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**Guidelines for the toxicological
and clinical assessment and
post-registration surveillance
of steroidal contraceptive drugs**

Special Programme of Research,
Development and Research Training
in Human Reproduction



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Preface

The World Health Organization's Special Programme of Research, Development and Research Training in Human Reproduction was established in December 1971, and from the outset one of its major objectives has been to develop new, safe and effective methods of fertility regulation. In pursuit of this objective, the Special Programme has since its inception monitored the testing of contraceptive steroids.

It has now become clear that there are major differences in the reproductive physiology (and hence in the responsiveness of target organs to different steroidal drugs) between man and laboratory animals. Furthermore, epidemiological studies have revealed that owing to these differences it is impossible to predict reliably the effects of contraceptive steroids in the human on the basis of animal studies alone. These findings have highlighted the importance of meticulous early studies in humans and careful and prolonged post-registration surveillance of contraceptive steroidal drugs.

The International Conference on Population held in Mexico City in August 1984 unanimously passed a resolution which included the recommendation that:

"Modernization and updating of the official requirements for the preclinical and clinical assessment of new fertility regulating agents and a strengthening of the research capabilities of developing countries in these areas are also urgently needed."

In response to this recommendation, the Special Programme convened in Geneva, Switzerland, from 2 to 5 February 1987, a Symposium on Improving Safety Requirements for Contraceptive Steroids. The Symposium was attended by basic and clinical scientists and representatives of drug regulatory agencies, of the pharmaceutical industry and of consumer groups.

The main questions before the participants were:

- (1) How predictive have animal studies been regarding the safety of contraceptive steroids in the light of subsequent epidemiological investigations?
- (2) What measures could be taken to improve the validity of animal models used in toxicological studies?
- (3) What improvements could be made to the design and organization of clinical trials of new contraceptive steroids?

(4) What should be done to ensure the identification of long-term sequelae or rare adverse events that may be associated with new contraceptive steroids?

On reviewing the results from extensive animal testing of contraceptive steroids, the participants concluded that such studies had given little guidance in predicting certain side-effects that have subsequently been elicited through epidemiological research. Hence, the participants stressed the need to rationalize the existing testing requirements, particularly those relating to the testing of drugs in animals, with the selection of animal models being based on the pharmacokinetics and pharmacodynamic characteristics of the drug. Furthermore, it was concluded that there are valid reasons for questioning the usefulness of some of the current requirements, for example, for long-term carcinogenicity studies, namely 7-year studies in the beagle dog and 10-year studies in non-human primates.

With regard to clinical trials, it was agreed that many of the current requirements were satisfactory but attention needed to be paid to the importance of relating clinical trials to the results of pharmacokinetic studies in animals, appropriateness of the sample size, and the significance of differences among populations. Finally, it was emphasized that a systematic approach should be adopted in order to identify long-term or uncommon adverse effects.

The views and recommendations of the participants are summarized in this document. The scientific papers presented at the Symposium are contained in the Proceedings of the Symposium (1).

This booklet describes the current approaches to the evaluation of safety of contraceptive steroids as a prelude to making recommendations for improvements. The recommendations contained here should increase the relevance, reliability and timeliness of data on the pre-clinical and clinical assessment of the safety and efficacy of contraceptive steroidal drugs and on their post-registration surveillance. Moreover, it is hoped that both the Proceedings of the Symposium (1) and these Guidelines will help national authorities, in both developed and developing countries, in the review and formulation of regulations relating to the development, testing and use of new steroidal fertility regulating agents.

1. TOXICOLOGICAL TESTING IN ANIMAL AND OTHER NON-HUMAN SYSTEMS

1.1 Introduction

All new drugs are tested in animal and other non-human systems prior to their use in people. In the first tests in animals, the potential toxicity of new drugs and their metabolites is assessed. If these animal tests suggest the drug to be non-toxic in man, studies of the pharmacodynamics and pharmacokinetics of the new drug or compound are carried out in a small number of subjects (10-20 volunteers) to determine the dose of drug to be used in further human and animal studies.

This section outlines the procedures currently used in the pharmacodynamic, pharmacokinetic, and toxicological studies of new hormonal contraceptive drugs. In addition, developments in the field of toxicological testing, a historical overview of toxicological tests as applied to contraceptive steroids, and a description of the results of long-term studies in animals are also presented. Finally, recommendations are made for improving current toxicological testing of new contraceptive steroids in animal and other non-human systems.

