MELENGESTROL ACETATE*

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ADDENDUM

To the monograph and its addendum prepared by the 54th and 58th meetings of the Committee and published in FAO Food and Nutrition Papers 41/13 and 41/14, respectively.

INTRODUCTION

Melengestrol acetate (17α-acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione acetate; MGA) is a progestogen that is used as an animal feed additive to improve feed efficiency, increase the rate of weight gain, and suppress oestrus in beef heifers. MGA is fed at daily doses of 0.25 – 0.50 mg per heifer for 90 to 150 days prior to slaughter. The Committee at its 54th meeting (Annex 1, reference 146) recommended temporary MRLs of 5 μg/kg for cattle fat and 2 μg/kg for cattle liver, and requested information on an analytical method suitable for quantifying residues of MGA in liver and fat tissue (JECFA, 2000). At its 58th meeting (Annex 1, reference 157), the Committee concluded that the analytical method submitted for evaluation had been validated for monitoring compliance with the MRLs, and recommended that the temporary MRLs for cattle liver and fat be made permanent (JECFA, 2002).

At its 54th meeting, the Committee was provided with insufficient information to characterise the structure and activity of the metabolites of MGA. The Committee therefore assumed that the metabolites were equipotent to MGA in terms of progestogenic activity in elaborating the temporary MRLs. At its 62nd meeting the Committee considered new data on the metabolism of MGA in vitro , which provided the structural identities of the major metabolites of MGA, as well as a report describing the results of in vitro transcriptional activation/reporter assays , which were used to determine the relative hormonal activities of MGA and its metabolites.

METABOLISM

The extensive metabolism of MGA in several animal species and in humans was documented in previous reports. In the present studies, the metabolic profile of MGA was characterized by means of the generation and isolation of metabolites in test systems in vitro, since the concentrations of metabolites in tissues and excreta from cattle fed with MGA were too low for this purpose. The test systems investigated used hepatic microsomes, hepatic S9 fractions, and liver slices, all of which were prepared from beef heifers. The metabolites were separated by semi-preparative HPLC and their structures characterized by HPLC, HPLC-MS and nuclear magnetic resonance (NMR).

Preliminary in vitro experiments were conducted to optimise the conditions for generating the greatest relative yield of metabolites. Typically, microsomes (0.5 mg/mL protein) or S9 fractions (1 mg/mL protein) were incubated at 37oC with the desired concentration of MGA and 1 mM NADPH. Reactions were terminated by the addition of ice-cold acetonitrile and the samples centrifuged at approximately 1000 g for 10 min. The supernatants were recovered and analysed by HPLC.

Incubation time for the in vitro generation of metabolites was optimised using batched liver microsomes prepared from several heifers. Pooled microsomes (0.5 mg/mL microsomal protein) were incubated with 100 μM MGA and 1 mM NADPH for 0, 1, 3, 5, 10, 20, 30, 60, and 120 min. Recovered supernatants were analysed by HPLC-UV. Based on the number and quantities of metabolites produced, an incubation time of 120 min was selected for experiments designed to generate metabolites. Metabolites were labelled A through E, according to the order in which they eluted on chromatography. The conversion of MGA and the formation of metabolites in liver microsomes prepared from heifers are given in Table 1. The trace amounts of Metabolite A generated were insufficient to quantify or characterise this metabolite.

Table 1 Conversion of MGA and formation of metabolites in bovine liver microsomes incubated for 120 minutes.

MGA converted (%)	Metabolite B formed (%)	Metabolite C formed (%)	Metabolite D formed (%)	Metabolite E formed (%)
25	1	5	2	15

* During the editing of the monograph for melengestrol acetate (MGA) some inconsistencies in the approach to derive the activity weighing factors for MGA-related residues where detected which could be corrected partially. To address all of them requires a revision at the next meeting of JECFA that will assess residues of veterinary drugs.

The effect of MGA concentration on the metabolite profiles was investigated in a separate experiment. Pooled liver microsomes from heifers were incubated for 30 min with MGA at 1, 12.4, 31, and 100 μM with at least 3 replicates per concentration. Sufficient amounts of Metabolites B, C, D, and E for quantification were generated at a concentration of 100 μM MGA (Table 2), the concentration chosen for subsequent experiments.

