

Toxicological evaluation of certain veterinary drug residues in food

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MELENGESTROL ACETATE (addendum)

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1. EXPLANATION

Melengestrol acetate (17 α -acetoxy-6-methyl-16-methylene-4,6-pregna-diene-3,20-dione; MGA) is a synthetic progestogen that is active after oral administration. It is administered as a feed additive to female beef cattle (heifers) to improve the efficiency of feed conversion, promote growth and suppress estrus. The range of approved doses is 0.25–0.50 mg/heifer daily, and it is fed for the duration of the fattening/finishing period, usually 90–150 days.

MGA has been reviewed previously by the Committee at its fifty-fourth, fifty-eighth, sixty-second and sixty-sixth meetings (Annex 1, references 146, 157, 169 and 187). An acceptable daily intake (ADI) of 0–0.03 g/kg body weight (bw) was established at the fifty-fourth meeting of the Committee. Maximum residue levels (MRLs) were initially recommended at the fifty-fourth meeting of the Committee and

subsequently reconsidered at the sixty-second and sixty-sixth meetings based on new metabolism studies that identified specific metabolites and determined their progestogenic activity relative to that of MGA. The sixty-sixth meeting recommended MRLs of 18 µg/kg in fat, 10 µg/kg in liver, 2 µg/kg in kidney and 1 µg/kg in muscle tissue of cattle. These MRLs are expressed as MGA equivalents and cover both MGA itself, as the marker residue, and its metabolites. The biological activity of the metabolites (non-MGA fraction of the tissue residues) was converted to MGA equivalents, taking into account their known, lower progestogenic activity and converting the marker residue to total residue, based on the fraction attributable to marker residue in each of the relevant tissues. The analytical methods were extensively reviewed at the fifty-eighth meeting of the Committee, at which time the Committee noted that the available high-performance liquid chromatography–mass spectrometry (HPLC-MS) method submitted by the sponsor was suitable to measure residues in food animal tissues.

At the 17th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) (Codex Alimentarius Commission, 2007), CCRVDF recommended that MGA be reconsidered based on a commitment of new scientific data on toxicity and hormonal effects to be provided by the European Commission, including reference to the specific issues to be addressed. Scientific data have now been submitted by the European Commission, comprising a number of papers published in the scientific literature or submitted as unpublished original research reports to the European Commission between 1996 and 2007. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was asked to reconsider MGA taking into account the more recent data on analytical methods, measurement of residues in tissues, toxicity and hormonal effects of MGA.

In the covering letter included in the European Commission submission, the Committee was asked in particular:

- To reconsider important end-points, such as the effects on pre-pubertal children (and other subpopulations that may be exposed) on the immune system, endocrinological effects etc.
- To discuss data indicating that hormones do not only act via hormone-mediated receptors but other mechanisms (e.g. direct and indirect genotoxic effects).
- To re-calculate the data from residues in meat from animals treated with these hormones for growth promotion purposes and to take into account data coming in particular from improved analytical capacities.
- To describe precisely what authorised use of *good practice of use of the veterinary drugs* it considers appropriate in the evaluation and their respective impact on potential exposure and risk estimate.

These issues were considered by the Committee at its present meeting, taking into account relevant earlier information as well as the submitted data. The Committee also searched the literature for any new publications on MGA since its last toxicological review in 2000 (Annex 1, reference 146). The majority of the papers and reports submitted by the European Commission concerned actions of hormones or substances with hormone-like activity in general. There is only limited new or additional information on MGA itself, covering developments in analytical

methods for residue analysis, further information on plasma concentrations of MGA and MGA residues in tissues of heifers, the mode of action of MGA in accelerating onset of puberty in heifers, lack of effect of MGA in bulls, further in vitro studies on interactions of MGA with hormone receptors and downstream effects, pathological effects in zoo animals with long-term contraceptive MGA implants, and effects of prenatal, juvenile and adult exposure to MGA in rabbits.

The main focus of this re-evaluation is on MGA itself. The Committee did not consider it necessary to review the considerable amount of information that is now available from studies on experimental animals, wildlife and humans concerning the possible effects of a wide range of hormonally active chemicals on development, physiological function and disease (endocrine disrupter effects), as a number of general reviews have addressed these issues (see, for example, [Damstra et al., 2002](#); [Daston et al., 2003](#); [Tabb & Blumberg, 2006](#); [Hotchkiss et al., 2008](#); [Phillips & Foster, 2008](#)). This re-evaluation of MGA is predicated on an understanding that the present state of the science provides strong evidence for effects of endocrine disrupters in laboratory animals. However, while there are serious concerns for wildlife and human health effects, the causal links between exposure and endocrine disruption in these populations remain unclear. As others have pointed out, attempting to relate events during fetal and neonatal life to subsequent function and adult disease is an exceedingly difficult challenge for epidemiology (Vom Saal, 2007). The Committee also noted that there is still no consensus in the scientific community as to whether low-dose exposures to endocrine disrupters cause adverse effects in humans.

Since MGA has both progestogenic and glucocorticoid activity, the Committee gave specific consideration to background data on the possible role of progesterone and interactions of progestogens with progesterone receptors in development and disease and to the possible influence of glucocorticoids on immune function. The Committee noted that while MGA undoubtedly has been shown to have hormonal activity, as demonstrated by in vitro experiments and in vivo studies in laboratory animals and treated cattle, an important consideration in the risk assessment is whether human exposure to residues in meat at the upper bound of the ADI (0.03 µg/kg bw) would be expected to have any effect.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

MGA was administered to heifers at doses of 0, 0.5, 1.5 and 5 mg MGA/day (two heifers per dose group) for 8 weeks, at which time they were slaughtered and concentrations of MGA measured by enzyme immunoassay in plasma, liquid chromatography–mass spectrometry (LC-MS) in kidney and muscle, and gas chromatography–mass spectrometry (GC-MS) in perirenal fat. The results showed that MGA is lipophilic and accumulates in fat, with concentrations about 200-fold above plasma levels. Next highest concentrations were found in liver, about 20- to

40-fold above plasma levels, and lowest amounts in kidney and muscle, approximately 5-fold above plasma levels. Concentrations in perirenal fat were 6.5 and 8.4 µg/kg, 24.1 and 33.9 µg/kg, and 56.3¹ and 60.9 µg/kg after 0.5, 1.5 and 5 mg/day, respectively. Concentrations in liver were 0.8 and 1.0 µg/kg, 2.3 and 7.7 µg/kg, and 5.1 and 7.6 µg/kg after 0.5, 1.5 and 5 mg MGA/day, respectively. Concentrations in kidney and muscle were at or below 2 µg/kg in all three treated groups (Daxenberger et al., 1999). These results indicate considerable variability in tissue levels. Further information on these same tissue samples was reported by the same group in a later publication, for fat samples analysed by both enzyme immunoassay and GC-MS and for muscle samples analysed by both enzyme immunoassay and LC-MS. The results for each tissue/dose from the two analytical methods were in reasonable agreement, with mean coefficients of variation of 7.1% and 13.9% for fat and muscle, respectively (Hageleit et al., 2001). In each of the above studies, a further group of two heifers was given 0.5 mg MGA/day for 8 weeks, then subjected to a 48-h withdrawal period before slaughter. This made little difference to the amounts retained in fat. The authors pointed out that longer withdrawal periods cannot be used, because estrus would be triggered within 3–7 days of cessation of MGA treatment, and transporting heifers during estrus is not recommended (Daxenberger et al., 1999).

The passage of MGA across the placenta has been investigated in two rabbits given MGA orally in corn syrup at a dose of 0.5 mg/kg bw per day on gestation days 14–27. In the dams, MGA was detectable in the nanogram per millilitre range in plasma and was measured at 0.29 and 0.70 µg/kg in muscle, 190 and 160 µg/kg in liver, 2.80 and 2.50 µg/kg in kidney, and 28 and 72 µg/kg in fat (tissue origin not specified). Amounts in corresponding tissues in four fetuses were as follows: muscle, 0.88–0.10 µg/kg; liver, 5.10–7.10 µg/kg; kidney, 0.60–1.10 µg/kg; and fat, 3.10–7.10 µg/kg. Concentrations in the placenta ranged from 0.69 to 0.95 µg/kg. Concentrations in all tissues in controls were below the limit of detection. This study shows significant, but limited, passage of MGA across the placenta (Lange et al., 2002).

The data reviewed by the Committee in its previous evaluation indicate that MGA is extensively metabolized in the liver, with formation of numerous metabolites in most of the species studied, including humans (Annex 1, reference 147). Progesterone itself is rapidly metabolized and cleared in humans and other species (Golub et al., 2006), and low doses administered by the oral route are almost completely metabolized in one pass through the gastrointestinal mucosa and liver (Simon, 1995).

2.1.2 Biotransformation

No new studies on the biotransformation of MGA have been identified since the last evaluation in 2004, in which the major monohydroxy and dihydroxy

¹ This figure is actually quoted as 6.3 in Daxenberger et al. (1999), but data on the same samples analysed by GC-MS were also quoted in Hageleit et al. (2001), and there the value is given as 56.3, which seems the more likely value.

metabolites were identified from in vitro studies using hepatic microsomes from heifers, rats and humans (Annex 1, reference 169).