1.2 Strategies currently used in the toxicological testing of contraceptive steroids in animal and other non-human systems

The procedures currently employed for the toxicological testing of drugs have been described in detail in several reports and publications (2-3). There are also specialized monographs that describe how tests of carcinogenicity, mutagenicity, and teratogenicity, are conducted (4-6). Comprehensive historical reviews and a detailed comparison of toxicological requirements for clinical testing of contraceptive steroids in different countries are available (7-8).

In the early 1960s, when contraceptive steroids were first introduced, there were few explicit requirements for proof of safety of drugs in humans prior to registration. Responsibility for safety testing and the choice of the types of test to be performed and the interpretation of the results rested with manufacturers. In the mid and late 1960s, important changes in requirements for the safety

testing of new drugs occurred, mostly stimulated by the human experience with thalidomide.

Already in the early 1960's the requirements for animal toxicology had been formalized in national legislation, particularly in the USA, and this lead was later followed by a number of other countries. In the United States, changing attitudes towards safety testing of new drugs culminated in the enactment in 1963 of the Kefauver-Harris Amendment to the Food, Drug and Cosmetic Act. The law established a statutory requirement that results of investigations in animals be submitted to the United States Food and Drug Administration (FDA) for validation as proof of safety. The FDA was authorized to establish guidelines for toxicity tests. Following the passage of the Amendment, detailed requirements for safety testing prior to marketing of new drugs were developed. The initial requirements for safety testing did not specify any special tests for contraceptive steroids which were not introduced until the late 1960's (9).

Table 1 shows the general sequence of animal and other non-human toxicological tests for contraceptive steroids required by the FDA for administration to women (7). Many other countries have adopted similar requirements. The purpose of the various toxicological studies in animals are described in Table 2.

Table 1. The present FDA requirements for animal tests for contraceptives of the hormonal type and for progestogens and estrogens for prolonged non-contraceptive use

Clinical study	Animal toxicity study requirements
Phase I (limited to a few subjects for up to 10 days' administration)	90-day studies in rats, dogs, and monkeys
Phase II (approximately 50 subjects for 3 menstrual cycles)	1-year studies in rats, dogs, and monkeys
Phase III (clinical trial)	2-year studies in rats, dogs, and monkeys Initiation of 7-year dog and 10-year monkey studies prior to start of Phase III
New drug application	No further requirements, but must include up-to-date progress reports on long-term dog and monkey studies

The most noteworthy special requirements for contraceptive steroids are those for long-term tests for the assessment of long-term adverse effects, including carcinogenicity. These requirements include tests in one rodent species, the dog, and the monkey. While

Table 2. Purposes of various toxicological tests in animals

Study	Purpose	Species usually used
<i>Pharmacodynamic studies</i>		
Pharmacological profile	To measure effects on the central nervous system, smooth muscle, circulation, respiration, etc.	rat
Endocrinological profile	To measure effects on pituitary hormone release, steroidogenesis, development of genital organs, selected responses to estrogens, androgens, progestogens, thyroid function and lactation	rat, mouse
Metabolic profile	To determine possible effects on carbohydrate metabolism, electrolyte balance, serum lipids	rat
<i>Pharmacokinetic studies</i>		
	To determine the metabolic pattern of the drug in the species to be used in later studies	rat, mouse
	To evaluate, in later studies, the blood levels at various doses, the rate of drug elimination, and the drug's principal metabolites	rat
<i>Single-dose toxicity</i>		
<i>Repeated-dose toxicity</i>		
Over a 3-month period	To evaluate the cumulative effects on organ function, general well-being, blood composition, clotting, endocrine system	rat, dog*, monkey*
Over a 12-month period	To determine if cumulative effects appear after longer periods of time	rat, dog*, monkey*
<i>Long-term studies</i>		
	To determine if there are long-term adverse effects, including carcinogenicity	rat, dog*, monkey*
<i>Reproductive studies</i>		
General	To assess effects on fertility and progeny	rodent
Teratogenesis	To determine if there are embryotoxic and teratogenic effects	rodent

* Tests in these species are required by the US FDA and some other drug regulatory agencies.

rodents are required to be observed for two years, dogs and monkeys require observation for 7 and 10 years, respectively; groups of at least 12 dogs and 12 monkeys are recommended for these studies. Doses of between ten and twenty-five times the anticipated human contraceptive dose calculated on the basis of a milligram-per-kilogram equivalent of body weight are required for studies in beagle bitches, and doses of between ten and fifty times the human contraceptive dose are required for studies in monkeys, again calculated on

the basis of body weight. Finally, the use of a low dose of one to two times the equivalent of the human dose is also considered advisable (7).