Table 2 Conversion of MGA and formation of metabolites in bovine liver microsomes incubated for 30 minutes.

MGA conc μM	MGA converted (%)	Metabolite B formed (%)	Metabolite C formed (%)	Metabolite D formed (%)	Metabolite E formed (%)
1	65	9	35	0	50
100	10	<2	20	12	65

The metabolite profiles of MGA from S9 fractions and liver microsomes from several beef cattle were compared. S9 fractions prepared from the livers of several male and female beef cattle were pooled and mixed in approximately equal sized batches for heifers and steers. Metabolites C and E were the most abundantly produced metabolites; Metabolite D was also formed. Other metabolites from the microsomal incubations were not observed in the S9 fractions.

A comparison of the metabolite profiles and examination for additional metabolites were undertaken in batches of mixed-sex bovine liver microsomes and from heifer-only liver microsomes. Similar metabolite profiles were generated for both batches and no new metabolites requiring characterisation were produced.

MGA metabolism was also investigated in liver slices prepared from two beef heifers. Metabolites C, D, and E, of which Metabolite C was the most abundant, was detected on HPLC analysis. At least three other peaks were present on the chromatograms, but were unrelated to MGA.

Additional studies with human microsomes, rat microsomes and human cytochrome P450 were performed to provide a better understanding of the historical data from comparative in vivo metabolism and toxicology studies of MGA.

A procedure similar to that described above for the bovine in vitro test systems was used with human and rat microsomes. Pooled microsomes from humans and rats were incubated with 100 μM MGA and 1 mM NADPH for 60 or 120 min. In human microsomes, Metabolite E was the most abundant metabolite, while significant quantities of Metabolites C, D, A, and B were produced. By comparison, in rat microsomes Metabolite C was the most abundant, Metabolites D and E were major metabolites, and Metabolites A and B were minor metabolites. Additional minor metabolites, identified by LC/MS as monohydroxy and dihydroxy metabolites, were produced by both human and rat microsomes but were present in insufficient quantities for further characterisation.

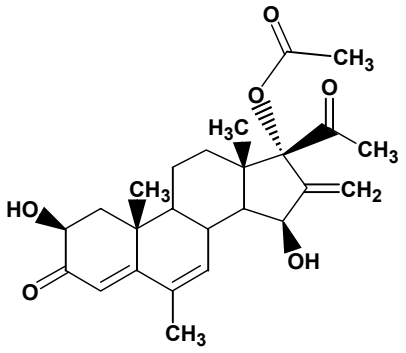
Human recombinant CYP450 isoenzymes, which were purchased as a pre-manufactured mixture and as individual isoenzymes of 1A2, 2C8, 2C9, 2C19, 2D6, and 3A4, were incubated under conditions identical to those described above. The results indicated that CYP450 metabolism of MGA is attributed primarily to 3A4. All major metabolites observed in human microsome incubations and in the isoenzyme mixture were produced by 3A4 in the same proportions, with Metabolite E being the most abundant metabolite. In contrast Metabolite C was the primary metabolite observed with 1A2, 2C8, 2C9, 2C19, and 2D6, with only a small amount of Metabolite E being produced.

MGA and its metabolites produced from the in vitro test systems were analysed by reverse phase HPLC with UV detection. Compounds were separated on a C-18 column and the eluants monitored by photodiode array detection at 285 nm. Different linear solvent gradient programs were used to analyse samples collected from liver microsomes and liver slices. A flow rate of 1 mL/min was used in both cases.

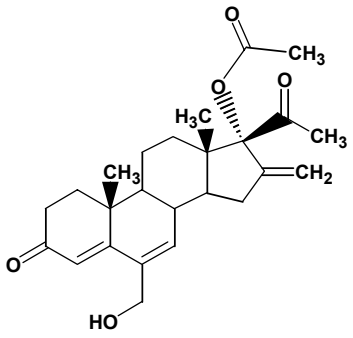
An experiment was undertaken to generate, isolate, and prepare MGA metabolites from heifer liver microsomes. Metabolites were generated using a 40x scale-up of the procedure described above for microsomes. Metabolite isolation was accomplished by reversed phase semi-preparative HPLC on a C18 column, a linear solvent gradient program, at a flow rate of 4 mL/min. Microsomal samples were loaded onto the column and 1-minute fractions collected. The MGA metabolites were then prepared for chemical structure characterisation.

