MELENGESTROL ACETATE

First draft prepared by
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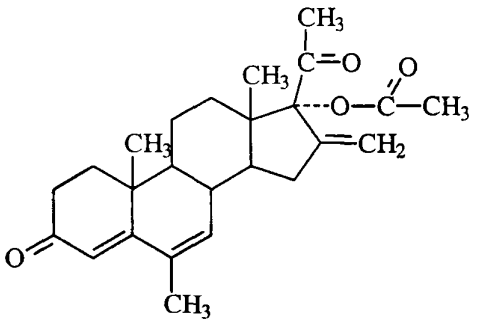
IDENTITY

Chemical name: 17α-Acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione acetate (Chemical Abstracts name); CAS No. 2919-66-6

International nonproprietary name: Melengestrol Acetate
Manufacturer's code, PNU-21240

Chemical structure:



Molecular formula: $C_{25}H_{32}O_4$

Molecular mass: 396.53

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White powder
Color: Off-white to light yellow
Melting point: 224-226 °C

Optical rotation: $[\alpha]_D^{23}$ -127° (c = 0.31 in chloroform)

Ultraviolet maximum: 287nm (in ethanol;log ε 4.35)
Solubility: 1.06 mg/L in water

RESIDUES IN FOODS AND THEIR EVALUATION

Conditions of use

Melengestrol acetate (MGA) is an orally active progestogen. It is used to improve feed conversion efficiency, promote growth and suppress estrus in female beef cattle fed for slaughter. The range of approved doses is 0.25 to 0.50 mg/heifer/day. Melengestrol acetate is fed for the duration of the fattening/finishing period, usually 90 to 150 days. Melengestrol acetate has not previously been evaluated by the Committee.

PHARMACOKINETICS AND METABOLISM

Studies with radio-labeled melengestrol acetate were carried out to determine its fate in feedlot heifers (**Krzeminsky *et al.*** 1981). Young Angus-Hereford heifers, housed in individual box stalls, received complete ground ration

supplemented with 0.5 mg of unlabelled melengestrol acetate/animal/day for four months. The animals were moved to metabolism stalls and acclimated. Three animals then received daily oral doses (in capsules) of approximately 100 µCi (equivalent to approximately 0.5 mg) of ^{3}H-melengestrol acetate, labeled in the 6-methyl group, for 21 days. One animal received daily oral doses (in capsules) of 254 µCi (equivalent to approximately 0.5 mg) of ^{14}C-melengestrol acetate, labeled in the 6-methyl group and in the 16-methylene group, for seven days. Radioactivity was determined in urine and faeces. Each heifer was slaughtered 6 h after the last capsule had been administered and total residues in body fluids and tissues were determined.

Significant losses of the ^{3}H - label (formation of ^{3}H-labeled water from the methyl group in 6-position) were observed in this study. The radioactivity was eliminated from the heifers via the faeces and urine in a 6:1 ratio. Independent studies of animals with bile cannulation showed that biliary excretion closely parallels total faecal output. In the present study, the highest concentration of total residue was found in liver; but the highest percentage of parent melengestrol acetate was found in fat. The concentrations found in visceral fat, omental fat and perirenal fat were similar. Whereas the results obtained for fat and liver were reproducible in all four treated animals, the concentrations found in muscle were variable because they were at or below the limit of detection of the radioactivity 6 h after the last treatment. Individual metabolites were not identified in the study because of their low concentrations. The individual results are given in Table 1.

Table 1. Total residue (µg/kg) and percent radio-labeled melengestrol acetate in tissues at steady state.

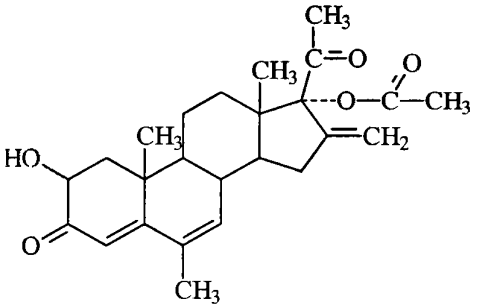
	Animal 1		Animal 2		Animal 3		Animal 4	
Tissue	Total residue	% ^{3}H-MGA	Total residue	% ^{3}H-MGA	Total residue	% ^{3}H-MGA	Total residue	% ^{14}C-MGA
Perirenal fat	7.5	78	7.7	86	8.0	94	3.6	75
Muscle	0.6	31	1.0	72	0.5	40	NS	45
Liver	12	30	15	30	9.0	28	8.2	37
Kidney	1.7	24	1.8	34	1.2	130	1.6	30

NS, Not significantly above background.
The limit of quantification is about 0.5 µg/kg in this study

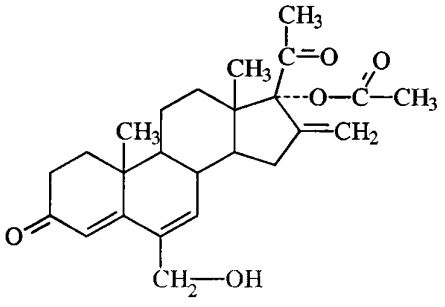
Under conditions of *in vitro* incubation of ^{3}H-melengestrol acetate with liver homogenates the 6-methyl- ^{3}H - label was reported to be stable (37°C, 3 hours). About 40% of radioactivity in the homogenate represented melengestrol acetate. The remainder of the radioactivity represented several unidentified degradation products (**Jaglan**, 1975a).