In addition to the tests shown in Table 2, it has become usual to perform potential mutagenicity tests prior to the initiation of human efficacy studies. The objective of these mutagenicity tests is to get an indication as to whether the compound being tested damages the genetic material; the tests are performed both *in vivo* and *in vitro*. There are two principal test systems for mutagenicity: (a) those for the detection of gene (point) mutations; and (b) those for the study of chromosomal aberrations. Current regulations on the appropriate models for testing potential mutagenicity are under constant review but, in general, include both bacterial and mammalian gene mutation studies, as well as *in-vivo* and *in-vitro* cytogenetic studies.

Along with the assessment of mutagenicity risks, these tests also aim at developing systems which will be predictive of carcinogenicity and hence replace the long-term animal studies. However, these tests lack specificity and their predictive value of the carcinogenic risk in the human is unclear (10).

Reproduction studies became mandatory for all drugs as a result of the experience with thalidomide. Data currently required include those on: (a) fertility and reproductive performance in treated female and male animals; (b) embryotoxicity; (c) teratogenicity; and (d) effects on peri- and post-natal development in the offspring.

1.3 Historical review of toxicological testing of contraceptive steroids in animal and other non-human systems

1.3.1 Acute single-dose toxicity testing

A problem with acute toxicity testing of contraceptive steroids in animals is that very large doses cannot always be delivered through the route that is anticipated for use in humans. This difficulty has in the past been overcome by dissolving the steroid in various vehicles or by administering the drug by another route, or both. However, the vehicle in which the drug is dissolved, in order to make it possible to give very large doses of the compound, may itself be toxic. There is no evidence that acute single-dose toxicity testing of steroids at very large doses (e.g., 500-1000 times the anticipated human dose) has been useful in predicting toxic effects in humans.

1.3.2 *Pharmacokinetic and pharmacodynamic studies*

The recent development of sensitive and specific radio-immunoassays for various contraceptive steroids has revealed much new information on the pharmacokinetics of these compounds (11), which could be used to improve their safety testing.

Current pharmacokinetic studies on contraceptive steroids include tests in animals. At the same time, it has become possible to identify species (such as the rhesus monkey) in which steroid pharmacokinetics are comparable to those in man. Differences between species have been highlighted in studies with, for example, d,l-norgestrel, in which the highest oral dose was 37.5 µg/kg/d given to dogs and 75 µg/kg/d to rhesus monkeys. It was shown that these dogs had only 30% of the mean daily levonorgestrel plasma level of women taking 150 µg levonorgestrel (approx. 2.5 µg/kg/d), but in the monkeys approximately the same plasma levels as in women were reached (12).

Comparable advances have also been made in the study of the pharmacodynamics of contraceptive steroids. Early tests of the pharmacodynamic effects of steroids were limited to the determination of end points such as pregnancy maintenance in rodents, and, in clinical studies, delay of menses, daily basal body temperature, and urinary pregnanediol measurements. To these tests have been added assays of the binding of steroids to various receptors and the effects of the administration of contraceptive steroids on other hormones such as gonadotrophins, progesterone and estradiol. A large amount of data on the comparative pharmacodynamics of different steroids in man and in several animal species has been accumulated. These data have been useful in reviewing the relevance of animal models to humans (1, 24).

1.3.3 *Repeated-dose toxicity studies*

Requirements for toxicity testing vary from one drug regulatory authority to another but in general the duration of testing is determined by the length of the period for which the drug is to be administered in the human. For example, in European Economic Community countries the general requirements are as follows (3):

*Duration of administration of
the drug in the human*

Required testing period in animals

1 single dose	1-2 weeks
1 week	4 weeks
4 weeks	3 months
> 4 weeks	6 months

In the USA and Canada, however, 12 months of testing is required for the last mentioned category.

In animal studies, three dose levels (low, intermediate, and high) are commonly used to demonstrate a dose-dependency of effects. The low dose is usually a simple multiple (2-5 times) of the anticipated human dose calculated on a milligram-per-kilogram-of-body-weight basis. The high dose is the lowest dose that shows animal toxicity (e.g., weight loss, reduced food consumption). The intermediate dose is between the two doses on a logarithmic scale.