2.1.3 Hormonal activities of MGA

Early studies on the hormonal activities of MGA showed that it exerts both progestational and glucocorticoid activity (Lauderdale et al., 1977). In vitro, MGA shows strong progesterone receptor binding affinity, as measured by displacement of a progesterone analogue, 16 α -ethyl-21-hydroxy-19-nor[6,7-³H]pregn-4-ene-3,20-dione, from bovine uterine progesterin receptor in cell cytosol preparations; the relative binding affinity of MGA was 526% of that of progesterone, whereas the affinity of three MGA metabolites generated from bovine hepatocytes was between 25% and 85% of that of progesterone (Bauer et al., 2000). In a different assay using the MCF-7 human breast cancer cell line in which the test compounds are taken up into whole cells, MGA showed an 11-fold increase, compared with progesterone, in relative binding affinity to the human progesterone receptor, which has 90% homology with the bovine progesterone receptor (Perry et al., 2005). The progestational activity of MGA in vivo in cattle has been shown to be about 125 times that of progesterone, when both compounds are given parenterally and measured by estrous cycle inhibition (Lauderdale et al., 1977; Lauderdale, 1983).

The possibility that MGA possesses estrogenic activity has been investigated in vitro. Le Gueval & Padkel (2001) tested MGA in two different bioassays—a recombinant yeast assay expressing trout estrogen receptor, which is sensitive down to 0.1–1 nmol estradiol/l, and an assay that measures induction of vitellogenin gene expression (normally triggered by estradiol) in cultures of rainbow trout hepatocyte aggregates, which is sensitive down to about 10 nmol estradiol/l. In the recombinant yeast assay, MGA was inactive at 0.1 and 1 μ mol/l (~40 000 and 400 000 pg/ml) but was active at 10 μ mol/l (~4 000 000 pg/ml), whereas estrogenic steroids such as 17 β - and 17 α -estradiol, estrone, diethylstilbestrol, 17 α -ethynylestradiol, zearalenone and related compounds were active at concentrations at least 2 orders of magnitude lower than MGA. MGA was inactive in the vitellogenin gene expression assay at 1 and 10 μ mol/l (~400 000 and 4 000 000 pg/ml). In another in vitro bioassay utilizing cell proliferation in MCF-7 cells as a marker of estrogenic activity, MGA did not show any activity at picomole to nanomole per litre concentrations (10^{-11} – 10^{-9} mol/l: ~4–400 pg/ml), but did show small, but statistically significant, increases in cell proliferation at higher (nanomole to micromole per litre) concentrations (10^{-8} – 10^{-6} mol/l: ~4000–400 000 pg/ml), although it was again without activity at 10 μ mol/l (~4 000 000 pg/ml) (Perry et al., 2005). The concentrations showing estrogenic activity in vitro are considerably higher than the plasma concentrations of MGA achieved in vivo of about 25–50 pg/ml in heifers treated with 0.5 mg MGA daily (Daxenberger et al., 1999; Hageleit et al., 2000; Pfaffl et al., 2002).

There are no data on plasma concentrations of MGA following human dietary exposure. However, in the studies in heifers reported above (Daxenberger et al., 1999; Hageleit et al., 2000; Pfaffl et al., 2002), the daily dose of MGA administered in the feed corresponds to approximately 1.6 μ g/kg bw. Assuming similar absorption

and metabolism in cattle, rats, rabbits and humans, the plasma concentration in humans after ingestion of 0.03 µg MGA/kg bw (i.e. equivalent to the upper bound of the ADI) would be 0.5–1 pg/ml, some 4000-fold below the minimum concentration necessary to stimulate proliferation in MCF-7 cells. In addition, the study in rabbits reported above (Lange et al., 2002) found nanogram per millilitre concentrations of MGA in plasma following an oral dose of 0.5 mg/kg bw. This is in the same range as the minimum concentration necessary to produce effects on the estrogen receptor in MCF-7 cells. Given that the dose in this study is some 17 000-fold greater than the maximum dose that would be ingested, assuming consumption at the upper bound of the ADI, even allowing for the uncertainty introduced by species differences (rabbit versus human), it is not anticipated that dietary residues of MGA will have any estrogenic effects in humans consuming meat from animals treated with this drug.

MGA does not have antiestrogenic activity in MCF-7 cells (Perry et al., 2005). Nor does it have significant androgenic activity, as measured in assays of relative binding affinity to recombinant human androgen receptors or to human sex hormone binding globulin, when compared with a natural ligand such as dihydrotestosterone. In contrast, the active metabolite of the androgenic anabolic steroid trenbolone acetate, 17β-trenbolone, has a relative binding affinity to recombinant human androgen receptors similar to that of dihydrotestosterone (Bauer et al., 2000).

The anabolic mode of action of MGA in heifers is not firmly established, but is assumed to be by the stimulation of ovarian synthesis of endogenous estradiol, caused by follicular development; although there is follicular development, it is accompanied by inhibition of ovulation, likely due to blocking of the positive feedback on gonadotrophin release that would normally be exerted by endogenous estrogen during an untreated estrous cycle (Henricks et al., 1997; Hageleit et al., 2000). The plasma concentrations of MGA that suppress estrus in heifers, when given at the recommended dose of 0.5 mg MGA/day in the feed, are in the range 25–50 pg/ml (Daxenberger et al., 1999; Hageleit et al., 2000; Pfaffl et al., 2002). Endogenous plasma estradiol concentrations increase from average values of about 1 pg/ml before treatment to about 5 pg/ml during treatment with 0.5 mg MGA/day (Henricks et al., 1997; Hageleit et al., 2000; Pfaffl et al., 2002). Mean plasma levels of luteinizing hormone (LH) increase slightly and the frequency of LH pulses increases during treatment with 0.5 mg MGA/day (Hageleit et al., 2000). If the amounts of MGA in the feed are increased to 1.5 or 5 mg MGA/day, which are 3 and 10 times, respectively, above the maximum recommended rate for addition to feed for growth promotion, there is a change in the profile of activity: there is negative feedback on pituitary gonadotrophin secretion, with a fall in mean plasma LH concentration, absence of follicular development, low plasma estradiol concentrations of 1–2 pg/ml and no anabolic effect (Hageleit et al., 2000; Meyer, 2001; Pfaffl et al., 2002).

Pfaffl et al. (2002) investigated gene expression in heifers following hormone receptor activation by MGA in order to better define the mode of action of MGA in cattle. In heifers given 0, 0.5, 1.5 or 5 mg MGA/day for 8 weeks in the feed (two heifers per dose group), messenger ribonucleic acid (mRNA) expression for androgen, progesterone and estrogen (ERα and ERβ) receptors and for insulin-like

growth factor-1 (IGF-1) and its receptor was measured in liver and in neck and shoulder muscles using reverse transcription followed by real-time polymerase chain reaction (RT-PCR) amplification. IGF-1 is an important growth regulator in many tissues, and IGF-1 gene expression is known to be stimulated by estradiol. Plasma concentrations of MGA, IGF-1, estradiol and progesterone were also measured. In the lowest dose group of 0.5 mg MGA/day, corresponding to the maximum amount that is given for growth promotion in heifers, plasma estradiol and IGF-1 concentrations were significantly increased, whereas progesterone concentrations were significantly reduced compared with controls. At the higher doses of 1.5 and 5 mg/day, plasma estradiol and progesterone concentrations were reduced compared with controls. Linear regression analysis of expression data against increasing doses of MGA showed significant ($P < 0.05$) increases in androgen receptor and IGF-1 receptor expression in liver and in IGF-1 receptor expression in neck muscle. ER α in liver and neck muscle showed a dose-related trend of increasing expression, which achieved statistical significance only for liver. This study indicates that the anabolic properties of MGA may be mediated by IGF-1 and that MGA has some weak activity with respect to expression of the genes for androgen receptor and ER α in heifers in vivo.

2.2 Role of progesterone and progesterone receptors in human development and disease

The role of progesterone and progesterone receptors in human development and disease is relevant in considering exposures to MGA and its metabolites, given their progestogenic activity.

2.2.1 Breast development and breast cancer

The human mammary gland is not completely formed at birth but begins to develop in early puberty, when the primitive network of ductal structures enlarges and branches, forming more complex lobular structures at the ends of the terminal ducts, known as the terminal ductal lobular units (TDLUs) (Russo & Russo, 1987). The TDLUs are lined by a continuous layer of luminal epithelial cells, and most human breast tumours appear to be derived from TDLUs and have the morphological and biochemical characteristics of luminal epithelial cells. Human breast tumours also contain estrogen receptors and progesterone receptors that, in the normal breast, are expressed only in the luminal epithelial cells.