The chemical structures of Metabolites B, C, D, and E were characterised using NMR and HPLC/MS; Metabolite A was not characterised since it was generated only in trace amounts. NMR data were acquired using a Varian INOVA 500 MHz NMR spectrometer operating at a proton observation frequency of 499.79 MHz and equipped with a Nalorac MIDTG 3-mm NMR probe. LC/MS analysis of Metabolites B, C, D, and E was performed on a ThermoFinnigan TSQ-Quantum triple quadrupole mass spectrometer operating in the positive-ion ESI mode. Separation by the LC/MS system was performed on a C18 column with a linear solvent gradient program at a flow rate of 1 mL/min. The eluant was monitored by photodiode array detection at 190-800 nm and MS detection of 150-900 amu.

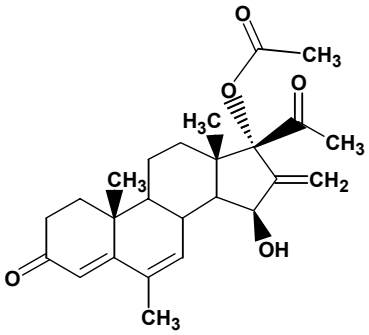
Structural assignments for the metabolites of MGA were based upon combined data from HPLC, LC/MS and NMR. Structures were assigned as follows:



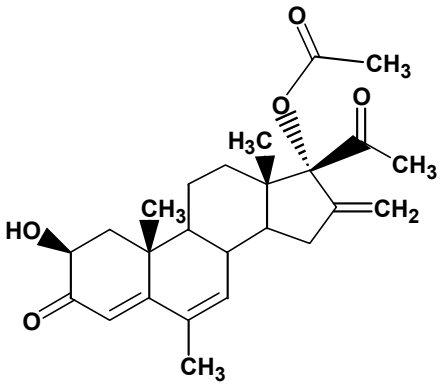
Metabolite B (2ß,15ß-dihydroxy-MGA)



Metabolite C (6-hydroxymethyl-MGA)



Metabolite D (15ß-hydroxy-MGA)



Metabolite E (2ß-hydroxy-MGA)

The metabolic pathway proposed for the biotransformation of MGA involved mono-hydroxylation of MGA to Metabolites C, D, and E. Metabolite B was presumed to form from Metabolite D (15β-hydroxy-MGA) by hydroxylation at C2, but not from Metabolite E (2β-hydroxy-MGA). This is consistent with the formation of Metabolite B in microsome incubations in which Metabolite D and not Metabolite E was isolated.

MGA metabolite steroid receptor specificity and relative potency

Samples of MGA, melengestrol, and Metabolites B, C, D, and E were prepared for testing by dilution in absolute ethanol to final concentrations of 100 μM for MGA and melengestrol, 1 μM for Metabolite B, and 10 μM for Metabolites C, D, and E. The purity of each metabolite, MGA and melengestrol as used in the in vitro transcriptional activator/reporter gene assay was determined to be >95% by HPLC-UV. Activity was compared against progesterone, R5020 (a synthetic progestin) and medroxyprogesterone acetate (MPA) in progesterone receptor assays; dexamethasone, hydrocortisone and medroxyprogesterone acetate in glucocorticoid receptor assays; dihydrotestosterone, R1881 (a synthetic androgen), progesterone and medroxyprogesterone acetate in androgen receptor assays; and 17β-estradiol, ethinyl estradiol (17α-ethinyl-17β-estradiol) and medroxyprogesterone acetate in oestrogen receptor assays. Comparator compounds were selected either because they exhibit hormonal activity in vivo due to their ability to act as agonists of specific hormone receptors, or they have close structural similarity to MGA. Melengestrol, while not detected as a metabolite in bovine metabolism studies, was included in this experiment since it is a potential enzymatic cleavage product of MGA.