Experience with these studies, however, shows that they do not reveal toxicity, but rather an exaggerated pharmacological response to excessive doses of estrogens and/or progestogens. These responses are manifested in the rat by a deterioration of the general body condition, an increase in pituitary adenomata and alterations in liver function and in the haematopoietic system; in the dog the responses are marked obesity, cushingoid syndrome, disturbed carbohydrate metabolism, altered liver function and changes in the haematopoietic system, pyometra and benign and malignant mammary gland tumours; in the monkey, alteration or suppression of the menstrual cycle and changes in liver function are seen (13).

1.3.4 Reproduction studies

Since the introduction of sex hormones as therapeutic and fertility regulating agents there has been concern over the possible effects on the offspring of their use during pregnancy (for therapeutic purposes) or as contraceptives. Thus, general reproductive and teratogenicity studies are carried out in one rodent species prior to early clinical studies in women of child-bearing age.

It has been reported that both embryotoxicity and teratogenic effects can be induced by steroidal compounds given at an early stage of gestation in the commonly used test animals, namely mice, rats and rabbits. In the case of progestogens, the type of embryotoxicity has also been found to vary with the type of compound tested.

Treatment in later stages of gestation with some progestogens has been shown to cause virilization of female fetuses (1, 14).

The use of rodents for testing long-acting agents presents numerous problems, since in these species in addition to the short embryonic and fetal period, many of the developmental events involving genital and hypothalamic differentiation occur postnatally. Hence, non-human primates, which have a similar reproductive process as man and long embryonic and fetal developmental periods, have been considered more suitable models for testing embryotoxicity of long-acting contraceptive steroids. However, the small number of offspring from each pregnancy compared to rodents and the very high cost of breeding and maintaining a non-human primate colony makes this approach impracticable.

1.3.5 *Mutagenicity studies*

Until very recently many drug regulatory authorities did not require mutagenicity testing and some still do not request it. There are insufficient data published to draw any firm conclusions as to whether hormonal contraceptive steroids are mutagenic or not in the conventional test systems used (6, 15-17). However, the available clinical information suggests that they are not mutagenic (18, 19).

1.3.6 *Long-term carcinogenicity studies*

The purpose of long-term carcinogenicity studies is to investigate the potential of compounds to induce proliferative changes or neoplasia in laboratory animals.

In general, the animals used are rodents (rats, mice and occasionally hamsters) and these are studied over 18-24 months. In the case of contraceptive steroids, however, the requirements were expanded to include studies in two non-rodent species, in the beagle dog, and in non-human primates; the latter two require 7 and 10 years, respectively, to complete. In view of the findings in the beagle dog, requirements for testing in this animal have been discontinued in many countries.

Information available from long-term carcinogenicity studies of steroidal contraceptives is consistent with the data from repeated-dose toxicity studies (see section 1.3.3). The majority of the neoplasia observed in the rat occur in endocrine organs (pituitary, ovary, etc.) and in hormone-dependent organs (mammary gland, uterus). The

occurrence of hepatic neoplasia varies depending on the strain and sex of the animal and the steroid being tested. Thus, as far as hepatic neoplasia is concerned, it is difficult, if not impossible, to predict its risk in man from animal studies.

In the beagle dog, an increased incidence of mammary tumours has been observed with all progestational steroids, including progesterone itself. This increased incidence is related to the duration of exposure and the bioavailability of the individual steroid (20). Other changes (diabetes mellitus, acromegalic syndrome) were consistent with gross overstimulation resulting in a profound functional disturbance of the hypothalamo-pituitary axis rather than toxicity *per se*.

Endometrial tumours—including some malignant ones—have been observed in monkeys treated for long periods with 50 times the equivalent human dose of depot medroxyprogesterone acetate and norethisterone enantate. The issue of the relevance of these findings in humans is still unresolved (21).

1.4 Recommendations for testing of new contraceptive steroids in animal and other non-human systems

The establishment of the safety of a new steroidal contraceptive is a shared responsibility of animal toxicology and clinical pharmacology. The following principles and recommendations guide the performance and interpretation of animal and other non-human toxicological tests.

- In order to extrapolate the findings from non-human toxicological tests to human beings, the following species-specific factors must be known: hormonal regulation of reproductive processes; pharmacodynamic effects and pharmacokinetics and metabolism of test substances.
- Toxicity of the test substance, including that resulting from its accumulation in the body, should be studied in a selected animal species, with the selection being justified on the basis of the pharmacodynamic and pharmacokinetic properties of the drug, the proposed human route of administration, and the reproductive characteristics of the species.
- In reproductive toxicity studies, the experimental design should be adapted to the specific pharmacodynamic and pharmacokinetic properties of the test drug with emphasis on exposure during the embryonic period.