There is considerable clinical and epidemiological evidence concerning the role of endogenous estrogens in normal breast development (Laron et al., 1989; ESHRE Capri Workshop Group, 2004). There is also considerable evidence for the role of both endogenous estrogens and exogenous estrogens (in the form of oral contraceptives and hormone replacement therapy) as primary risk factors in the development of breast cancer in women (IARC, 1999; Liehr, 2001; ESHRE Capri Workshop Group, 2004; Rogan & Cavalieri, 2004), with estrogens causing proliferation of epithelial cells via estrogen receptor-mediated processes (Feigelson & Henderson, 1996).

The role of progesterone in breast development has been far less studied (Anderson, 2002). Studies on progesterone receptor knockout mice suggest that, while estradiol stimulates ductal elongation, progesterone induces lobuloalveolar development (Humphreys et al., 1997). It is assumed that progesterone plays a similar role in normal human breast development, stimulating TDLU formation and expansion during puberty and pregnancy (Anderson, 2002). It has also been shown that the normal luminal epithelial cell proliferation that occurs in the breast during the menstrual cycle is caused by progesterone (Schairer, 2002).

Progesterone has two receptor isoforms, PRA and PRB. Unlike estrogen receptors (ER α and ER β), which are distinctly different receptors, in that the ER β gene is smaller, has a different chromosomal location and encodes a shorter protein, PRA and PRB are transcribed from the same gene by alternative promoters. Both function as ligand-dependent nuclear transcription factors, and it is now thought that both can activate gene transcription, but can be differentiated in terms of the overlapping, but partially distinct, profile of genes that they can activate (Conneely et al., 2007). For example, in the mouse, PRB, but not PRA, mediates the effects of progesterone on mammary gland development (Conneely et al., 2002). In normal human breast tissue, progesterone receptor is present in 15–30% of luminal epithelial cells, but is not found elsewhere in the breast, and PRA and PRB are co-expressed at similar levels in the epithelial cells, suggesting that both proteins may be required to mediate physiological progesterone signalling (Mote et al., 2007). Cells with progesterone receptor also all contain ER α , and in normal tissue, estradiol stimulation of ER α expression also regulates the synthesis of progesterone receptor (Lee et al., 2006). Steroid receptor-expressing cells are separate from, but often adjacent to, dividing cells that contain markers of proliferation (Anderson, 2002; Clarke et al., 2004). Thus, it has been hypothesized that estradiol and/or progesterone control the proliferation of luminal epithelial cells indirectly by the receptor-containing cells acting as sensors and secreting positive or negative paracrine growth factors that influence the proliferative activity of nearby cells, according to the prevailing estradiol/progesterone concentrations (Anderson, 2002; Lee et al., 2006).

In normal breast tissue, ER α expression is inversely related to proliferation, whereas in the early stages of breast tumour development, there appears to be dysregulation, in that increasing ER α expression is accompanied by proliferation and progression towards malignancy (Anderson, 2002). In tumorigenesis, there is an increase in ER α expression at the early stages of ductal hyperplasia, with further increases in expression with increasing atypia. The role of progesterone and progesterone receptor is much less well understood (Wiebe, 2005), but human studies suggest that expression of progesterone receptor also increases with increasing atypia, and approximately 60% of invasive breast carcinomas express PRA and/or PRB (Allred et al., 2001). Enhanced ER α and progesterone receptor expression in premalignant epithelium would be expected to enhance the sensitivity to their respective ligands (Anderson, 2002).

There is evidence that human breast tissue is generated from stem cells and that it is the longevity of these stem cells that explains the extensive further development of the breast in puberty and pregnancy (Clarke et al., 2004; Lamb

et al., 2007). The pool of stem cells in breast tissue has also been suggested as a critical factor linking early life exposures with later development of cancer (Baik et al., 2005), the longevity of stem cells making them susceptible to genetic change and consequent transformation (Lamb et al., 2007). The pool of stem cells is known to contain some cells that are ER α /progesterone receptor positive, and they self-renew (Clarke et al., 2004). In normal tissue, progesterone acts by the secreted factor WNT4 to cause side branching in the mammary gland. WNT is also linked to self-renewal of adult stem cells; thus, it has been hypothesized that progesterone may regulate the mammary stem cell population (Lamb et al., 2007). The *WNT* family of genes is also associated with cellular responses such as carcinogenesis (IARC, 2007). In mammary tumorigenesis, it is hypothesized that accumulated mutations in stem cells lead to uncontrolled self-renewal of stem cells with aberrant differentiation (Dontu et al., 2004). Thus, progesterone as well as estrogen may play a role by increasing the number and rate of cell divisions, which in turn will increase the likelihood of mutational damage, or, after mutational change has occurred, by promoting the growth and survival of tumorigenic cells in progesterone receptor-positive phenotypes (ESHRE Capri Workshop Group, 2004).

Concerning the development of breast cancer in women, epidemiological evidence that has emerged during the last decade suggests that exogenous progestogens (medroxyprogesterone acetate [MPA], norethisterone or levonorgestrel), used together with estrogens in combined hormone replacement therapy, increase the risk of postmenopausal breast cancer to a greater extent than estrogen replacement therapy alone, with significant increases in relative risk for exposures both less than 5 years and more than 5 years (Ross et al., 2000; Schairer et al., 2000; Beral et al., 2003). In the most recent review of these data, the International Agency for Research on Cancer (IARC) commented that there are consistent epidemiological observations that the addition of a progestogen to estrogen in hormone replacement therapy for menopause confers a small, but significant, increase in the risk of breast cancer, which is greater than that in users of estrogen alone (IARC, 2007). The increase in risk in menopausal women is largely confined to current or recent users. The available evidence was considered by IARC to be inadequate to evaluate whether or not the risk for breast cancer varies according to the type of progestogen, its dose or the number of days each month it is added to the estrogen therapy. IARC concluded that there is sufficient evidence in humans for the carcinogenicity of combined estrogen–progestogen menopausal therapy in the breast. IARC also concluded that there is sufficient evidence in humans for the carcinogenicity of combined oral estrogen–progestogen contraceptives in the breast among current and recent users. IARC classified both types of preparation as “carcinogenic to humans (Group 1)” (IARC, 2007).

Some epidemiological studies have also identified an increase in the risk of breast cancer in association with red meat consumption, although the studies overall are inconsistent (Missmer et al., 2002; Cho et al., 2006), and it is notable that the reports of the World Cancer Research Fund, which has twice reviewed the evidence on the association between food categories and cancer at various sites, while identifying red meat as possibly associated with breast cancer in its 1997 report, did not do so in its 2007 report (WCRF, 1997, 2007). In a 12-year prospective

investigation, with follow-up to 2003 of over 90 000 premenopausal women enrolled in the United States Nurses Health Study II, greater red meat intake was strongly associated with an elevated risk of breast cancers that were estrogen receptor– or progesterone receptor–positive (512 such cancers out of a total of 1021 cases of invasive breast carcinoma), but not those that were estrogen receptor– or progesterone receptor–negative (Cho et al., 2006). The authors discussed the possible components of (cooked) red meat that might be carcinogenic, including not only residues of growth hormones in meat, but also heterocyclic amines, *N*-nitroso compounds, polycyclic aromatic hydrocarbons, haem iron and fat. Estrogenic mechanisms are mentioned for some of these components.

In established breast cancer, the results of use of progestogens in treatment regimens have been conflicting, with some reports showing no effects and others showing either stimulation or inhibition in human tumours or in human breast cancer cell lines (Wiebe, 2005). This is presumably a consequence of the heterogeneity of human breast tumours with respect to estrogen receptor and progesterone receptor responsiveness. In vitro metabolism studies in both normal tissue and human breast tumour tissue and in non-tumorigenic and tumorigenic breast cell lines have shown that they all convert progesterone into two groups of metabolites, the 5 α -reduced and the 4-ene metabolites, which have marked breast cancer modulating activities. The 5 α -reduced group stimulates cell proliferation, and the 4-ene group suppresses cell proliferation. In tumour tissue and tumorigenic cell lines, there is a marked change in the ratio of the metabolites towards increased 5 α -reductase activity and lower production of 4-ene metabolites (Wiebe, 2005). The possible effects of metabolites of exogenous progestogens have not been investigated, but the studies on progesterone suggest that metabolites may influence tumour progression.

Animal models using progesterone receptor knockout mice also suggest that the progesterone receptor has a role in growth stimulation and tumour promotion in mammary carcinogenesis (Schairer, 2002; Conneely et al., 2003). Zoo felids are at high risk for mammary cancer, but the risk is further increased by the use of MGA in the form of contraceptive implants, and it has been noted that in most felid mammary tumours, estrogen receptor expression is low, but progesterone receptor expression persists (Munson & Moresco, 2007).