Incubation of ^{3}H-melengestrol acetate in fresh bovine rumen fluid *in vitro* for 24 and 96 hours at 37°C also resulted in no loss of ^{3}H - label from the molecule. All acetonitrile extractable radioactivity co-chromatographed with melengestrol acetate. However, when rumen fluid was collected from a heifer slaughtered six hours after the final dose of a 23-day treatment period with ^{3}H-melengestrol acetate, 28.5% of the recovered radioactivity was incorporated into water (**Jaglan**, 1975b).

In women melengestrol acetate was metabolized to at least thirteen distinct metabolites (**Cooper**, 1968). The only metabolites identified in experimental animals and in humans were the 2α-hydroxy- and 6-hydroxymethyl -metabolites, respectively, of the parent compound. 2α-hydroxy-melengestrol acetate was excreted in the urine of women and rabbits. 6-hydroxy-methyl -melengestrol-acetate was only identified in urine of rabbits.



2α-hydroxy-melengestrol acetate



6-hydroxymethyl-melengestrol acetate

When 4.0 mg of ^{3}H-melengestrol acetate was given orally as a single dose to a Holstein heifer, the radioactivity was quantitatively recovered from urine and faeces within seven days following administration. Eight consecutive daily oral doses of 4.0 mg were quantitatively recovered within 14 days after the last dose (**Neff and Thornton**, 1964a).

Continuous daily oral administration of 4.0 mg of [^{3}H]-melengestrol acetate over 15 days to three Holstein heifers led to a "steady state" and 83 ± 13% of the daily dose was recovered in the urine and faeces on the same day. The animals were killed 1, 4, and 10 days after the last dose and the total residue contents of selected edible tissues were determined (**Neff and Thornton**, 1964b). The results confirmed that the concentrations of total residues in perirenal fat, visceral fat, and omental fat were similar and depleted at similar rates. Even at this eight-fold overdosing no residues above the limit of detection of the radioactive label were found in muscle (see Table 2).

Table 2. Depletion of residues of ^{3}H-labeled melengestrol acetate in selected tissues of heifers.

Withdrawal time (days)	1	4	10
Tissue	Melengestrol acetate-equivalents (µg/kg)		
Liver	43	14	4
Visceral fat	43	22	6
Perirenal fat	43	--	9
Omental fat	42	22	4
Kidney	6	*	*
Heart	2	*	*
loin muscles	*	*	*
Round steak muscles	*	*	*

Note: * = below the limit of quantification (LOQ).

In an attempt to obtain a first estimate of the proportions of the concentrations of the residues in edible tissues, an animal was treated with 1000 mg orally (corresponding to 2000 times the highest recommended dose) for five days. In addition, 500 mg was injected subcutaneously on the fifth day and another 500 mg were injected intramuscularly. The animal was killed on the sixth day and the following concentrations of residues of parent melengestrol acetate were found: fat 3300 µg/kg, liver 880 µg/kg, muscle 220 µg/kg, and kidney 120 µg/kg. These results are in line with the findings that residues in muscle were not detectable in studies when the animals were treated with recommended doses.