- The mutagenic potential of new contraceptive steroids should continue to be screened by selected *in-vivo* and *in-vitro* tests.
- Long-term carcinogenicity studies should be performed in rodent species to differentiate between proliferative and neoplastic changes due to continued stimulation of hormonal target organs and neoplastic changes caused by mechanisms not related to the pharmacodynamic properties of contraceptive steroids. The reversibility of proliferative responses should be investigated.
- When established steroidal contraceptives are administered through new delivery systems, animal safety studies should concentrate on potential interactions of the drug with the system and on the possibility of adverse effects of the delivery system *per se*, unless the safety of the materials used in the system is known.
- Post-registration surveillance together with clinical assessment should be considered as an integral part of the risk assessment process, allowing re-interpretation and re-evaluation of the animal data.

1.4.1 *Pharmacokinetic and pharmacodynamic studies*

The extrapolation of toxicological effects of a drug observed in one species to another, particularly to man, is extremely difficult primarily due to variations between species with regard to the absorption, distribution and metabolism of drugs. This is especially true for contraceptive steroids. The major advantage of conducting pre-clinical assessment of steroidal compounds is that a detailed picture becomes available of their hormonal (pharmacodynamic) effect in several appropriate laboratory animal species. Pharmacokinetic studies in non-human primates are of particular value.

There are characteristics of reproductive physiology in different species of laboratory animals that *a priori* limit their comparison to the human. In addition, there are differences between species in the responsiveness of endocrine organs to gonadal and pituitary hormones and to steroidal contraceptives, as there are species-specific peculiarities in the feedback mechanisms of steroids.

In spite of such difficulties, there is scope for improvement in the extrapolation of data from animal species to the human. Sensitive and specific techniques for the determination of plasma and tissue levels of drugs and their metabolites are now available. Such

determinations are reliable and should be used to estimate the body burden of a steroid in different species as well as to describe the details of its pharmacokinetics. A variety of data on the pharmacodynamic effects of steroidal contraceptives are also now available. These data should be used in parallel with pharmacokinetic data in the human to plan further animal studies. This approach would avoid the use of doses that result in high body burdens of the drug in test animals, which give rise to adverse effects that cannot be extrapolated to humans.

1.4.2 *Initial toxicological studies*

Animal toxicity studies are required prior to the initiation of early clinical trials intended to establish general tolerance, pharmacokinetics, and selected endocrine (pharmacodynamic) effects. In general, these animal studies should be conducted in two species selected on the basis of available pharmacological and other data. For conducting limited repeated-dose toxicity studies, the length of the test period is decided on the basis of the intended use of the compound during early clinical trials and the pharmacokinetics of the compound.

Single-dose (acute) toxicity studies should be limited to one rodent species. In most cases, a "limit test", i.e., the determination of an approximate lethal dose, should be sufficient. There is no scientific justification for conducting acute, single-dose studies of the LD-50 type.

The use of very high doses of contraceptive steroids in long-term tests in animals results in an excessive pharmacological response which precludes the extrapolation of the findings to humans. The pharmacokinetic and pharmacodynamic characteristics provide a more rational basis for the selection of the dose to be used than the approach based solely on the principle of milligram per kilogram of body weight.

1.4.3 *Repeated-dose toxicity studies (long-term toxicity tests)*

Studies designed to detect cumulative toxicity should be performed in two animal species. In designing the study and selecting the species, consideration should be given to the reproductive physiology of the proposed animal model, the pharmacodynamic and pharmacokinetic characteristics of the compound, and the

proposed route, frequency and duration of its administration in the human. The duration of treatment should be not more than 6 months. However, in some cases (e.g., a long-acting steroid that is not completely eliminated from the body within six months), owing to the special chemical, pharmacokinetic and pharmacodynamic characteristics of the test drug or findings obtained in other toxicological models, it may be necessary to administer the drug for a longer period.

1.4.4 *Reproduction studies*

The predictive value of reproduction studies in forecasting possible adverse effects on reproduction in the human is regarded to be generally low. However, as indicated before, if the selection of dosage is based on the drug's pharmacokinetics, the predictive value of these studies could be improved.

Since steroidal compounds have pharmacological effects on the endocrine and reproductive systems, special attention should be paid in reproduction studies to the potential risk of embryonic and fetal exposure. Furthermore, their potential adverse effect on subsequent maternal fertility and on the development and future fertility of the offspring should also be studied.