2.2.2 Uterine (endometrial) cancer

A number of reports on captive animals in zoos have described uterine endometrial pathology, including cancer, induced by MGA given as a contraceptive implant over several years in species belonging to the families Felidae (cats), Canidae (dogs) and Viverridae (civets) (Chittick et al., 2001; Munson et al., 2002). However, in women, the addition of a progestogen to estrogen for menopausal therapy prevents the development of endometrial hyperplasia and reduces the rate of endometrial cell proliferation induced by treatment with estrogen alone (IARC, 2007). The addition of a progestogen to estrogen therapy is also associated with a reduced incidence of uterine cancer compared with the elevated risk from use of estrogen alone, with five out of eight studies in which progestogen was added every day showing relative risks below unity, compared with never users of hormone replacement therapy, irrespective of the specific type or dose of progestogen used

(IARC, 2007). Taking all the results of combined estrogen–progestogen menopausal therapy together, IARC (2007) concluded that they “are consistent with the view that the addition of progestagens to estrogen therapy lessens the risk associated with the use of estrogens alone, and that the greater the number of days per month that progestagens are added, the greater is the reduction in risk”. The most commonly used progestogens in combined menopausal therapy are MPA, norethisterone and levonorgestrel; of the three, MPA is the closest to MGA in chemical structure and hormonal activity profile. IARC concluded that there is convincing evidence in humans that combined oral estrogen–progestogen contraceptives also have a protective effect against carcinogenicity in the endometrium (IARC, 2007).

2.2.3 Ovarian cancer

Use of combined estrogen–progestogen oral contraceptives reduces the risk of ovarian cancer compared with never users, with the reduction in risk persisting for at least 20 years after cessation of use. The data on combined estrogen–progestogen hormone replacement therapy and ovarian cancer are inadequate for evaluation (IARC, 2007).

2.2.4 Cervical cancer

The risk for cervical cancer is increased with increasing duration of use of combined estrogen–progestogen oral contraceptives. The data on combined estrogen–progestogen hormone replacement therapy and cervical cancer are inadequate for evaluation (IARC, 2007).

2.2.5 Precocious puberty

Available data do not allow evaluation of the possible influence of environmental exposures, including endocrine disruptors, on the timing of onset of puberty in humans (Lee et al., 2001), but speculation about any such influence has concerned exposure to xenoestrogens and not progestogens (Partsch & Sippell, 2001). However, even in the case of estrogens, it has been commented that published data suggest that the composition of the diet is a less important determinant of pubertal events than is attainment of a certain body size or fat mass (de Muinich Keizer & Mul, 2001).

2.2.6 Gynaecomastia

Two outbreaks of gynaecomastia possibly associated with food contaminated with growth promoters have been described in the literature. An epidemic of gynaecomastia in 1977 in prepubertal boys and girls in a private school in Milan, Italy, has been described (Fara et al., 1979). Although estrogenic contamination of meat in the school canteen was suspected as the cause of the outbreak, it was never proven (Chiumello et al., 2001). In a follow-up case–control study of the affected children 20 years after the outbreak (Chiumello, 1997), a slightly earlier onset of puberty in girls and a slightly later onset of puberty in boys were found, when case groups were compared with controls; in boys, testicular

volume was slightly reduced and testicular hypoplasia more frequent in the case group compared with controls. However, none of these changes were statistically significant at the $P = 0.05$ level. Fertility as measured by paternity was significantly reduced in the boys who were aged 11–14 years at the time of the outbreak. Chiumello et al. (2001) also described the reports from Puerto Rico in the 1980s of high incidences of gynaecomastia, premature thelarche, precocious puberty and polycystic ovaries found in a large number of children, said to be in excess of 10 000. The effects were said to be associated with estrogen contamination of poultry and with environmental pollution from factories making estrogen–progestogen medicines (Chiumello et al., 2001). There have been no reports of gynaecomastia in association with progestogenic contamination of meat.

2.2.7 Prenatal development, including the reproductive tract

The effects of progesterone and, to a limited extent, progestogens on fetal development, including fetal sexual development, have been reviewed by Golub et al. (2006). A Cochrane review is also available that describes a meta-analysis of 15 randomized controlled trials of the outcome of progestogen therapy to prevent spontaneous abortion during the first 20 weeks of pregnancy (Haas & Ramsey, 2008). The information in this section is drawn from these reviews.

Only limited work has been done on progesterone receptor expression in the embryo. In human fetuses, progesterone receptor expression (mainly PRB) is seen in weeks 11–21 of gestation in a wide variety of reproductive and non-reproductive tissues. Later in gestation, expression is limited to reproductive organs, pancreas and intestinal cells.

Concern about adverse prenatal effects of exposure to progestogens has emerged from the use of progestogens as a therapeutic agent during pregnancy. Early case-reports of hypospadias in progestogen-treated pregnancies triggered subsequent epidemiological investigations, but reports of an association between exposure to progestogens during gestation and hypospadias in male offspring have been conflicting, and meta-analyses and reviews have not been able to confirm an association. Laboratory animal studies on the influence of progesterone and other progestogens on anogenital distance have produced conflicting findings (Golub et al., 2006).

Virilizing effects on female genitalia of prenatal exposure to some progestogens have been reported in both animals and humans, but the progestogens concerned were those known to also have androgenic activity. MGA is devoid of androgenic activity.

The Cochrane review assessed miscarriage, preterm birth, stillbirth, neonatal death, low birth weight, fetal genital anomalies (including virilization and hypospadias), teratogenic effects and admissions to special care units. There was no significant effect of progestogen treatment on any of these adverse outcomes. The progestogens used in the trials were MPA, cyclopentyl enyl ether of progesterone, dydrogesterone, hydroxyprogesterone caproate, allylestrenol and progesterone, given orally, intramuscularly or by vaginal suppository (Haas & Ramsey, 2008).

Northen et al. (2007) examined surviving children of mothers participating in a multicentre, placebo-controlled trial of intramuscular 17 α -hydroxyprogesterone caproate for prevention of recurrent miscarriage (mean age at follow-up, 48 months). The children were screened for general health by a questionnaire to the guardian and given a physical examination and a developmental screen, and gender-specific roles were assessed. There were no significant differences between progestogen-exposed children ($n = 194$) and the placebo group ($n = 84$). 17 α -Hydroxyprogesterone is one of the major circulating metabolites of natural progesterone in women.

Laboratory animal studies on the effect of exposure to high doses of progesterone either prenatally or between birth and weaning on male sexual development suggest that it can suppress androgen production and alter male sexual development.

2.3 Genotoxicity

The genotoxicity of substances used as growth promoters became a high-profile issue during the last decade following publication of experimental studies showing that natural estrogenic hormones, such as estradiol, estrone and estriol, can damage deoxyribonucleic acid (DNA). It has now been established that the genotoxic agents formed by these estrogens are quinones, which form DNA adducts. The quinones are derived by aromatic hydroxylation at the C-2 and C-4 positions by cytochrome P450 enzymes, followed by peroxidation to form catechol estrogens, then oxidation to the corresponding 2,3- and 3,4-quinones (Cavalieri & Rogan, 2004; IARC, 2007).

The genotoxicity of hormonal steroids used in human medicinal drugs has been reviewed by Joosten et al. (2004). Progestogens were considered in several separate groups, depending on their chemical structures—progesterone itself, the norethisterone group, the norgestrel group, the MPA group and the spironolactone group. MGA was not among those considered, but it is most closely related to the MPA group in terms of its structure and hormonal profile, although it should be noted that several steroids in the MPA group, including MPA itself and cyproterone acetate, have hormonal profiles that differ somewhat from that of MGA, in that they are also anti-androgenic. Several steroids in the MPA group, but not MPA itself, have genotoxic potential. Cyproterone acetate, a member of the MPA group, was negative in vitro in tests for gene mutation in bacteria (Ames tests) and in hamster V79 cells, but was positive for gene mutation in vivo in liver of the Big Blue rat. Cyproterone acetate was also positive in several indicator tests for DNA damage using human or rat hepatocytes, including measurement of DNA adducts using the ³²P-postlabelling technique, unscheduled DNA synthesis and formation of DNA single strand breaks. Joosten et al. (2004) considered that the genotoxicity is likely to be attributable to the highly reactive carbonium ion metabolite that is formed from the unstable sulfonated 3-hydroxy metabolite of cyproterone acetate; the carbonium ion is short-lived and so active only when formed within a target cell. DNA adduct formation in rat liver in vivo and in human hepatocytes in vitro has also been shown with other members of the MPA group, chlormadinone and megestrol acetate (MA) (the latter being the member of this MPA group structurally most closely related to

MGA). In tests for chromosome breakage, cyproterone acetate gave an equivocal response in cultured rat hepatocytes but was negative in cultures of human lymphocytes; in vivo, cyproterone acetate was negative in a mouse bone marrow micronucleus test but positive in rat liver. Similarly, chlormadinone and MA were negative for chromosomal abnormalities in cultured human lymphocytes but positive for micronucleus induction in rat liver in vivo. The more recent review of estrogens and progestogens by IARC (2007) did not reveal any additional tests on steroids from the MPA group.