TISSUE RESIDUE DEPLETION STUDIES

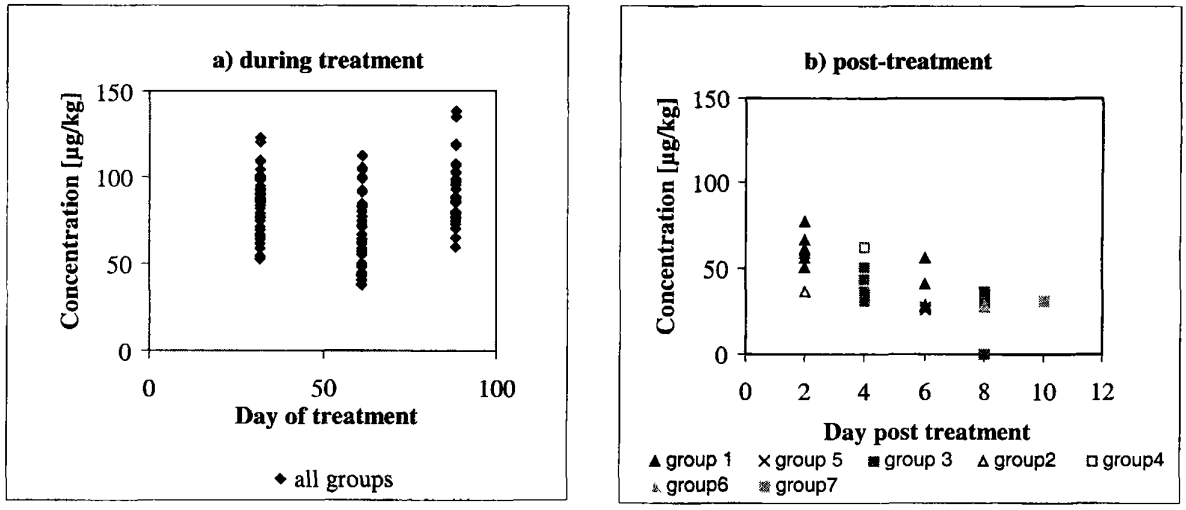
Muscle, liver, kidney and fat obtained from 5 heifers (initial body weights: 234-280 kg; final body weights 320-386 kg) fed 0.5 mg melengestrol acetate/animal daily for 126 days and slaughtered after two days withdrawal contained no quantifiable residues of melengestrol acetate (**Krzeminsky *et al.***, 1971a). The reported LOQ of the method was 25 µg/kg. Similar results were obtained in two similarly designed studies where five beef heifers received 0.5 mg melengestrol acetate/animal daily for 142 and 143 days, respectively, in combination with oxytetracycline and chlorotetracycline, respectively (**Krzeminsky *et al.***, 1971b; **Krzeminsky *et al.***, 1971c). In these studies animals were slaughtered following a two-day withdrawal period. The method used for quantification was GLC with electron capture detection in both studies (**Krzeminsky *et al.***, 1970). The reported LOQ was 25 µg/kg.

In a study with thirty-five Angus beef heifers with an initial average body weight of 241 kg, seven groups each comprising 5 animals were fed 10.0 mg melengestrol acetate/animal daily for 113 days. Biopsies of perirenal fat were taken at 32, 61, 88 days during treatment and 2,4,6,8, and 10 days post-treatment (**Krzeminsky et al.**, 1971d). The results of the quantitative determination of melengestrol acetate residues are given in the Figures 1a and 1b. The concentrations of several samples were below the LOQ of 25 µg/kg (1/10 on day four post-dose; 5/10 on day eight post-dose and 9/10 on day ten post-dose). During treatment the following concentrations were reached (Table 3):

Table 3. Melengestrol acetate in samples of perirenal fat in heifers "on treatment" with 10 mg per day.

Sampling time (days)	arithmetic mean	geometric mean	median
32	85.2	83.3	86.5
61	71.3	68.7	71.0
88	92.6	90.8	95.5
32 to 88	82.9	80.4	82.9
zero withdrawal time (extrapolated)		78.0	

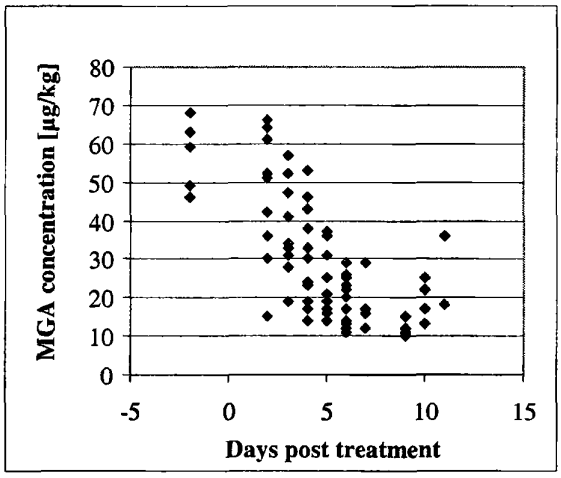
Figure 1a, 1b. MGA residues in peri-renal fat during and post-treatment in cattle receiving 10 mg per day



Eight groups of beef heifers comprising a total of seventy-nine animals were fed 0.4 mg melengestrol acetate/animal daily for 48 days. With forty-seven animals the treatment was continued for 14 days. The 32 remaining animals received reduced doses of 0.25 mg of melengestrol acetate/animal daily during the same period of time. Fat biopsy samples were taken and analyzed at various times (0,1,2,4 and 6 days post treatment in the 0.4 mg dose group and 0,1 and 2 days post treatment in the 0.25 mg dose group). No residues above the LOQ of the method (10 µg/kg) were found in any fat sample (**Krzeminsky *et al.***, 1973a).

One hundred and fifteen beef heifers were fed 3.0 mg melengestrol acetate/animal daily. Biopsies of perirenal fat were taken from six animals after 13 days of treatment. The drug was withdrawn after 15 days of treatment and biopsy samples were taken 2,3,4,5,6,7,8,9,10,11, and 12 days post-treatment (**Krzeminsky *et al.***, 1974b). The samples were analyzed by a GLC/ECD method with an LOQ of 10 µg/kg (**Krzeminsky and Cox**, 1974c). The results are given in Figure 2. The concentrations found during treatment were [µg/kg]: arithmetic mean, 59; median, 61; value at zero withdrawal time (extrapolated by semi-log linear regression from data obtained between days 2 and 6 post-dose), 69.