In vitro transcriptional activation/reporter gene assays were used to determine the activities of compounds as agonists for the human progesterone receptor B-subtype (PR), the human glucocorticoid receptor (GR), the human androgen receptor (AR), and the human oestrogen receptor α-subtype (ERα). The B-subtype of the human progesterone receptor was chosen because it is the dominant subtype in humans. Monkey kidney CV-1 cells were transiently co-transfected with the designated human steroid receptor expression vector and a luciferase reporter vector containing the appropriate hormone response element. The mouse mammary tumour virus-luciferase reporter vector (MMTV-Luc) was used for PR, GR, and AR, whereas the oestrogen response element-luciferase reporter vector (ERE-Luc) was used for ERα. The response of each hormone receptor to test substance was determined by steroid receptor-mediated transcription of MMTV-Luc for PR, GR, and AR, or ERE-Luc for ERα, to produce luciferase and thus yield a measurable signal upon the introduction of luciferin.

Details of the PR, GR, AR, and ERα assay procedures were provided but are not discussed here. Each assay experiment was conducted over 3 days (ERα) or 4 days (PR, GR, and AR).

MGA, melengestrol, and Metabolites B, C, D, and E were evaluated in each hormone receptor assay. The test concentrations chosen for these experiments were consistent with the approach used in classic pharmacodynamic dose-response studies, in which the response is mathematically expressed by the standard Hill equation describing receptor-mediated responses, and the response is linearly related to the logarithm of concentration or dose (Novotny, 2001).

Each assay experiment was repeated three times in separate weeks for GR, AR, and ERα, and eight times for PR. Each test substance and each comparator compound was analysed in duplicate at each concentration. Control blanks comprising tissue culture media without steroid test substances and a pCMV5(p5) empty expression vector control were included. The latter lacked activity in the absence and presence of a control agonist, thereby demonstrating that transcriptional activity resulted from the expressed receptor and was independent of any receptor endogenous to CV1 cells.

The relative activity of each compound for each receptor was reported as the minimum effective concentration resulting in 50-100% maximal transactivation, as indicated by luciferase activity. Calculations used the lowest reported value in cases in which a range was reported. Where luciferase activity was not detectable (less than 5-fold higher than the no ligand control), the highest concentration tested that did not yield a response was used in calculations. In cases in which the highest

concentration tested produced a detectable response but did not result in 50-100% maximal transactivation, the highest tested concentration was used in calculations.

The response of MGA, its metabolites, and of the comparator compounds in the hormone receptor assays are shown in Table 4. The responses reported in this table represent approximate orders of magnitude of relative biological activity, with each value being the most common empirical observation for each ligand in each assay, without regard to statistical evaluation.

Table 4 Minimum effective concentrations (nM) for compounds to induce 50-100% maximal transactivation of human hormone receptors

Compound	PR	GR	AR	ERα
Progesterone	0.1	---	>100	---
Medroxyprogesterone acetate	0.01	10	1	>100
R5020	0.01	---	---	---
Dexamethasone	---	1	---	---
Cortisol	---	10	---	---
Dihydrotestosterone	---	---	0.1	---
R1881	---	---	0.1	---
17β-Estradiol	---	---	---	0.01
Ethinyl estradiol	---	---	---	0.01
MGA	0.01	1	>100	>100
Melengestrol	>100	10	>100	>100
Metabolite B	5	>10	>10	>10
Metabolite C	10	>100	>100	>100
Metabolite D	10	>100	>100	>100
Metabolite E	0.1	10	>100	>100

The data show that MGA and its metabolites exert their biological action primarily as progestogens and secondarily as glucocorticoids. At relevant physiologic concentrations, no activity was demonstrated in either the AR or ERα assays.

Transcriptional activation of MMTV-Luc by full-length human PR was maximal in response to approximately 0.1 to 1 nM progesterone, 0.01 nM R5020, and 0.01 nM medroxyprogesterone acetate. These data indicate that all of the assays were performing correctly, exhibiting responses that were consistent with historical data and general biologic activity relationships. With MGA, 50-100% maximal transactivation of PR was observed at 0.01 nM but not at 0.005 nM. Metabolite E was the most active metabolite with activity (i.e. the minimum concentration resulting in 50-100% maximal transactivation) being reported at 0.1 nM but not at 0.01 nM. Metabolites B, C, and D were generally active at 1 to 10 nM, which represented much lower activity compared to MGA. Melengestrol began to exhibit activity at concentrations of 1-100 nM but response was <50-100% maximal at these concentrations.

