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Distribution list

RE: Therapeutic Products Programme Guidelines for the  
Toxicological Evaluation of Contraceptive Steroids

Please find attached the Guidelines for the Toxicological Evaluation of Contraceptive Steroids.

These guidelines have been developed by a Therapeutic Products Programme's Working Group for the Toxicological Assessment of Contraceptive Steroids in close consultation with several experts in the fields of toxicology, pathology and reproductive physiology. Furthermore, the guidelines have taken into consideration the recommendations resulting from the 1987 World Health Organization symposium on *Safety Requirements for Contraceptive Steroids*.

The guidelines provide specific recommendations on the design and conduct of preclinical studies that are essential to confidently assess the toxicity of contraceptive steroids. As such, these guidelines complement the existing Therapeutic Products Programme guidelines and policies related to the content and format of New Drug Submissions (e.g. "Chemistry and Manufacturing: New Drugs" and "Toxicologic Evaluation").

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Few comments were received in response to my letter of April 3, 1998. They were generally supportive and few editorial changes were incorporated into the current guidelines.

The guidelines are effective immediately. If you have any questions or comments, please do not hesitate to contact Dr. Peter Grosser at (613) 954-4594.

**Original signed by**

Dann M. Michols  
Director General

Enclosure

**GUIDELINES FOR THE  
TOXICOLOGICAL EVALUATION OF  
CONTRACEPTIVE STEROIDS**

*Division of Endocrinology, Metabolism and Allergy  
Bureau of Pharmaceutical Assessment  
Therapeutic Products Programme  
Health Canada*

*March 21, 2000*

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**I. PREAMBLE**

When oral contraceptives were first introduced, there were no specific toxicology testing requirements for new drugs in Canada. Generally, it was left to the discretion of the sponsor to perform tests in animals to support drug safety, and contraceptives were treated in a similar manner.

In 1966, an investigational hormonal steroid, ethynone or MK-665, produced mammary nodules during a toxicology study in beagle dogs, thereby raising the possibility that contraceptive steroids might be carcinogenic in humans. Since the mammary nodules produced by MK-665 represented the potential for considerable risk in an otherwise healthy population, extraordinary measures were taken to evaluate the degree of risk to women taking contraceptive steroids.

As a result of the MK-665 experience, long-term studies (7 years\* duration in beagles and 10 years\*duration in monkeys) were mandated by the FDA to study long-term toxicity for all steroidal contraceptives. The beagle was chosen as one species primarily because it was the species in which MK-665 had produced tumors; the monkey because it is similar to the human in most aspects of reproductive physiology. Canada likewise adopted these guidelines governing the data requirements for New Drug Submissions to the Therapeutic Products Programme.

In February of 1987, the World Health Organization's Special Programme of Research, Development and Research Training in Human Reproduction sponsored a symposium on *Improving Safety Requirements for Contraceptive Steroids* to develop alternative guidelines for the steroidal contraceptives that more closely reflect current scientific opinion.

The Therapeutic Products Programme Working Group on Toxicity Testing Requirements for Steroid Contraceptives, in consultation with two external expert advisors, met on numerous occasions in late 1994 and 1995 to consider the acceptability of revised guidelines for steroidal

contraceptives based on those proposed by the World Health Organization. The updated Guidelines recommended by the Working Group are described in detail in the remainder of this document. These Guidelines differ from previous requirements stipulated by the Therapeutic Products Programme for pre-clinical testing in the following ways:

- the elimination of the monkey 10 year carcinogenicity test
- the elimination of the dog carcinogenicity test
- the inclusion of more detailed pharmacokinetic data

These Guidelines should be used in conjunction with the Guidelines for Toxicological Evaluation as published by the Therapeutic Products Programme. It should be noted that these Guidelines do not constitute requirements, and that the Therapeutic Products Programme maintains the right to request such additional data as is seen fit on a case by case basis.