Since the previous evaluation by the Committee (Annex 1, reference 147), additional in vitro studies on the genotoxicity of MGA have been conducted by Metzler & Pfeiffer (2001), although few details of the studies were given. They reported that MGA did not induce *HPRT* gene mutations in V79 cells at concentrations between 50 and 100 µmol/l, micronuclei in V79 cells at 20–100 µmol/l or *lacI* mutations in *Escherichia coli* bacteria at 400 µmol/l. It was also noted that there were clear signs of apoptosis in the nuclei of the V79 cells at concentrations of 75 and 100 µmol/l. Subsequent DNA gel electrophoresis confirmed the typical DNA ladder of apoptotic cells. When the commercial sample of MGA used was subjected to HPLC analysis, it was revealed that there were a number of impurities that had mass spectra identical to those of some of the MGA metabolites. Separation of these impurities and retesting showed that pure MGA was devoid of apoptotic activity, whereas the sample of the impurities induced apoptosis.

2.4 Reproductive and developmental toxicity

The developmental effects of exposure to 0.5 mg MGA/kg bw per day given orally in corn syrup to rabbits have been investigated following administration during gestation and lactation (gestation day 15 through to postnatal week 4), the “adolescent” period (postnatal weeks 4–12) or adulthood (12 weeks of exposure between the 1st and 2nd years of life). Comparable groups were given trenbolone acetate or zeranol subcutaneously. The numbers of male rabbits examined per dose group were 10, 10 and 8 for the gestation/lactation, adolescent and adulthood periods of exposure, respectively, but the numbers of dams from which the males were derived in the gestation/lactation study were not stated. No data were available in the report on the variance in the measurements. In the gestation/lactation and adolescent studies, the offspring were killed at 25 weeks of age. The objective of the study was to examine testicular function and sexual capacity at maturity. Body and organ weights were significantly increased in males exposed to MGA during adolescence, compared with controls. Significantly smaller testis weight was seen in males exposed to MGA during gestation/lactation. Cryptorchidism was not seen in the MGA-treated groups, but there were four cases in animals exposed to trenbolone acetate or zeranol during gestation/lactation or adolescence. Males exposed to MGA during adulthood showed a low degree of germinal epithelium loss in the testis. Baseline concentrations of follicle stimulating hormone and LH were lower at 6 weeks of age, but not at 12 weeks of age, in those exposed to MGA during adolescence, and estrone was significantly elevated at 6 and 12 weeks. Cauda epididymal sperm reserves were reduced in those exposed to MGA in adolescence, but this was an isolated finding among no changes in semen volume, sperm

concentration or sperm motility in ejaculate or in daily sperm production (Rajpert-De Meyts et al., 2001).

A study has examined the semen quality in 387 male partners of pregnant women in five cities in the USA between 1999 and 2005, in relation to the amount of beef their mothers reported consuming during pregnancy. Regression analysis showed that sperm quality was inversely related to the number of beef meals per week that their mothers reported consuming during pregnancy. In sons of high consumers (>7 beef meals per week), sperm concentration was 24.3% lower ($43.1 \times 10^6/\text{ml}$ versus $56.9 \times 10^6/\text{ml}$), and the proportion of sons with sperm concentrations below $20 \times 10^6/\text{ml}$ (the World Health Organization threshold for subfertility) was 3 times higher (17.7% versus 5.7%) than in sons whose mothers ate less beef. Both these differences were statistically significant. A history of previous subfertility was also significantly higher in sons of mothers who were high consumers of beef. Sperm concentrations were not significantly related to the mothers' consumption of other types of meat or to the consumption of meats of any type by the sons themselves. Sperm motility and morphology were not affected by mothers' beef consumption. The authors commented that the results may be subject to recall errors but that the mothers did not know their sons' sperm quality at the time of completing the questionnaire about food consumption and would be unlikely to suspect subfertility in their sons, given that the sons' partners were pregnant. The authors speculated that the reductions in sperm concentration observed may be due to prenatal exposure to anabolic steroid residues and/or to other xenobiotics in beef (Swan et al., 2007).

2.5 Immunotoxicity

The immunotoxicity of MGA was considered by the Committee in its evaluation of 2000 (Annex 1, reference 147), which can be summarized as follows. In the mammalian repeat-dose studies, immune system parameters, such as serum cortisol, leukocyte counts, and adrenal, thymus and spleen weights, were affected by MGA only at doses of $\geq 50 \mu\text{g/kg bw per day}$. Similarly, in the three special studies on immunotoxicity in the rat, MGA showed immunosuppressive activity only at doses of $\geq 5 \text{ mg/kg bw per day}$. In long-term clinical trials of MGA in humans, no adverse effects associated with immunosuppression were reported. In a human study, a dose of 20 mg/person reduced plasma cortisol concentrations to about 20% of pretreatment values, but there was no reduction in plasma cortisol at a dose of 10 mg/person ; MGA was reported to have a potency of only 1/40th of that of dexamethasone with respect to suppression of plasma cortisol levels.

In its previous evaluation (Annex 1, reference 147), the Committee also considered reports of effects on the immune system during long-term treatment of cattle. Heifers given 0.45 mg MGA/day for 2.5 months showed a 50% reduction in plasma cortisol levels compared with untreated controls, together with significant reductions in adrenal weight and in the width of the zona fasciculata. (Note that there is an error in Annex 1, reference 147: the dose was reported by JECFA as $0.2 \text{ mg/kg bw per day}$, whereas the dose used and reported by Purchas et al. [1971] was 0.45 mg/day .) MGA did not significantly alter the ability of the uterus to resist infection after infusion of *E. coli* (Lauderdale, 1971).

In a recent study, groups of 24 heifers were fed 0 or 0.5 mg MGA/day and after 14 days were inoculated with *Mannheimia haemolytica* (a bacterium causing bovine respiratory disease), which was described as a mild challenge. The animals were observed for clinical signs of respiratory disease and slaughtered at intervals up to 138 h after inoculation, and the lungs were examined. In those fed MGA, the response was increased, the lung lesions being of greater severity and present in a larger number of cattle at 138 h, compared with controls (Corrigan et al., 2007).

4. COMMENTS

The progestogenic activity of MGA in humans was reviewed by the Committee in 2000 (Annex 1, reference 147). Steroid receptor specificity and relative binding activity of MGA and its four major metabolites were also reviewed by the Committee in 2004 (Annex 1, reference 169), when it was concluded that MGA and its metabolites exert their biological action primarily as progestogens and secondarily as glucocorticoids, with no androgenic or estrogenic activity at relevant physiological concentrations. The Committee noted in 2004 that the most potent progestogenic metabolite of MGA, 2 β -hydroxy-MGA, had a potency of only 12% compared with that of MGA itself. In the present evaluation, additional *in vitro* studies on progesterone receptor binding affinity were reviewed, and they are consistent with the previous data. Overall, the studies indicate that the relative progesterone receptor binding affinity of MGA is greater than that of progesterone by around 5- to 11-fold, depending on the assay, whereas the metabolites of MGA have lower affinities than progesterone.

The first area the Committee was asked to reconsider was the important toxicological end-points in relation to particular subpopulations that may be exposed. The Committee considered the hormonal activity profile of MGA in relation to prenatal and postnatal development, genotoxicity, cancer and immunotoxicity. Potential pharmacological and toxicological effects have been considered.

The Committee noted that many of the general concerns that have been expressed concerning the possible effects of endocrine disrupters on fetal and child development relate to estrogenic, androgenic and anti-androgenic activity of substances. MGA is not considered to show estrogenic activity *in vivo* and has estrogenic activity only at high concentrations (nanomoles to micromoles per litre) *in vitro*. In this regard, the Committee noted that many of the data submitted by the European Commission referred to the known or possible effects of estrogens, which are not considered relevant for MGA. Nor is MGA considered to have anti-estrogenic activity, from its lack of activity in the human breast cancer cell line, MCF-7 cells, or (anti-)androgenic activity, from its lack of binding to the androgen receptor.

Concerning progestogenic activity, one way of assessing whether exposure to residues of MGA and its metabolites in meat would have any effect in adult humans is to compare the pharmacological activity of progestogenic steroids that are structurally related to MGA, as was also done earlier by the Committee (Annex 1, reference 147). MGA has not been used in human therapeutic medicine, but the closely related compounds MA and MPA are, or have been, used in human contraception and for the treatment of endometriosis and cancer (breast,

endometrium, ovary, testis). The amounts of MA or MPA used orally in endometriosis and cancer therapy are in the range of 30–800 mg daily. The amounts of MPA used orally for contraception are lower, in the range of 2.5–10 mg/day, whereas contraceptive doses of MA have been reported to be in the range of 0.35–0.5 mg/day. MGA has been estimated to be less potent than MA, based both on inhibition of menstruation in estrogen-primed women and on alteration of cervical mucus. The relative binding affinity of MPA to the bovine progesterone receptor, compared with that of progesterone, is 223%, whereas that of MGA is 526%. Data from humans and laboratory animals indicate that MGA is about 4 times more potent than MPA with respect to activity on the endometrium. This information, together with that on the lowest pharmacologically active doses of MPA in the range of 2.5–10 mg daily, suggests that amounts of orally ingested MGA in humans would need to be of the order of 0.5 mg/day (8 µg/kg bw for a 60-kg person) or more to exert any discernible pharmacological effect. This is supported by limited data showing that 7.5 or 10 mg MGA/day, but not 5 mg/day, delays the onset of menstruation in regularly ovulating women, and that 2.5 mg MGA/day induces withdrawal bleeding in estrogen-primed amenorrhoeic women. Ingestion of MGA at the level of the upper bound of the ADI (1.8 µg/day for a 60-kg person) would be around 300-fold below 0.5 mg/day, the estimate of the minimal or no-effect level for alteration of menstrual cycle hormones and endometrial effects of MGA in women. The ADI for MGA is derived by application of an uncertainty factor to the minimally effective progestogenic dose of 5 µg MGA/kg bw per day affecting the menstrual cycle in cynomolgus monkeys, which appear to be the most sensitive species.