Figure 2. Residues of MGA in peri-renal fat from animals receiving 3 mg per day



Seventy-one beef heifers were fed 0.4 mg of melengestrol acetate/animal daily. The animals were fed either a high roughage or a high concentrate diet for the first 41 days of treatment. Heifers on a high roughage diet had no residues above LOQ (10 µg/kg) in fat biopsy samples obtained on days 20 and 41 of the treatment. Of the non-pregnant animals fed a high concentrate diet, one of fourteen sampled animals had residues above LOQ on day 20 and one of sixteen animals sampled on day 41 had residues above the LOQ. Of the eleven pregnant heifers in the study (all on high concentrate diet), five had residues above the LOQ in fat biopsy samples on day 41. The study was continued for an additional 83 days with all animals on a high concentrate diet. The animals were then slaughtered at zero withdrawal time or two days after the last dose of melengestrol acetate. Residue concentrations in muscle, liver and kidney were all

below the LOQ. Only one fat sample out of seventy was above the LOQ. The numerical results are summarized in Table 4. Recovery of MGA was approximately 115% at levels between 10 and 30 µg/kg (**Krzeminsky *et al.***, 1973b).

Table 4. Residues of melengestrol acetate in fat biopsy samples of pregnant and non-pregnant animals

Day of treatment	Condition of the animal	MGA [µg/kg]
20	non-pregnant	13.1
41	non-pregnant	10.0
41	Pregnant	11.0
41	Pregnant	20.0
41	Pregnant	11.0
41	Pregnant	13.0
41	Pregnant	10.0
48 hours post-treatment	non-pregnant	11.5

The effects on melengestrol acetate residues in perirenal fat of the melengestrol acetate dose, the type of heifer and the melengestrol acetate withdrawal period was studied. Two doses (0.3 and 0.4 mg/head/day), three withdrawal periods (0, 2, 12 days) were used with light (289-350 kg body weight) and heavy (409-475 kg body weight) commercial feedlot heifers (10 feedlots from 5 States of the United States). Certain test groups received additional growth promotants, e.g., Synovex H/Rumensin, DES-Implant/Rumensin, Synovex H/Ralgro/Rumensin. (**Lauderdale *et al.***, 1977) The range of melengestrol acetate feeding periods is given in Table 5.

Table 5. Range of melengestrol acetate doses and feeding periods in heifers.

Animals	Dose [mg MGA/animal/day]	Feeding period [days]
Light heifers	0.3	131-187
	0.4	100-185
Heavy heifers	0.3	118-147
	0.4	110-180

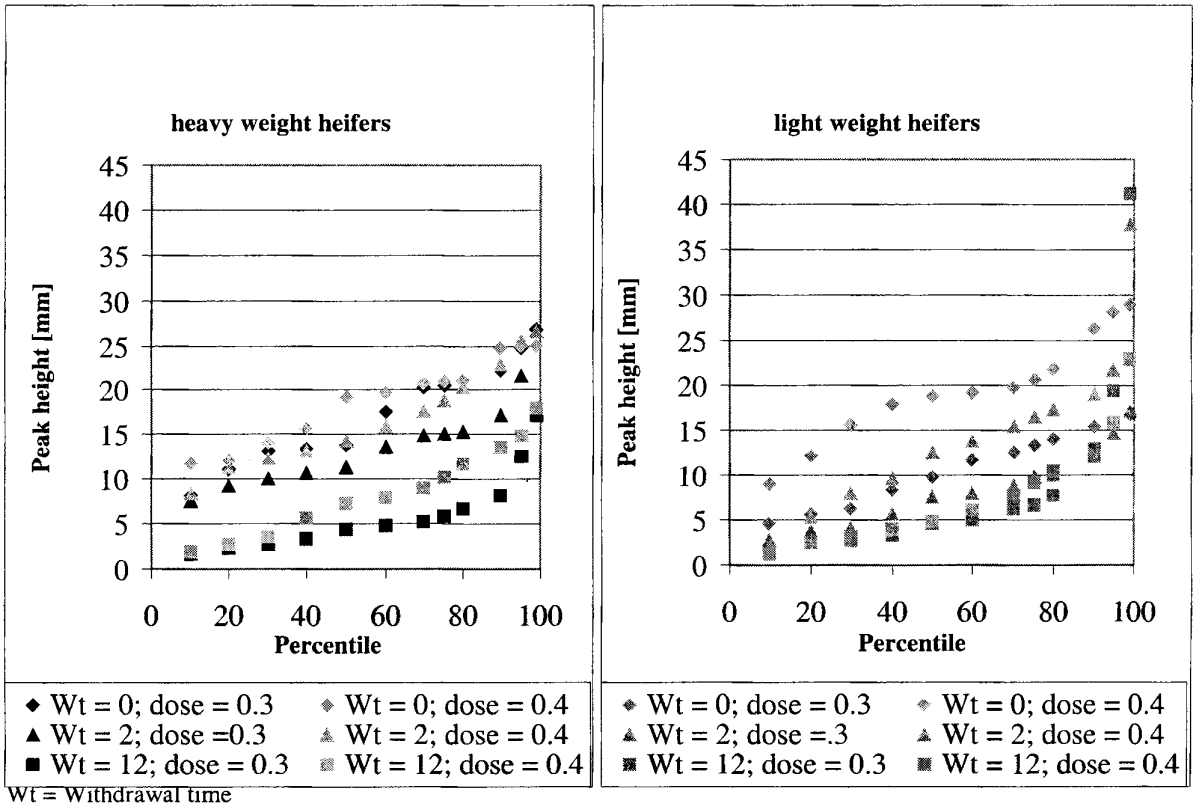
Perirenal fat samples were collected from a total of 481 heifers. The GLC/ECD method with a LOQ of 10 µg/kg was used. All observed peak heights for residues in fat samples were normalized relative to a 10 µg/kg melengestrol acetate standard (20 mm peak height under the specified experimental conditions). These data are summarized in Table 6.