## **II. PRE-CLINICAL /CLINICAL TESTING SCHEDULES**

The duration of animal toxicology studies in support of clinical testing should be at least equal to that of the proposed clinical trial (Table 1). Prior to Phase I clinical testing, single dose studies in rats and mice, and repeat dose studies (a minimum of one month) in rats and monkeys are necessary. Long-term toxicology studies are needed to support longer-term clinical studies. In line with current Therapeutic Products Programme Guidelines requiring two species for carcinogenicity testing of new drugs, the mouse has been included as the second species.

**Table 1.** The type and duration of preclinical studies required for each clinical phase in the development of an oral contraceptive steroid

Clinical Phase	Type and Duration of Pre-clinical
I	Single dose studies in rats and mice. Repeat dose studies in rats and monkeys of a minimum of one month.
II	Flexible, with a duration at least equal to the length of the proposed clinical trial to a maximum of 6 months (rat) or one year (monkey). Special studies of reproduction and return to fertility should be performed prior to the initiation of clinical trials in women who are at risk for pregnancy.
III	Same as for Phase II. Results of genotoxicity tests should be submitted prior to Phase III.
NDS	6-month toxicology (rat); 12-month toxicology (monkey); and 2-year carcinogenicity (rat/mouse)

The rat and monkey should be used as test species for repeat dose toxicology studies because a wealth of historical data exists for the rat; it is believed that, due to similar reproductive anatomy and physiology, the monkey represents the most logical non-rodent species in which to test drugs that affect human reproductive processes. The Division of Endocrinology, Metabolism and Allergy will consider the use of alternative species for toxicology testing on a case-by-case basis.

### **III. PHARMACOLOGY**

New contraceptive steroids must be evaluated both for efficacy and for other physiological effects. In evaluating pharmacology, the following points should be addressed:

- Potency of the drug on various reproductive and pharmacological endpoints, including *in vitro* endocrine tests (hormone receptor assays, bioassays, and hormone levels).
- Thorough investigation of the estrogenic, progestogenic, androgenic, and when necessary, glucocorticoid agonistic and antagonistic activities of the drug.
- A general pharmacological assessment to determine drug effects on neurological, cardiovascular, immunological, and other parameters.
- The pharmacology studies should ideally be done in rodents, but other species could substituted if appropriately justified.

### **III. A. PHARMACOKINETICS**

Pharmacokinetic parameters should be determined in rats and monkeys as well as in humans under steady state conditions and should include:

- the time at which maximum blood concentration occurs following dosing ( $t_{max}$ )
- the maximum concentration reached ( $C_{max}$ )
- the half life ( $t_{1/2}$ )
- the area under the concentration-time curve (AUC), including both area under the curve at a steady state between dosing intervals ( $AUC_x$ ) and the area under the curve once treatment ceases ( $AUC_i$ );
- the extent of plasma protein binding of the steroids in humans and test animals;
- a sensitive assay specific for the parent compound and using the same analytical methodology for all species, including humans.
- when estrogen/progestogen (E/P) combinations are used, plasma levels of both steroids should be measured.

### **IV. TOXICOLOGY TESTING**

The reader should use these Guidelines in conjunction with the Therapeutic Products Programme Guidelines for

Toxicologic Evaluation, 1990.

#### **IV. A. DOSE SELECTION**

The following criteria are important in selecting the dose for toxicity testing:

- Contraceptives containing E/P combinations should be tested for toxicity at the ratio to be marketed, or if they contain more than one ratio (a bi- or triphasic combination), testing should be done using a combination that reflects the lowest Estrogen to Progestogen ratio. For repeat dose testing in rats, the steroids should be given daily.
- Monkeys should receive the same dosing regimen as will be used in the clinical situation.
- Both male and female animals are usually evaluated in toxicity studies. However, if the drug is indicated only for a single sex, it is permissible to test in that sex only.