Data on the role of progesterone during embryonic and fetal development and on development of the reproductive tract in particular are sparse in comparison with the information that is available on estrogenic, androgenic and anti-androgenic substances. Although there are laboratory animal studies and occasional human case-reports of adverse effects of prenatal or perinatal exposure to high doses of progesterone and other progestogens, data from human trials on the use of progestogens in pregnancy (e.g. to prevent spontaneous abortions) do not provide any evidence of adverse effects on the embryo, fetus or young child.

Data on the effects of MGA on the immune system are limited, and no new information was submitted. The information reviewed by the Committee in 2000 (Annex 1, reference 147) indicated that there are measurable effects on adrenal gland size, cortisol secretion and susceptibility to infection in cattle given 0.5 mg MGA/day in the feed. Data from clinical trials in humans given MGA indicated that 10 mg/person (167 µg/kg bw) was without any effect on adrenal hormonal responsiveness, a no-observed-effect level (NOEL) that is at least 10 000 times higher than the exposure would be at the upper bound of the ADI (0.03 µg/kg bw). These exposure and dose considerations indicate that any indirect effect of MGA through glucocorticoid alteration of the immune system is unlikely. The Committee also noted that in the mammalian repeat-dose studies considered in its previous evaluation, immune system parameters, such as serum cortisol, leukocyte counts, and adrenal, thymus and spleen weights, were affected by MGA only at doses of 50 µg/kg bw per day or more. Similarly, in three special studies on immunotoxicity in the rat, also considered in the previous evaluation, MGA showed

immunosuppressive activity only at doses of 5 mg/kg bw per day or more. Overall, the data show that the effects of MGA on immune system parameters are apparent only at doses that are 6–600 times higher than the dose at which MGA is considered to have minimal progestogenic activity in humans (8 µg/kg bw).

The second area the Committee was asked to consider concerned the data indicating that hormones may act not only by interaction with hormone receptors and the consequent downstream effects, but also by other mechanisms, such as direct or indirect genotoxic activity. This is an important question, because it raises the possibility that adverse effects such as cancer might be incurred through toxic modes of action that are without a discernible threshold.

The Committee noted that in 2000 it had reviewed a comprehensive set of genotoxicity tests on MGA itself, including *in vitro* tests on gene mutation in bacteria and mammalian cells, unscheduled DNA synthesis in rat hepatocytes and single strand breaks in mammalian cells and an *in vivo* test for micronucleus formation in mouse bone marrow. The results were all negative, and the Committee had concluded that MGA was not genotoxic (Annex 1, reference 147). New data on genotoxicity submitted for the present evaluation have shown that MGA does not induce gene mutations or micronuclei in mammalian cells *in vitro*, nor does it induce *lacI* mutations in *E. coli*. A review published in 2004 on the genotoxicity of hormonal steroids showed that the majority of progestogens are not genotoxic. However, some progestogens that are structurally related to MGA, sharing the 17-hydroxy-3-oxo-pregna-4,6-diene structure, do have genotoxic potential. Since the genotoxic profile of MGA itself is uniformly negative, it appears to differ from some of those with which it shares a structural similarity. The Committee reaffirmed its previous view that the data on MGA show that it is not genotoxic. Thus, there is no evidence that MGA would increase the risk of cancer by a genotoxic mechanism.

However, progesterone, after binding to progesterone receptors and triggering downstream gene expression, clearly plays a role in, for example, human breast development and progression of some types of breast cancer. The Committee also recalled that several long-term studies in mice, reviewed in its previous evaluation, showed dose-related increases in the incidence of mammary adenocarcinoma, but that mechanistic studies had shown that the effect was attributable to promotion caused by stimulation of prolactin secretion, with a NOEL for mammary tumorigenesis of 0.5 mg/kg bw per day.

Epidemiological evidence from both combined estrogen–progestogen contraceptive use and combined estrogen–progestogen hormone replacement therapy shows that the addition of the progestogen confers a small, but significant, increase in the risk of breast cancer, which is greater than that in users of estrogen alone. Since duration of exposure is a significant risk factor for breast cancer in women taking combined oral contraceptives or combined hormone replacement therapy and the increase in risk for breast cancer is largely confined to current or recent users, this also suggests that the progestogenic component is acting more as a promoter than as an initiator of cancer, which is consistent with a non-genotoxic mode of action. Although the increase in risk of breast cancer is seen in association with pharmacologically active doses of progestogen, the doses used are such that

the data do not allow no-effect levels for the increase in risk to be established. However, exposure to MGA from residues in meat is several orders of magnitude lower than the pharmacologically active doses of a progestogen that would elevate the risk of breast cancer. Given the involvement of progesterone receptors in the carcinogenic effects of progestogens, there should be no increase in the risk of cancer at exposure levels below those that activate progesterone receptors *in vivo* in humans. An additional consideration is that absorption of low concentrations of MGA present as residues in meat would be followed by extensive and rapid metabolism in the liver and that the metabolites of MGA have less binding affinity for progesterone receptors than progesterone itself. The Committee also noted that very young infants, in whom metabolizing capacity is not fully developed, would not be consuming meat or other animal tissues in which MGA residues may be present.

It can be concluded that progestogens do not have a carcinogenic effect on the uterus, since all the available clinical and epidemiological data show that progestogens oppose the hyperplastic effect of estrogens in the endometrium and reduce the likelihood of endometrial cancer in women also exposed to exogenous estrogens. Use of combined estrogen–progestogen oral contraceptives reduces the risk of ovarian cancer and increases the risk of cervical cancer, whereas the data from use of combined estrogen–progestogen hormone replacement therapy are inadequate for evaluation with respect to these two cancer sites. The influence of the progestogen component with respect to these two cancer sites has not been separately analysed.

The fourth area the Committee was asked to consider was the authorized uses according to good practice in the use of veterinary drugs that it considers appropriate in the evaluation and their respective impact on potential exposure and risk estimates. Good practice in the use of veterinary drugs is the official recommended or authorized usage, including withdrawal periods, approved by national authorities of veterinary drugs under practical conditions (Codex Alimentarius Commission, 2007b). In line with established practice in JECFA, when assessing exposure to residues and risk, the Committee considers only maximum residues from approved treatments. In the case of MGA, the Committee considered only the approved use in heifers at the recommended dose range of 0.25–0.5 mg/heifer per day for recommending MRLs. The Committee did note that in the case of MGA, which is added to the feed, if the level of addition increases to 3 times the maximum recommended dose of 0.5 mg/heifer per day, then the hormonal activity profile changes, and the desired effect (growth promotion) does not occur.

4. EVALUATION

In reaching its conclusions, the Committee considered not only the data on MGA from its previous toxicological evaluation in 2000 and the few new studies on MGA that have been published or submitted since then, but also more general information on the role of progestogens in human reproduction, prenatal and child development, and cancer. The issue of immunotoxicity was also revisited. The Committee noted previously that MGA has both progestogenic and glucocorticoid activity, and that both earlier and new data confirm these as the principal hormonal

activities of MGA, with only weak estrogenic activity shown at relatively high concentrations in vitro.

There are no data on plasma concentrations of MGA following human dietary exposure. However, based on the comparative data considered in the Committee's previous evaluations (Annex 1, references 146 and 169) showing similarities in absorption and metabolism of MGA among cattle, rats, rabbits and humans, it can be estimated that the plasma concentration in humans after ingestion of 0.03 µg/kg bw (equivalent to the upper bound of the ADI) would be around 0.5–1 pg/ml. This is some 4000-fold below the minimum concentration necessary to stimulate proliferation in the human breast cancer cell line, MCF-7 cells, which is a sensitive indicator of estrogenic activity. In addition, a study in rabbits found concentrations of MGA in plasma in the low nanogram per millilitre range following an oral dose of 0.5 mg/kg bw. This is in the same range as the minimum concentration of MGA necessary to produce effects on the estrogen receptor in MCF-7 cells. Given that the dose in this study was some 17 000-fold greater than the maximum intake of humans exposed to residues in meat, assuming consumption at the upper bound of the ADI, even allowing for the uncertainty introduced by species differences (rabbit versus human), it is highly unlikely that dietary residues of MGA will have any estrogenic effects in humans consuming meat from animals treated with this drug. The Committee also noted that MGA is devoid of genotoxic activity both in vitro and in vivo; thus, non-thresholded mechanisms of carcinogenicity are unlikely to play any role.