Table 6 Gas-chromatographic peak heights at melengestrol acetate retention time [mm].

	Heavy heifers (409-475 kg bw)						Light heifers (289-350 kg bw)					
Withdrawal time (days)	0		2		12		0		2		12	
Dose (mg/head/day)	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4
Number of animals	12	17	40	60	40	59	18	15	60	50	60	50
Arithmetic mean	15.8	17.7	12.7	15.3	4.90	7.27	9.78	17.9	7.28	12.0	6.62	6.46
Geometric mean	18.8	17.0	11.9	14.4	3.84	5.27	8.73	16.7	6.15	8.90	4.18	4.67
Median	13.8	19.2	11.3	14.3	4.35	7.30	9.75	18.7	7.40	12.6	4.50	4.80
75th percentile	20.5	21.0	15.0	18.8	5.83	10.3	13.3	20.6	9.73	16.5	6.65	9.18
90th percentile	22.2	24.7	17.1	22.8	8.11	13.6	15.4	26.2	12.9	19.0	13.0	12.1
95th percentile	24.6	24.8	21.6	25.6	12.5	14.9	15.6	28.2	14.5	21.7	19.3	15.9
99th percentile	26.8	25.0	26.5	26.6	17.1	18.0	16.6	29.0	17.1	37.7	41.3	22.9

Both parameters, dose of melengestrol acetate and duration of withdrawal time prior to sampling significantly affected the results. The data also suggest a more rapid decline in light heifers if compared with heavy heifers in this experiment (see Figures 3a and 3b).

Figure 3a, 3b. Influence of dose and withdrawal time on MGA residues in heifers



Wt = Withdrawal time

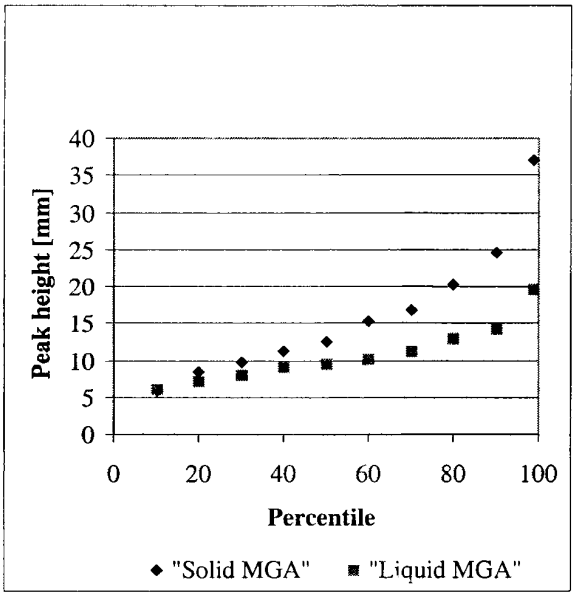
Three groups of heifers (12 animals per group) weighingfrom 300-386 kg were treated with melengestrol acetate, melengestrol acetate plus rumensin, and melengestrol acetate plus Synovex H implants, respectively for 49 days. The melengestrol acetate dose was 0.5 mg/animal daily. Perirenal fat was collected between 49 and 55 hours after complete consumption of the last melengestrol acetate feeding. The 90th percentile of all measured contents was slightly above 10 µg/kg. However, the number of above LOQ results was too small to compare the effects of the different treatments. This was also the case after continuation of treatment for another 36 or 46 days (**Krzeminsky *et al.***, 1977).

Similarly, residues in fat, liver, kidney and muscle were below the LOQ of 10 µg/kg when 0.4 mg melengestrol acetate/head/day was administered in combination with 0.5 ppm of salinomycin, equivalent to 100 mg/head/day (**Davis and Hamlow**, 1992).