In addition to the ICH Harmonized Tripartite Guideline, the following points are salient to proper dose selection:

- In toxicity studies done prior to collection of human pharmacokinetic data, doses should be based on multiples of the anticipated human dose on a mg/kg body weight basis.
- In keeping with past requirements, rats should be given up to 200 times the human dose and monkeys should be tested at a high dose of 50 times the human dose.
- After animal and human pharmacokinetic data are developed, toxicity studies of new contraceptives should use a high dose that produces plasma drug levels in animals at least 10 times higher than the human plasma drug levels as determined by the AUC of the parent compound. In the case of combination contraceptives, dose multiples should be selected on the basis of the progestational component.
- Data demonstrating that oral administration to animals cannot produce the required human multiple (ie. saturation of absorption) will be considered on a case-by-case basis.

- If, prior to reaching a 10- fold multiple of human exposure, dose-limiting toxicity is produced, the sponsor should submit the supporting data to the Programme for review prior to initiation of the long-term toxicology studies.

#### **IV. B. GROUP SIZE**

In addition to the Guidelines on Toxicologic Evaluation, the following points are pertinent to oral contraceptive steroid evaluation:

- Because of the limited availability of monkeys and the desire to minimize their use, the early, short-term toxicology studies may be conducted with 3 monkeys/sex/group (or if only one sex is studied, 6 of that sex).
- For the one year studies, 4 monkeys/sex/group are recommended (or 8 of only one sex).

#### **IV. C. SINGLE DOSE STUDIES**

Single dose toxicity should be evaluated in rats and mice and should be restricted to administration of a single dose that produces overt toxicity up to a maximum feasible dose (or 5 g/kg bw) using the route of administration intended for humans. Animals should be observed for 14 days after dosing to monitor clinical signs and body weight changes. Moribund or dead animals should undergo complete necropsy and the cause of death should be determined. Gross lesions should be examined microscopically.

#### **IV. D. REPEAT DOSE STUDIES**

The repeat dose toxicity studies should encompass all the parameters usually studied to characterize toxicity (hematology, clinical chemistry, urinalysis, gross and microscopic pathology). In the monkey, data on parameters affected by oral contraceptives such as serum cholesterol (including HDL and LDL), fibrinogen and antithrombin 3, as well as data on endometrial hyperplasia, ophthalmologic parameters, blood pressure, and menstrual cyclicity are necessary.

#### **IV. E. REPRODUCTION STUDIES**

Reproduction and return to fertility studies are pivotal to evaluating oral contraceptives. As these contraceptive are to be used in women, a Segment I reproduction study is not required. The following is required for evaluating reproductive toxicity of the compound:

- Reproduction testing Segments II and III should be performed with new contraceptive steroids (Segment III testing is particularly important for long-acting injectable contraceptives).
- Rats should be used for reproduction testing Segments II and III.

#### **IV. F. RETURN TO FERTILITY**

It is necessary to demonstrate that cessation of contraceptive steroid use results in eventual return to fertility in animals. Although some flexibility is available for the exact protocol, this should be an essential component of the submission package. An extra 10 female rats in the control and high dose groups of a multidose toxicity study should be included to determine the time required after cessation of dosing for the resumption of normal estrous cycles. Following the return of normal cycling, the rats should be mated with males of proven fertility and the number of pregnancies and other measures of fertility should be assessed.

#### **IV. G. CARCINOGENICITY STUDIES**

Carcinogenicity testing of hormonally active compounds in general, and steroids in particular, presents a tremendous challenge, primarily because estrogens and progestogens are known carcinogens in animals and estrogens are carcinogenic in humans. This tumorigenic action is believed to be through an epigenetic (non-genotoxic) mechanism. Continual overstimulation of responsive cells due to the inherent hormonal activity of these compounds can result in malignancy. Therefore, chronic exposure to high doses of these steroids produce neoplasms in a number of animal species.

The data obtained from rodent carcinogenicity bioassays can be used to compare one compound to another (assuming both are tested under similar and appropriate conditions) and to assess possible carcinogenicity in organ systems not related to the hormonal action of the steroids. The requirements for carcinogenicity testing for steroidal contraceptives are similar to the requirements for testing of other new drugs.