Concerning progestogenic activity, there is a small, but significant, increase in risk of breast cancer in humans exposed to progestogens in the form of combined estrogen–progestogen oral contraceptives or hormone replacement therapy, and the evidence suggests that the progestogenic component is acting more as a promoter than as an initiator of cancer. On the basis of comparative estimates of progestogenic activity, the exposure to MGA and its metabolites from residues in meat consumed at the upper bound of the ADI is 200–300 times lower than these pharmacologically active doses and below a dose that would produce any measurable effect on progesterone receptors. Although MGA causes mammary tumorigenesis in the mouse, this is attributable to stimulation of prolactin secretion having a promoter activity, and there is a clear NOEL of 0.5 mg/kg bw per day for this effect. This NOEL is more than 15 000 times higher than the exposure would be at the upper bound of the ADI. The Committee therefore concluded that residues of MGA and its metabolites would be unlikely to have any influence on the development of breast cancer.

Concerning glucocorticoid activity and effects on the immune system, there is no new information. However, the Committee noted that the NOEL for adrenal hormonal responsiveness to MGA in humans is at least 10 000 times higher than the exposure would be at the upper bound of the ADI. Similarly, the NOEL for immunosuppressive activity is at least 1000 times higher than the exposure would be at the upper bound of the ADI.

The Committee concluded overall that the new data do not provide any basis to reconsider the ADI. Human exposure to residues of MGA and its metabolites in

meat, resulting from the use of MGA as a feed additive in cattle at a daily dose of 0.25–0.5 mg/heifer, would be unlikely to have any adverse effects on adults, children, the embryo or the fetus.

5. REFERENCES

- Allred, D.C., Mohsin, S.K. & Fuqua, S.A.W. (2001) Histological and biological evolution of human premalignant breast disease. *Endocr. Relat. Cancer*, **8**, 47–61.
- Anderson, E. (2002) The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. *Breast Cancer Res.*, **4**, 197–201.
- Baik, I., DeVito, W.J., Ballen, K., Becker, P.S., Okulicz, W., Liu, Q., Delpapa, E., Lagiou, P., Sturgeon, S., Trichopoulos, D., Queensberry, P.J. & Hsieh, C.-C. (2005) Association of fetal hormone levels with stem cell potential: Evidence of early life roots of human cancer. *Cancer Res.*, **65**, 358–363.
- Bauer, E.R.S., Daxenberger, A., Petri, T., Sauerwein, H. & Meyer, H.H.D. (2000) Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progesterin receptor. *APMIS*, **108**, 838–846.
- Beral, V. & the Million Women Study collaborators (2003) Breast cancer and hormone replacement therapy. *Lancet*, **362**, 419–427.
- Cavalieri, E. & Rogan, E. (2004) A unifying mechanism in the initiation of cancer and other diseases by catechol quinones. *Ann. N.Y. Acad. Sci.*, **1028**, 247–257.
- Chittick, E., Rotstein, D., Brown, T. & Wolfe, B. (2001) Pyometra and uterine adenocarcinoma in a melengestrol acetate-implanted captive coati (*Nasua nasua*). *J. Zoo Wildlife Med.*, **32**, 245–251.
- Chiumello, G. (1997) *Long-term effect in children of exposure to estrogen-contaminated meat: A retrospective group study*. Original research report submitted to WHO by the European Commission.
- Chiumello, G., Guarneri, M.P., Russo, G., Stroppa, L. & Sgaramella, P. (2001) Accidental gynecomastia in children. *APMIS*, **109**, S203–209.
- Cho, E., Chen, W.Y., Hunter, D.J., Stampfer, M.J., Colditz, G.A., Hankinson, S.E. & Willet, W.C. (2006) Red meat intake and risk of breast cancer in premenopausal women. *Arch. Intern. Med.*, **166**, 2253–2259.
- Clarke, R.B., Spence, K., Anderson, E., Howell, A., Okano, H. & Potten, C.S. (2004) A putative human breast stem cell population is enriched for steroid receptor-positive cells. *Dev. Biol.*, **277**, 443–456.
- Codex Alimentarius Commission (2007a) *Report of the 17th Session of the Codex Committee on Residues of Veterinary Drugs in Foods, Breckenridge, CO, USA, 3–7 September 2007*. Rome, Italy, Food and Agriculture Organization of the United Nations (ALINORM 08/31/31; <http://www.codexalimentarius.net/web/archives.jsp?year=08>).
- Codex Alimentarius Commission (2007b) *Procedural manual*, 17th ed. Rome, Italy, World Health Organization and Food and Agriculture Organization of the United Nations (<http://www.fao.org/docrep/010/a1472e/a1472e00.htm>).
- Conneely, O.M., Mulac-Jericevic, B., DeMayo, F., Lydon, J.P. & O'Malley, B.W. (2002) Reproductive functions of progesterone receptors. *Rec. Prog. Horm. Res.*, **57**, 339–355.
- Conneely, O.M., Mulac-Jericevic, B.M. & Lydon, J.P. (2003) Progesterone receptors in mammary gland development and tumorigenesis. *J. Mammary Gland Biol. Neoplasia*, **8**, 205–214.
- Conneely, O.M., Mulac-Jericevic, B. & Arnett-Mansfield, R. (2007) Progesterone signalling in mammary gland development. *Ernst Schering Found. Symp. Proc.*, **2007**(1), 45–54.

- Corrigan, M.E., Drouillard, J.S., Spire, M.F., Mosier, D.A., Minton, J.E., Higgins, J.J., Loe, E.R., Depenbusch, B.E. & Fox, J.T. (2007) Effect of melengestrol acetate on the inflammatory response in heifers challenged with *Mannheimia haemolytica*. *J. Anim. Sci.*, **85**, 1770–1779.
- Damstra, T., Barlow, S., Bergman, A., Kavlock, R. & Van der Kraak, G., eds. (2002) *Global assessment of the state-of-the-science of endocrine disruptors*. Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety (WHO/PCS/EDC/02.2).
- Daston, G.P., Cook, J.C. & Kavlock, R.J. (2003) Uncertainties for endocrine disrupters: Our view on progress. *Toxicol. Sci.*, **74**, 245–252.
- Daxenberger, A., Meyer, K., Hageleit, M. & Meyer, H.H.D. (1999) Detection of melengestrol acetate residues in plasma and edible tissues of heifers. *Vet. Q.*, **21**, 154–158.
- de Muinich Keizer, S.M. & Mul, D. (2001) Trends in pubertal development in Europe. *Hum. Reprod. Update*, **7**, 287–291.
- Dontu, G., El-Ashry, D. & Wicha, M.S. (2004) Breast cancer, stem/progenitor cells and the estrogen receptor. *Trends Endocrinol. Metab.*, **15**, 193–197.
- ESHRE Capri Workshop Group (2004) Hormones and breast cancer. *Hum. Reprod. Update*, **10**, 281–293.
- Fara, G.M., Del Corvo, G., Bernuzzi, S., Bigatello, A., Di Pietro, C., Scaglioni, S. & Chiumello, G. (1979) Epidemic of breast enlargement in an Italian school. *Lancet*, **2**, 295–297.
- Feigelson, H.S. & Henderson, B.E. (1996) Estrogens and breast cancer. *Carcinogenesis*, **17**, 2279–2284.
- Golub, M.S., Kaufman, F.L., Campbell, M.A., Li, L.-H. & Donald, J.M. (2006) “Natural” progesterone: Information on fetal effects. *Birth Defects Res. B Dev. Reprod. Toxicol.*, **77**, 455–470.
- Haas, D.M. & Ramsey, P.S. (2008) Progestogen for preventing miscarriage. *Cochrane Database Syst. Rev.*, **2008**(2), CD003511.
- Hageleit, M., Daxenberger, A., Kraetzel, W.-D., Kettler, A. & Meyer, H.H.D. (2000) Dose-dependent effects of melengestrol acetate (MGA) on plasma levels of estradiol, progesterone and luteinising hormone in cycling heifers and influences on estrogen residues in edible tissues. *APMIS*, **108**, 847–854.
- Hageleit, M., Daxenberger, A. & Meyer, H.H.D. (2001) A sensitive immunoassay (EIA) for the determination of melengestrol acetate (MGA) in adipose and muscle tissues. *Food Addit. Contam.*, **18**, 285–291.
- Henricks, D.M., Brandt, R.T., Jr, Titgemeyer, E.C. & Milton, C.T. (1997) Serum concentrations of trenbolone-17 β and estradiol-17 β and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17 β . *J. Anim. Sci.*, **75**, 2627–2633.
- Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., Foster, P.M., Gray, C.L. & Gray, L.E. (2008) Fifteen years after “Wingspread”—Environmental endocrine disrupters and human and wildlife health: Where we are today and where we need to go. *Toxicol. Sci.*, **105**(2), 235–259.
- Humphreys, R., Lydon, J., O'Malley, B. & Rosen, J. (1997) The use of PRKO mice to study the role of progesterone in mammary gland development. *J. Mammary Gland Biol. Neoplasia*, **2**, 343–354.
- IARC (1999) *Hormonal contraception and postmenopausal hormone therapy*. Lyon, France, International Agency for Research on Cancer, pp. 474–530 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 72).
- IARC (2007) *Combined estrogen-progestogen contraceptives and combined estrogen-progestogen menopausal therapy*. Lyon, France, International Agency for Research on Cancer (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 91).
- Joosten, H.F.P., van Acker, F.A.A., van den Dobbelsteen, D.J., Horbach, G.J.M.J. & Krajnc, E.I. (2004) Genotoxicity of hormonal steroids. *Toxicol. Lett.*, **151**, 113–134.