Due to a lack of sensitivity of the method applied in some early studies, no results above the LOQ of 25 µg/kg were observed in edible tissues when beef heifers were treated with melengestrol acetate in the presence of zinc bacitracin (**Krzeminsky *et al.***, 1969a). Similarly no residues above the LOQ of 25 µg/kg were found in edible tissues of 201 Hereford yearling heifers which had received 0.5 mg melengestrol acetate/head/day in combination with 350 mg chlorotetracycline/head/day and from which tissues were collected two days post-treatment (**Krzeminsky *et al.***, 1969b). Similarly no residues above the 25 µg/kg LOQ were found in tissue samples of animals treated with a combination of 0.5 mg melengestrol acetate and 75 mg Oxytetracycline per head per day over a period of 100 days and slaughtered after 2, 3, and 4 days withdrawal, resp. (**Krzeminsky *et al.***, 1969c). In another study in which the animals were treated with 0.5 mg melengestrol acetate/head/day in the presence of 350 mg and 70 mg Chlorotetracycline for the first 30 and subsequent 110 days, resp. no tissue residues above the LOQ of 25 µg/kg were found (**Krzeminsky *et al.***, 1969d).

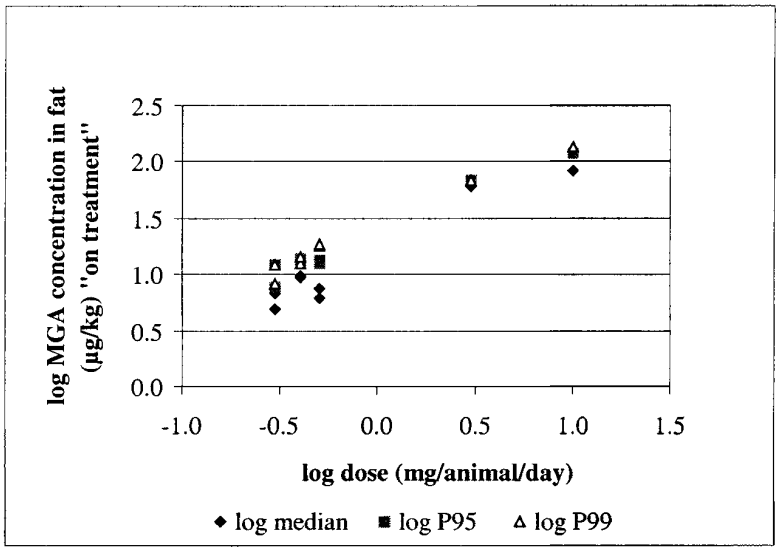
Analyses were carried out on 174 samples of perirenal fat obtained from 25 feedlots where heifers were fed 0.5 mg of melengestrol acetate/animal daily via a conventional supplement. In addition, another 84 fat samples were obtained from 12 feedlots where 0.5 mg of melengestrol acetate/animal daily was fed using a liquid formulation of melengestrol acetate that was delivered into the complete feed by a metering machine (**Krzeminsky *et al.***, 1983). Beef heifers were predominantly a mixed breed. Of the 37 groups of cattle, 27 were slaughtered at less than 10 hrs after the last melengestrol acetate feeding, 8 were slaughtered between 11 and 16 h after the last melengestrol acetate feeding and 2 were slaughtered at 18 and 27.5 h after the last melengestrol acetate feeding. Peak heights were significantly lower (see Figure 4) in samples from animals treated with a liquid formulation poured on feed compared to the administration of the solid melengestrol acetate formulation mixed into feed.

Figure 4. MGA formulation effects on peak height in the GLS/ECD chromatographic analysis of fat samples.



For the population sampled in this study, the 99th percentile of the concentrations of melengestrol acetate was about 18 µg/kg. The median concentration was about 6 µg/kg. A relationship between the administered dose and the concentrations of melengestrol acetate residues found "on treatment" could be established on the basis of the results of the above discussed studies that were conducted over a range of doses from 0.3 to 10.0 mg melengestrol acetate/animal per day (see Figure 5). Using this relationship it was estimated for the lower approved dose of 0.25 mg/head per day, that the corresponding upper 99th percentile would be about 10 µg of parent melengestrol acetate/kg of fat.

Figure 5. Relationship of the administered dose of MGA and concentration of residues in fat.



Marker Residue and Target Tissues

The average theoretical daily intake of "total residues" resulting from the treatment of heifers with 0.5 mg melengestrol acetate/animal per day and slaughtering at zero withdrawal time can be estimated from the data given in Table 1. The results of these calculations are given in Table 7a. It is evident that on the basis of the JECFA food basket liver would be the main dietary source of intake of total residues. A similar calculation based on parent compound melengestrol acetate is carried out in Table 7b. From these tables it becomes evident that liver and fat are equally important as dietary sources of melengestrol acetate parent drug residues. The contribution of muscle and kidney is less significant

compared with liver and fat. The parent melengestrol acetate contents in fat are the highest of all four standard edible tissues. Therefore, parent melengestrol acetate and fat (most data are from perirenal fat) are the suitable pair of marker residue and target tissue, respectively.