Activity data resulting from MGA, its metabolites and melengestrol in the PR assay were analysed statistically using an analysis of variance, with the objective of determining the relative bioactivity of each compound compared to parent MGA. The results are shown in Table 5. The comparator compounds progesterone, R5020 and MPA, were not included in the statistical analysis.

Table 5 Relative activity of MGA metabolites versus MGA based upon the PR assay

Compound	Relative Activity (%)	95% Confidence Interval (%)
Metabolite B	0.16	0.03, 0.89
Metabolite C	0.23	0.05, 1.05
Metabolite D	0.09	0.02, 0.39
Metabolite E	8.59	1.88, 39.30
Melengestrol	0.85	0.02, 47.08

Large 95% confidence intervals were reported for Metabolite E and melengestrol (Table 5). With Metabolite E, this is attributed primarily to one of eight assay results where the 50-100% maximal response of the human PR occurred at 0.0005 nM MGA, in contrast to 0.01 nM for the other assays. The activity of Metabolite E in the PR assays was consistent at 0.1 nM with one isolated exception of 0.01-0.1 nM. When melengestrol was used as the test substance in PR assays, activity was reported to range from 0.001 nM to >100 nM. Such pronounced variability contributed to a large 95% confidence interval, which is not a concern since melengestrol demonstrated < 0.01% of the progestogen activity of MGA (Table 4).

The progestogenic activity of Metabolite E was further examined using a modelling approach. This approach differs from the statistical approach described above since it does not rely on discrete values, but instead utilises all data from the assays to interpolate activity between the discrete values. The induction level was determined for each experimental observation as the ratio of the observed optical unit response to the average baseline. These induction data were analysed using a mixed effects model analysis of variance from which the concentration least squares means for the compound were determined and converted to percentages of maximum MGA induction. For each compound, the percent of maximum induction values were then fitted to a logistic model. The predicted concentrations for 10%, 50% and 90% maximum induction for MGA and for Metabolite E were determined from the model. The ratio of Metabolite E to MGA was used to determine the relative biologic activity of Metabolite E at each induction level (10%, 50%, and 90%).

The least squares means with standard errors for MGA and Metabolite E are summarised in Table 6 and graphically illustrated in Figure 1.

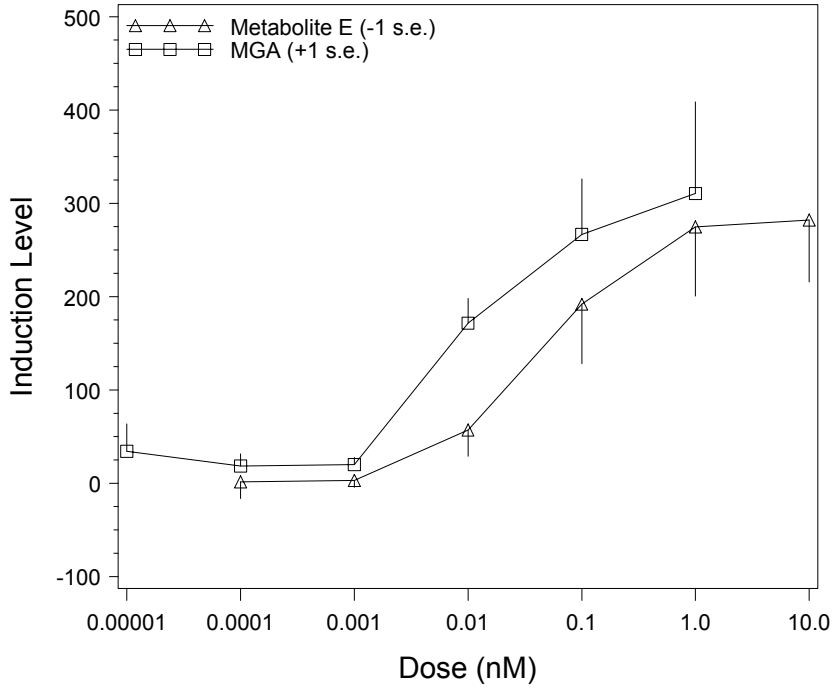
Table 6 Summary statistics for induction levels for MGA and Metabolite E