- Lamb, R., Harrison, H. & Clarke, R.B. (2007) Mammary development, carcinoma and progesterone: Role of Wnt signalling. *Ernst Schering Found. Symp. Proc.*, **2007**(1), 1–23.
- Lange, I.G., Daxenberger, A., Meyer, H.H.D., Rajpert-De Meyts, E., Skakkebaek, N.E. & Veeramachaneni, D.N.R. (2002) Quantitative assessment of foetal exposure to trenbolone acetate, zeranol and melengestrol acetate, following maternal dosing in rabbits. *Xenobiotica*, **32**, 641–651.
- Laron, Z., Pauli, R. & Pertzelan, A. (1989) Clinical evidence on the role of estrogens in the development of the breasts. *Proc. R. Soc. Edinb. B1*, **85**, 29–43.
- Lauderdale, J.W. (1971) Influence of melengestrol acetate on acute responses of the bovine uterus inoculated with *Escherichia coli*. *Am. J. Vet. Res.*, **32**, 1033–1038.
- Lauderdale, J.W. (1983) Use of MGA® (melengestrol acetate) in animal production. In: Meisssonier, E. & Mitchell-Vigneron, J., eds. *Proceedings of the Symposium on Anabolics in Animal Production: Public Health Aspects, Analytical Methods, and Regulation*, 15–17 February 1983, Paris. Paris, France, Office International des Épidémiologies, pp. 193–212.
- Lauderdale, J.W., Goyings, L.S., Krzeminski, L.F. & Zimbelman, R.G. (1977) Studies of a progestogen (MGA) as related to residues and human consumption. *J. Toxicol. Environ. Health*, **3**, 5–33.
- Lee, P.A., Guo, S.S. & Kulin, H.E. (2001) Age of puberty: Data from the United States of America. *APMIS*, **109**, 81–88.
- Lee, S., Mohsin, S.K., Mao, S., Hilsenbeck, S.G., Medina, D. & Allred, D.C. (2006) Hormones, receptors and growth in hyperplastic enlarged lobular units: Early potential precursors of breast cancer. *Breast Cancer Res.*, **8**, R6.
- Le Gueval, R. & Padkel, R. (2001) Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. *Hum. Reprod.*, **16**, 1030–1036.
- Liehr, J.G. (2001) Genotoxicity of steroidal estrogens estrone and estradiol: Possible mechanism of uterine and mammary cancer development. *Hum. Reprod. Update*, **7**, 273–281.
- Metzler, M. & Pfeiffer, E. (2001) Genotoxic potential of xenobiotics growth promoters and their metabolites. *APMIS*, **109**, 89–95.
- Meyer, H.H.D. (2001) Biochemistry and physiology of anabolic hormones used for improvement of meat production. *APMIS*, **109**, 1–8.
- Missmer, S.A., Smith-Warner, S.A., Spiegelman, D., Yaun, S.S., Adami, H.O., Beeson, W.L., van den Brandt, P.A., Fraser, G.E., Freudenheim, J.L., Goldbohm, R.A., Graham, S., Kushi, L.H., Miller, A.B., Potter, J.D., Rohan, T.E., Speizer, F.E., Toniolo, P., Willett, W.C., Wolk, A., Zeleniuch-Jacquotte, A. & Hunter, D.J. (2002) Meat and dairy food consumption and breast cancer: A pooled analysis of cohort studies. *Int. J. Epidemiol.*, **31**, 78–85.
- Mote, P.A., Graham, J.D. & Clarke, C.L. (2007) Progesterone receptor isoforms in normal and malignant breast. *Ernst Schering Found. Symp. Proc.*, **2007**(1), 77–107.
- Munson, L. & Moresco, A. (2007) Comparative pathology of mammary gland cancers in domestic and wild animals. *Breast Dis.*, **28**, 7–21.
- Munson, L., Gardner, I.A., Mason, R.J., Chassy, L.M. & Seal, U.S. (2002) Endometrial hyperplasia and mineralisation in zoo felids treated with melengestrol acetate contraceptives. *Vet. Pathol.*, **39**, 419–427.
- Northern, A.T., Norman, G.S., Anderson, K., Moseley, L., Divito, M., Cotroneo, M., Swain, M., Bousleiman, S., Johnson, F., Dorman, K., Milluzzi, C., Tillinghast, J.A., Kerr, M., Mallett, G., Thom, E., Pagliaro, S. & Anderson, G.D.; National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network (2007) Follow-up of children exposed in utero to 17 alpha-hydroxyprogesterone caproate compared with placebo. *Obstet. Gynecol.*, **110**, 865–872.
- Partsch, C.-J. & Sippell, W.G. (2001) Pathogenesis and epidemiology of precocious puberty. Effects of exogenous oestrogens. *Hum. Reprod. Update*, **7**, 292–302.

- Perry, G.A., Welshons, W.V., Bott, R.C. & Smith, M.F. (2005) Basis of melengestrol acetate action as a progestin. *Domest. Anim. Endocrinol.*, **28**, 147–161.
- Pfaffl, M.W., Daxenberger, A., Hageleit, M. & Meyer, H.H.D. (2002) Effects of synthetic progestagens on the mRNA expression of androgen receptor, progesterone receptor, estrogen receptor α and β , insulin-like growth factor-1 (IGF-1) and IGF-1 receptor in heifer tissues. *J. Vet. Med. A*, **49**, 57–64.
- Phillips, K.P. & Foster, W.G. (2008) Key developments in endocrine disrupter research and human health. *J. Toxicol. Environ. Health B*, **11**, 233–244.
- Purchas, R.W., Pearson, A.M., Pritchard, D.E., Hafs, H.D. & Tucker, H.A. (1971) Some carcass quality and endocrine criteria of Holstein heifers fed melengestrol acetate. *J. Anim. Sci.*, **32**, 629–635.
- Rajpert-De Meyts, E., Veeramacheneni, D.N.R., Andersson, A.-M. & Skakkebaek, N.E. (2001) *Reproductive sequelae of developmental exposure to zeranol, trenbolone acetate and melengestrol acetate with special emphasis upon differentiation and neoplastic transformation of germ cells*. Final report to the European Commission (FAIR CT 98-4753, 31 December 2001).
- Rogan, E.G. & Cavalieri, E.L. (2004) Estrogen metabolites, conjugates and DNA adducts: Possible biomarkers for risk of breast, prostate and other human cancers. *Adv. Clin. Chem.*, **38**, 135–149.
- Ross, R.K., Paganini-Hill, A., Wan, P.C. & Pike, M.C. (2000) Effect of hormone replacement therapy on breast cancer risk: Estrogen versus estrogen plus progestin. *J. Natl. Cancer Inst.*, **92**, 328–332.
- Russo, J. & Russo, I.H. (1987). Development of the human mammary gland. In: Neville, M. & Daniel, C.W., eds. *The mammary gland, development, regulation and function*. New York, NY, USA, Plenum Press, pp. 67–93.
- Schairer, C. (2002) Progesterone receptors—animal models and cell signalling in breast cancer. Implications for breast cancer of inclusion of progestins in hormone replacement therapies. *Breast Cancer Res.*, **4**, 244–248.
- Schairer, C., Lubin, J., Troisi, R., Sturgeon, S., Brinton, L. & Hoover, R. (2000) Menopausal estrogen and estrogen–progestin replacement therapy and breast cancer risk. *JAMA*, **283**, 485–491.
- Simon, J.A. (1995) Micronised progesterone: Vaginal and oral uses. *Clin. Obstet. Gynecol.*, **38**, 902–914.
- Swan, S.H., Liu, F., Overstreet, J.W., Brazil, C. & Skakkebaek, N.E. (2007) Semen quality of fertile US males in relation to their mothers' beef consumption during pregnancy. *Hum. Reprod.*, **22**, 1497–1502.
- Tabb, M.M. & Blumberg, B. (2006) New modes of action for endocrine-disrupting chemicals. *Mol. Endocrinol.*, **20**, 475–482.
- Vom Saal, F. (2007) Could hormone residues be involved? *Hum. Reprod.*, **22**, 1503–1505.
- WCRF (1997) *Food, nutrition and the prevention of cancer: A global perspective*. Washington, DC, USA, American Institute for Cancer Research, World Cancer Research Fund.
- WCRF (2007) *Food, nutrition, physical activity and the prevention of cancer: A global perspective*. Washington, DC, USA, World Cancer Research Fund, American Institute for Cancer Research (<http://www.dietandcancerreport.org/>).
- Wiebe, J.P. (2005) Role of progesterone metabolites in mammary cancer. *J. Dairy Res.*, **72** (Special Issue), 51–57.