Table 7a. Estimation of the dietary total residue intake of melengestrol acetate

Tissue	Content of "total residues" expressed as µg/kg MGA equivalents				Food consumption [g/person/ day]	Intake [µg/person/ day]	% of total daily intake
	animal 1	animal 2	Animal 3	mean			
Fat	7.5	7.7	8	7.7	50	0.39	20.5
Muscle	0.6	1	0.5	0.70	300	0.21	11.1
Liver	12	15.4	9	12.1	100	1.21	64.3
Kidney	1.7	1.8	1.2	1.6	50	0.08	4.1
All tissues						1.9	100.0

Table 7b. Estimation of the dietary parent drug intake of melengestrol acetate.

Tissue	Content of MGA [µg/kg]				Food consumption [g/person/ day]	Intake [µg/person/ day]	% of total daily intake
	animal 1	animal 2	Animal 3	mean			
Fat	5.85	6.62	7.52	6.66	50	0.33	39.5
Muscle	0.19	0.72	0.20	0.37	300	0.11	13.1
Liver	3.60	4.62	2.52	3.58	100	0.36	42.4
Kidney	0.41	0.61	1.51	0.84	50	0.04	5.0
All tissues						0.8	100.0

METHODS OF ANALYSIS

The analytical methods submitted to the Committee were developed before the introduction of GLP. A text published in the US Federal Register (**FDA**, 1968) describes in full detail the original analytical method for determination of residues of melengestrol acetate in lean muscle and fatty tissues. Following the extraction of residues, interfering substances are removed from the extract by several solvent partition and thin layer chromatographic clean-up steps. Final separation and detection is performed by GLC-FID (Flame Ionisation Detector) using a 3% QF-1 phase in an all-glass packed column. Quantification is performed on the basis of peak height measurements . Melengestrol acetate can be quantitatively determined at a level of 25 µg/kg with negligible interference from tissues or reagents. Observed recovery and estimated standard deviation at 25 ppb in muscle , liver and fat is 74.4 ± 8.0 %.

This method has been further developed and GLC-FID detection has been replaced by electron capture detection (GLC-ECD). Furthermore, a GLC-MS method using 25 g of fat and the ions with m/z 311, 321, 336, 337, and 354 amu has been described for confirmation (**Krzeminsky *et al.***, 1974a). A collaborative study of the modified GLC-ECD method with seven participating laboratories (**Krzeminski *et al.***, 1976) gave the results summarized in Table 8.

Table 8. Summary characteristics of the collaboratively studied method for the determination of melengestrol acetate residues in bovine tissues.

Sample		Analytical procedure	Validation parameters		
Matrix	Sample weight [gram]		Concentration [µg/kg]	Limit of quantification [µg/kg]	Recovery at 10 and 20 µg/kg [%]
Kidney	25	Extraction, solvent partition, gas-liquid column chromatography, electron capture detection.	0, 10, 20	10	76-105
Liver	25				78-142
Muscle	25				74-98
Fat	25				88-108

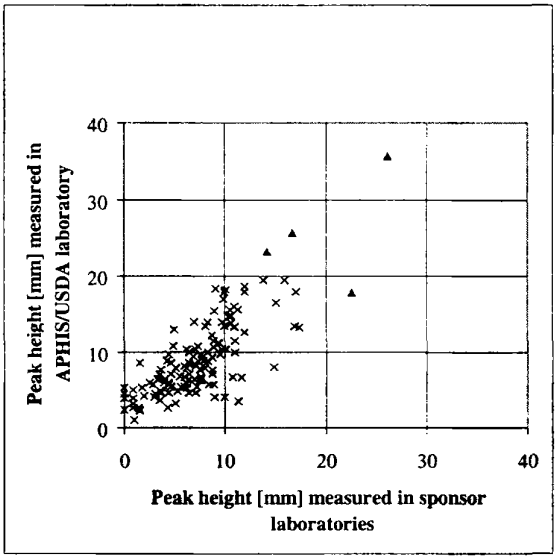
For all four tissues fortified samples were tested at the 0, 10 and 20 µg/kg level; for fat, incurred samples from heifers were tested at the 0, 10 and 20 µg/kg level. Apparent melengestrol acetate concentrations [µg melengestrol acetate equivalents per kg] observed in the zero level control tissues (blanks) ranged between 0.0 – 4.6 (kidney), 0.0 – 4.2 (liver), 0.0 – 1.1 (muscle) and 0.0 – 2.2 (fat). From statistical analyses it was concluded that the true concentration of melengestrol acetate for the incurred fat samples was in the range 6.9 –13.8 µg/kg for the sample at the 10 µg/kg level and in the range 13.4 – 26.4 µg/kg for the sample at the 20 µg/kg level. It was concluded that the method could distinguish samples with residues at the 10 µg/kg level from samples not containing melengestrol acetate residues, but that the method could not fully differentiate between samples with residues at the 10 µg/kg and the 20 µg/kg level. This collaborative study resulted in the AOAC Official Method 976.36 (**AOAC International**, 1995) applicable to animal tissues (kidney, liver, muscle, fat; sample weight 25 gram each) down to 10 µg/kg melengestrol acetate with a calibration range between 10 and 30 µg/kg (first action in 1976 and final action 1978).

