is used as the internal standard (Shearan *et. al.*, 1989).

A recently published method employs the oxidation of 21-hydroxycorticosteroids to glyoxals with cupric acetate followed by condensation with 1,2-diamino-4,5-methylenedioxybenzene to yield intensely fluorescent products. These are conveniently separated and determined by HPLC using fluorescence detection. To date, the method has only been validated for serum (Yoshitake *et. al.*, 1989)

Gas Chromatography - Mass Spectrometry (GC-MS)

A rapid and highly sensitive method for the quantitative determination of dexamethasone in plasma, synovial fluid and tissues by combined gas chromatography - negative-ion chemical-ionisation mass spectrometry has been developed by Girault et al. (1990). The tri-trimethylsilyl derivative of dexamethasone was found to give an intense fragment ion at *m/z* 446 and the limit of detection of 0.1 ng/ml. Flumethazone was utilised as an internal standard. However, attempts to adapt this method to food commodities were unsuccessful because a single consistent and stable derivative of dexamethasone could not be reproducibly formed in attempts to validate the assay (Bette, 1993a).

Many methods for the determination of 21-hydroxycorticosteroids are based on the protection of ketone groups with methylhydroxylamine followed by hydroxyl protection via TMS derivatives. GC-MS determination with single ion monitoring enables the detection and quantitation of corticosteroids in the low μg/kg range in urine and plasma. These methods have not been validated for the determination of dexamethasone in a range of animal tissues. However they represent a potential method which is sensitive and could be adapted for use in a wide range of regulatory laboratories (Yap *et. al.*, 1992).

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

Several important corticosteroids were quantitatively determined in plasma and urine of horses by micro-liquid chromatography-mass spectrometry (micro-LC-MS). Plasma and urine were extracted with a mixture of ether, methylene chloride and isopropanol and initial sample clean-up achieved by thin layer chromatography. Final sample clean-up was performed by micro-liquid chromatography and the column eluent introduced directly into a mass spectrometer. This highly sensitive method was used to determine the metabolism of dexamethasone in horses discussed in an earlier section (Skrabalak et. al., 1984).

An assay has been developed on behalf of Boehringer Ingelheim Vetmedica GmbH International, Ingelheim, Germany (BIV-I) and Intervet International B.V., Boxmeer, The Netherlands (Intervet). It determines dexamethasone residues in tissues, milk and plasma by HPLC with mass spectrometric detection using a thermospray interface with positive filament ionisation. The method has been validated by determining the specificity, accuracy, precision and limits of quantification and detection. This method has been nominated as the preferred analytical method for regulatory control (Bette, 1993a).

Validation of the procedure was established for kidney, liver, muscle, fat, plasma and milk from cattle and pig skin. The further applicability of the method to liver, kidney, muscle, fat and plasma from pig and horse was also confirmed.

The method was specific to dexamethasone in control matrices at the limit of quantitation. This was found to be 2.5 ng/g for liver and 0.5 ng/g or ng/ml for all other tissue types and plasma. For milk it was shown to be 0.25 ng/ml. A tabulated summary of the performance characteristics of the method is presented in Table 14.

Table 14. Performance Data for HPLC-MS Methodology for the Quantitation of Dexamethasone in Animal Tissues and Milk

Cattle Tissues, Milk, Plasma and Pig Skin

Matrix	Intra day accuracy %	Inter day accuracy %	Precision %	Repeatability %
Muscle	92 - 101	90 - 104	5 - 19	7 - 26
Liver	90 - 107	94 - 109	5 - 18	7 - 31
Kidney	90 - 110	96 - 108	4 - 13	3 - 14
Fat	94 - 106	92 - 108	6 - 12	2 - 31
Milk	91 - 104	95 - 110	5 - 25	4 - 18
Plasma	79 - 114	93 - 113	4 - 12	7 - 18
Pig Skin	83 - 103	77 - 104	5 - 31	6 - 27

Other Species - Intra Day Results

	Pig		Horse	
Matrix	Accuracy %	Precision %	Accuracy %	Precision %
Muscle	96 - 118	6 - 13	82 - 105	5 - 28
Liver	92 - 103	8 - 18	89 - 116	6 - 20
Kidney	95 - 112	5 - 12	89 - 112	9 - 18
Fat	94 - 106	11 - 24	91 - 111	5 - 13
Plasma	79 - 114	3 - 8	84 - 105	5 - 17

In summary, it has proved to be a reliable method for the determination of dexamethasone residues in a range of selected food commodities.

APPRAISAL

Dexamethasone esters are very efficiently and rapidly hydrolysed in both *in vitro* systems and *in vivo* by esterases. Therefore, the relative dexamethasone residue concentrations in edible tissues following the administration of ester formulations will depend on the bioavailability of the ester from the injection site. However, the rate of absorption of the ester from the injection site will vary between different ester formulations and it will be the absorption rate which determines the availability of dexamethasone.

The concentration of dexamethasone at the injection site at a particular time point post injection will depend on the dexamethasone ester used and will significantly exceed levels in all other edible tissues at the same time point until totally absorbed.

Recent findings that tritiated dexamethasone is subject to tritium exchange *in vivo* render any residue depletion

data based on radiolabeled studies before 1992 open to challenge. Residue depletion data for dexamethasone and its esters are therefore somewhat restricted. Recent data have been supplied by the sponsors for the depletion of various esters in cattle and swine tissues. Results indicate that different ester preparations lead to significantly different dexamethasone depletion rates.

Results in cattle and swine show that:

- Dexamethasone residues are quickly eliminated from muscle and cow's milk,

- Residues do not occur in fat in free form although no studies on the potential retention of dexamethasone esters in fat have been carried out, and

- Depletion of dexamethasone residues is slowest from liver which is therefore the target tissue of choice.

Except for conjugates of dexamethasone itself, the major metabolic excretion pathway in all species studied commences with 6-hydroxylation of the steroid ring. This, together with minor metabolic pathways, results in a large decrease in corticosteroid activity. The low NOEL which has been established is based on the pharmacological activity of the free drug, absent in oxidized metabolites. Therefore the parent, dexamethasone, is proposed as a marker residue.

A method for dexamethasone determination by HPLC-MS is available with a lower limit of quantitation of 2.5 μg/kg for liver, 0.5 μg/kg for kidney and muscle, and 0.25 μg/kg for milk.

Maximum Residue Limits

Based on the ADI of 0-0.015 μg/kg body weight for parent drug established by the Committee, the permitted daily intake of parent drug is 0.9 μg.

The Committee recommends MRLs of 0.5 μg/kg for muscle and kidney, and 2.5 μg/kg for liver of cattle and pigs, and 0.3 μg/l for cattle milk, expressed as parent drug.

Using these values for MRLs, and daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat and 1.5 l of milk, the maximum ingested residue of dexamethasone is 0.875 μg/day.

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