- If the drug is indicated solely for a single sex it should be tested in that sex only.
- For mice and rats, 100 animals/group should be dosed for 2 years.
- The choice of rodent strain should be left up to the discretion of the sponsor
- Adult rats should be fed a caloric-restricted diet containing approximately 65 percent of the calories consumed by rats fed *ad libitum*. Parallel non caloric-restricted control and high dose groups should also be included unless the sponsor can justify the adequacy of the caloric-reduced diet model through other parallel studies, historical data, or by other means.

#### IV. G. 1. Carcinogenicity Dose Selection Considerations

In the past, oral contraceptives were tested in animals at fixed multiples of the human dose on a mg/kg body weight basis. However, some contraceptive steroids, such as norgestrel, desogestrel, or gestodene, are far less orally bioavailable in rats than in humans. As a result, some oral contraceptives were tested in rats at plasma levels of parent compound (or active metabolite) below the plasma levels in women taking the recommended dose. The converse can also occur at higher doses. For example, DMPA acetate given at a dose 33 times higher than the human dose (on a mg/kg bw basis) produced a plasma steroid  $C_{max}$  in rats 126 times higher than the  $C_{max}$  in women. When progestogens are tested in combination with an estrogen, high doses of steroid often produce lethal pituitary tumors within a relatively short time. For these reasons the following guideline for dosing is given:

- the high dose group should result in plasma levels of parent steroid approximately 10 times higher than the mean plasma drug levels in women given a therapeutic dose (as measured by AUC of the parent progestational steroid);
- an extra group of rats and mice should be included which receive the high dose of the progestogen only; and,
- if pharmacokinetic studies reveal that the doses necessary to produce plasma drug levels 10 times the human level are excessive (and have not reached a plateau or produced toxicity in earlier studies) to the point that the duration of the study may be compromised, the sponsor should consult with the Bureau prior to initiating carcinogenicity studies.

#### **IV. H. GENOTOXICITY TESTING**

All new contraceptive steroids should undergo a battery of tests to assess potential genotoxicity. To ensure consistency in testing, the following tests results should be submitted as part of the NDS:

- an *in vitro* mammalian cell gene mutation assay with and without metabolic activation;
- an *in vitro* chromosome aberration test in mammalian cells with and without metabolic activation; and,
- the mouse micronucleus test.

#### **V. INJECTABLE CONTRACEPTIVES**

Single and multiple entity long-acting injectable contraceptive steroids should be assessed for toxicity and carcinogenicity in a manner similar to oral contraceptives.

An important factor to consider in testing injectables is the steady-state pharmacokinetic profile to ensure that drug release patterns are similar between animals and humans (e.g., there is no excessive steroid accumulation during long-term animal tests).

Some progestogens, which were not carcinogenic in animals when given orally, produced a significant increase in tumors when given parenterally to animals. As this effect

is likely due to greater bioavailability of the injected steroid resulting in higher plasma concentrations, the following guidelines are given:

- thorough clinical pharmacokinetic studies should be performed to ensure that the plasma levels of parent drug are no greater than those achieved by oral dosing and that the plasma profile of drug metabolites is not significantly different between the two routes of administration;
- if either of these conditions are not met, the steroid should undergo a two-year carcinogenicity study in rats using injection as the route of administration;
- if an approved steroid is to be given intravaginally, additional toxicology tests will be necessary to assess the effects of the drug locally on the reproductive system; and,
- if physiologically-based pharmacokinetic models are available for the compound(s), these can be used by the sponsor to justify dose-response extrapolation.

## **VI. HORMONE REPLACEMENT THERAPY**

Oral contraceptive steroidal agents are sometimes used in hormone replacement therapy. Hormone replacement therapeutics are usually used at lower dosage levels than those used for contraception. For this reason the following guideline is provided:

- studies used for oral contraceptive registration can be used for hormone replacement therapy submissions provided that the ratios of the compounds are the same as, and the doses are less than, those used for contraception in humans.

## **VII. MALE CONTRACEPTIVES**

Nonclinical testing requirements of steroids for male contraception are similar to those for women. New esters of testosterone will need a thorough pharmacokinetic examination in animals and man to determine what additional toxicology testing is warranted.