The method uses large volumes of organic solvents, amongst others benzene, a solvent the use of which is prohibited in many parts of the world due to its carcinogenic properties. It uses packed column GLC-ECD for final separation and quantification of the melengestrol acetate residue. However, improvements to GLC-ECD instrumentation and its implicit analytical signal processing varied considerable in the past 25 years. The GLC-ECD conditions as used in the collaboratively tested method are no longer operational or even available in contemporary regulatory laboratories performing residue analyses. In consequence the sponsors method as such does no longer exist. The validation data obtained from the 1974 AOAC collaborative study are no longer applicable for contemporary method performance.

As early as the mid seventies various independent attempts were made to develop regulatory methods with better performance characteristics than the sponsors method, especially with regard to improved specificity and a lower limit of quantification (see for example the GLC-ECD method published by **Ryan & Dupont**, 1975). The Committee also noted that a variety of potentially suitable modern regulatory residue methods is available which are *in-house-validated* according to recent requirements (**FAO/IAEA**, 1998).

A collaboratively studied GLC/ECD method (**Krzeminsky *et al.***, 1976) was used in the most relevant residue studies. The results of the GLC/ECD method can be confirmed by a GLC-mass spectrometric method with a LOQ of 10 µg/kg in fat (**Krzeminsky *et al.***, 1974a). Further comparative studies have been conducted in order to correlate the results obtained with field samples collected in several U. S. States and using this method in two or more laboratories, e.g., USDA/APHIS and the sponsor. In one such study (**Neff**, 1977), 130 fat samples obtained from beef heifers were analyzed in the two laboratories. A coefficient of correlation of r=0.791 was obtained. The results are shown in Figure 6 on the basis of normalized peak heights measured (20 mm peak height corresponds to 10 µg/kg melengestrol acetate).

Figure 6. Correlation of the results of MGA assays in fat carried out in two laboratories.



The results of the sponsor laboratories had not been corrected for recovery. The triangles in Figure 6 highlight results that were above the LOQ in at least one of the two laboratories involved in the study. The good correlation between results even at levels below the established lower limit of reliable measurement suggests that melengestrol acetate was present in nearly all fat samples and that the 99th percentile of the residue levels found in these field samples was slightly above 10 µg/kg.

APPRAISAL

A large data base was made available to the Committee to estimate the median and higher percentiles of "on treatment" concentrations of residues of parent melengestrol acetate in fat of animals treated with the upper recommended dose (0.5 mg/animal per day. There were also sufficient data from several studies where doses ranging from 0.3 to 10 mg of melengestrol acetate/animal per day had been used to extrapolate the 99th percentile of "on treatment" concentrations of residues of parent melengestrol acetate in fat of animals treated with the lower recommended dose (0.25 mg of melengestrol acetate/animal per day). There were three studies that included four different dose rates where it was also possible to estimate rate constants for the depletion of parent melengestrol acetate in fat. However, information on concentrations of residues of melengestrol acetate in the remaining three standard edible tissues (muscle, liver and kidney) was limited. Knowledge of the ratio between concentrations of "total residue" and of parent melengestrol acetate was based on a study conducted with four animals. In this study ^{3}H-labeled as well as ^{14}C-labeled melengestrol acetate was used. No information on both the nature and quantities of individual metabolites with potential progestogenic activity was available.

MAXIMUM RESIDUE LIMITS

Therefore, in recommending MRLs, the Committee took into account the following:

- An ADI of 0-0.03 µg/kg of body weight corresponding to an upper limit of acceptable daily intakes of 1.8 µg for a 60 kg body weight person
- Only fat and liver contain routinely quantifiable concentrations of the marker residue; methods with limits of quantification above 0.3 µg/kg are unlikely to quantify residues in muscle of incurred tissues from animals treated with recommended doses of melengestrol acetate. The sponsors did not make available a suitable analytical method for monitoring the MRLs at this concentration.
- Due to lack of information on structure and activity, metabolites had to be treated as if they were equally potent progestogens.

On this basis, the Committee recommended Temporary Maximum Residue Limits of 5 µg/kg of parent melengestrol acetate in fat and of 2 µg/kg of parent melengestrol acetate in liver.

Taking into account the ratio between concentrations of total residue and of marker residue in the four standard edible tissues (see Tables 1, 7a and 7b) a Theoretical Maximum Daily Intake (TMDI) of 1.2 µg of total residue expressed as melengestrol acetate equivalents per 60 kg person would result from the consumption of 300 g of muscle, 100g of liver, 50g of kidney and 50g of fat. The contribution of muscle and kidney to the TMDI is in the order of only 0.15 µg of total residue for muscle and 0.05 µg for kidney. The contribution to this TMDI of the residues of parent melengestrol acetate in all four tissues is about 0.6 µg per person. Therefore, the Theoretical Maximum Daily Intakes of parent melengestrol acetate as well as of its unidentified metabolites remains well below the ADI recommended by the Committee.

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