Concen-tration* (nM)	MGA				Metabolite E			
	n	LSMean	Std Err	Percent†	n	LSMean	Std Err	Percent†
0.00001	2	34.15	29.35	11.0	-	-	-	-
0.0001	6	18.43	12.86	5.9	3	1.49	18.04	0.5
0.001	7	19.99	7.60	6.4	7	3.04	7.60	1.0
0.01	8	171.57	26.33	55.3	7	57.00	28.15	18.4
0.1	8	266.65	59.62	85.9	7	191.79	63.74	61.8
1	4	310.45	98.12	100	7	274.57	74.19	88.4
10	-	-	-	-	4	282.12	66.43	90.9

* MGA was tested at 0.0005, 0.005, 0.05 and 10 nM in only one assay and because of lack of replication were not included in these analyses.

† Percent of the maximum MGA response in the LSMeans (these are the data used in the logistic models).

Figure 1 Plot of the least squares means +/- standard error for MGA and metabolite E (lines are simple line segments connecting each point)



The fit of the logistic models is shown in Figure 2 while the concentration to reach induction levels of 10%, 50% and 90% and the relative bioactivity (potency) of Metabolite E are shown in Table 7. The potency of Metabolite E relative to MGA was 12.2% at the 10% induction level, 12.0% at the 50% induction level, and 11.8% at the 90% induction level.

Figure 2 Plot of the logistic model fits and the LSMeans for MGA and Metabolite E.

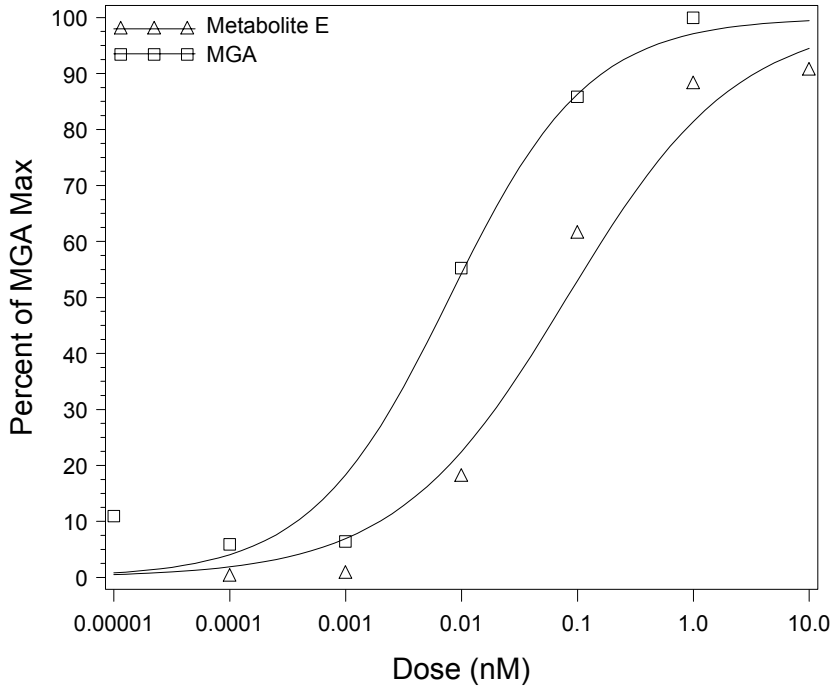


Table 7 The relative bioactivity of Metabolite E to MGA at various points on the curves (based on data from the logistic model).

% of Max MGA	Concentration of MGA* (nM/L)	Concentration of E* (nM/L)	Relative Activity of E to MGA
10	0.0005	0.0038	12.2%
50	0.0088	0.0733	12.0%
90	0.1677	1.4162	11.8%
*	As predicted by the models, the dose at which the % of maximum MGA is reached.		

APPRAISAL

Previous studies reported the extensive metabolism of MGA in several animal species and in humans. In vivo studies that investigated the fate of MGA have been conducted in cattle (Krzeminski et al, 1981), rabbits (Cooper et al, 1965), and women (Cooper et al, 1967). None of the metabolites of MGA formed in cattle were identified, whereas 6-hydroxymethyl-MGA and 2α-hydroxy-MGA were identified in the urine of rabbits. At least thirteen metabolites were generated in the urine of women, however, only one of these, 2α-hydroxy-MGA, was identified. In vitro studies into the metabolism of MGA have also been reported. Early experiments with bovine liver homogenates and rumen fluids were conducted using 3H-MGA (Janglan 1975a, 1975b). It was not possible to characterise the metabolites of MGA in these studies due to the limitations of analytical methodologies at the time. More recently, the extensive oxidative metabolism of MGA by hepatic microsomes prepared from rats, bovine, and human liver has been reported (Pfeiffer and Metzler, 2001). Although seven mono-oxygenated and five dioxygenated metabolites were observed in these studies, none of the metabolites was characterised further.

In the present studies, the metabolic profile of MGA was characterised following the generation and isolation of metabolites in in vitro test systems prepared from beef heifers. The metabolites were separated by HPLC and their structures characterised by HPLC, HPLC/MS and NMR. Three monohydroxy metabolites, one dihydroxy metabolite, and several trace metabolites were generated in bovine liver microsomes. Metabolites, from greatest to least abundance, were 2β-hydroxy-MGA (Metabolite E), 6-hydroxymethyl-MGA (Metabolite C), 15β-hydroxy-MGA (Metabolite D), and 2β,15β-dihydroxy-MGA (Metabolite B). The 2 β stereochemistry assigned to the hydroxyl moiety of Metabolite E differs from the 2α stereochemistry assigned arbitrarily by Cooper (1968), presumably reflecting the modern technology utilised in the present studies. Since Metabolite A was generated only in trace amounts, its structure could not be determined. Additional metabolites formed in trace amounts by bovine liver microsomal systems were identified as monohydroxy and dihydroxy products. Furthermore, no conjugation products or additional metabolites of MGA were observed in bovine liver slices or bovine liver S9 fractions.

Rat microsomes, human microsomes and human recombinant cytochromes P450 generated Metabolites B, C, D, and E, and additional minor metabolites. The latter were identified as monohydroxy and dihydroxy products. However, there were insufficient amounts for complete structure elucidation. Human P450 metabolism of MGA was shown to be primarily attributable to the CYP3A4 isoenzyme.

Using metabolites separated by semi-preparative HPLC in an in vitro cell receptor and gene expression system, the present studies concluded that MGA and its metabolites exert their biological action primarily as progestogens and secondarily as glucocorticoids. At relevant physiologic concentrations, no activity was demonstrated in either the androgen (AR) or oestrogen (ERα) receptor assays.

Since MGA and its metabolites were demonstrated to function primarily as progestogens, PR assay data were used to determine the relative bioactivity or potency (mg/kg dose resulting in equal pharmacological effect) of each compound compared to MGA. Metabolite E was shown to be the most potent of the metabolites when the pharmacodynamic data were analysed statistically using analysis of variance. The potency of Metabolite E relative to MGA, as measured by minimum induction concentrations that resulted in 50-100% maximal response, was estimated as 8.6% (i.e. 11.6-fold the dose of Metabolite E was required to achieve similar progestogenic activity as MGA). By comparison, the mean progestogen activities relative to MGA were 0.16% for Metabolite B, 0.23% for Metabolite C and 0.09% for Metabolite D.

The relative progestogenic activities of Metabolite E and MGA were subsequently compared by fitting concentration-effect curves using logistic modelling. In this analysis, all data from the assays were used to interpolate activity between the discrete test values. The concentration-effect curves for MGA and Metabolite E were parallel, indicating that both compounds act through the same receptor. The predicted concentrations of MGA and Metabolite E for 10%, 50% and 90% maximum response were determined. The potency of Metabolite E relative to MGA was 12.2% at the 10% induction level, 12.0% at the 50% induction level, and 11.8% at the 90% induction level.

The 54th meeting of the Committee noted that MGA, which is the marker residue, accounted for 85% of the total residues in fat and 33% of the total residues in liver (JECFA, 2000). Moreover, the ratio in total residues that was used to establish the MRLs for fat and liver was based upon radiolabelled metabolism studies in animals slaughtered under conditions consistent with zero-day withdrawal (6 hours after the last dose). The ratio of MGA residues in fat versus liver was 1.6:1. Based on the new information, the toxicological significance of the metabolites of MGA in tissue residues was considered further. Metabolite E, the most active metabolite, demonstrated on average 12% of the progestogenic potency of MGA i.e. on average requiring 8.8-fold the dose of MGA to achieve equipotent progestogenic activity. The relative potency of Metabolite E was then used to define the biological activity of the entire non-MGA fraction in the tissue residue, which potentially may be present in food for human consumption. This is conservative since the other metabolites (Metabolites B, C and D) had negligible activities ranging from 0.09% to 0.23% versus MGA. On the basis of the relative potency of Metabolite E, the non-MGA residues (fat 15%; liver 67%) were converted to MGA activity equivalents by reducing the percentage by a factor of 8.8. As shown in Table 8, 2.07% of the total progestogenic activity was attributable to non-MGA residues in fat and liver, respectively.

Table 8 Activity Weighting Factors for MGA-related residues in tissues

Tissue	% of total radioactive residue attributable to:		% of total progestogenic activity attributable to[b]:		
	MGA[a]	Non-MGA residues	MGA	Non-MGA residues	Sum of progestogenic residues
Fat	85	15	$\frac{85 \times 1 \times 100}{85 + (0.12 \times 15)}$	$\frac{15 \times 0.12 \times 100}{85 + (0.12 \times 15)}$	97.93 + 2.07 = 100
Liver	33	67	$\frac{33 \times 1 \times 100}{33 + (0.12 \times 67)}$	$\frac{67 \times 0.12 \times 100}{33 + (0.12 \times 67)}$	80.4 + 19.6 = 100
Kidney	<<LOQ	<<LOQ	-	-	-
Muscle	<<LOQ	<<LOQ	-	-	-

[a] Data from 54th JECFA

[b] The % of progestogenic activity of MGA-related residues is calculated by applying a weighting factor of 1 to MGA and of 0.12 (corresponding to the relative potency of Metabolite E) to all non-MGA metabolites, respectively, in fat and in liver.

The MRLs were subsequently derived by apportioning the ADI to the corrected total residues in fat and liver, in a ratio of 1.6:1 (Table 9). Accordingly, MRLs for cattle in fat and liver of 8 μg/kg and 5 μg/kg, respectively, were proposed.

Table 9 Theoretical maximum daily intake of MGA residues

Tissue	MRL (μg/kg)	Marker residue/ total residue[a]	Total residue (μg/kg)	Diet (kg)	Intake of residues ((μg)
Fat	8	0.979	8.2	0.05	0.41
Liver	5	0.804	6.2	0.1	0.62
TMDI					1.03

[a] This ratio is based on % of total progestogenic activity of the marker residue MGA as shown in column 6 in Table 8.

MAXIMUM RESIDUE LIMITS

In reaching its decision on MRLs for MGA, the Committee took the following factors into account:

- The established ADI is 0-0.03 μg/kg bw, which is the equivalent to up to 1.8 μg for a 60-kg person.
- The metabolites of MGA in in vitro test systems prepared from female cattle were identified as 2β,15β-dihydroxy-MGA (Metabolite B), 6-hydroxymethyl-MGA (Metabolite C), 15β-hydroxy-MGA (Metabolite D), and 2β-hydroxy-MGA (Metabolite E).
- Activation by MGA and its metabolites in in vitro test systems was most selective for the human progesterone receptor, which is consistent with historical in vivo data.
- Based on the submitted data, the biological activity of MGA-related residues in edible tissues of MGA-fed beef heifers can be principally attributed to MGA.
- The most active metabolite of MGA, 2β-hydroxy-MGA (Metabolite E), is 9-times less potent than MGA.
- A suitable regulatory method is available.

The Committee recommended MRLs in cattle of 8 μg/kg for fat and 5 μg/kg for liver, expressed as MGA. From these values, the theoretical daily intake of residues as MGA equivalents is 1.03 μg per person or 57.2% of the allowable ADI.

References

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