In animals as in humans, the genital tract continues to develop after the organogenic period, and exposure to steroidal hormones in the last part of gestation could influence the normal development and subsequent sexual maturation of the offspring. Even if the possibility of exposure at this time in humans is remote, it should be studied in animals, recognizing that such studies might encounter technical difficulties in terms of interference with parturition when long-acting steroid preparations are tested. In most cases, a rodent species is appropriate.

1.4.5 *Mutagenicity studies*

When mutagenicity studies are required by the drug regulatory agencies, tests for gene mutations in micro-organisms and mammalian cells are performed together with *in-vivo* and *in-vitro* cytogenetic tests for chromosomal aberrations. It is expected that in the future increased understanding of mutations and chromosomal aberrations will lead to these tests being modified.

1.4.6 *Long-term carcinogenicity studies*

The dose levels for these studies should be chosen on the basis of the findings of the pharmacokinetics of the drug in a rodent species. All long-term carcinogenicity studies must: (a) use dosage regimens comparable to those proposed in the human; (b) have a meticulous study design, with special attention being paid to the feeding and housing of the animals; and (c) include consideration of the possibility that a reversal of proliferative changes could take place upon withdrawal of treatment.

These studies need only be conducted in a rodent species, for either 80 weeks in the mouse or two years in the rat.

1.4.7 *Sex of laboratory animals*

For most toxicological studies it is usually sufficient to use animals of the sex for which the drug is intended in the human. In carcinogenicity studies in rodents, tests may need to be conducted on both sexes because in these species steroids may cause changes in both hormone-dependent and other organs; it is possible that adverse effects on non-target organs may be more readily identifiable if both sexes are used.

1.4.8 *Established contraceptive steroids in new delivery systems*

Occasionally, a new contraceptive entity consisting of an established contraceptive steroid, i.e., a steroid which has completed the conventional toxicological studies and which has been accepted for use in people, is incorporated into a new delivery system, such as an implant or vaginal ring. If it can be shown that there is no pharmacological interaction between the drug and the delivery system and that the bioavailability of the steroid released is similar to that when administered by the "established" route, there is no need to carry out the toxicological assessment of the steroid again. On the other hand, where interaction is suspected between the drug and the components of the delivery system, or if the delivery system itself undergoes biological degradation, it will be necessary to undertake toxicological studies again.

In the case of new injectable or implantable preparations using established steroids, short-term toxicological studies to observe any local effects at or near the site of drug release are necessary. In order to avoid unanticipated and inexplicable toxicity in the test animal,

attention should be paid to the possible release of plasticizers, catalysts, monomers, dimers, etc. from the delivery system before toxicological studies are undertaken. If previous animal toxicology studies on the drug had been undertaken with the compound being administered by the oral route alone, further toxicological studies will be necessary using the proposed route of administration.

1.4.9 *New derivatives of established contraceptive steroids*

All new long-acting esters and other steroid derivatives must be treated in the same way as a new steroid. The only possible exceptions would be compounds for which it has been demonstrated that the sole pharmacological action is confined to the parent steroid. In these cases, it may be possible, after pharmacokinetic studies in an appropriate animal species, to go directly into human Phase I-IIA clinical trials. Following these studies, however, it will be necessary to undertake the long-term repeated-dose toxicity studies (section 1.4.3) and reproduction studies (section 1.4.4) based upon the pharmacokinetics of the compound itself.

1.4.10 *Toxicological testing of combinations of contraceptive steroids*

Previous experience with the toxicology of combined estrogen-progestogen preparations shows that there are marked differences between species in their responses to different ratios of these two steroids. For example, the optimum ratio of estrogen to progestogen for the induction of decidual reaction or transformation of the endometrium is 1:20 000 in the rat and 1:50 in women. Hence, when rats are given the human dose, the effect of estrogen is predominant, and consequently, pituitary tumours will be induced. Therefore, rodent toxicology studies of estrogen-progestogen combinations will not give results that can be extrapolated to man. Hence it may be more useful to design clinical studies to assess possible pharmacokinetic and metabolic interactions.

1.4.11 *Use of standard procedures*

All preclinical experiments must comply with the recommendations on good laboratory practice applicable in the country. The current guidelines for laboratory animal welfare must also be followed (22).

1.4.12 *Timing of animal toxicology and clinical trials*

Each phase of clinical studies is preceded by specific animal toxicology studies. Phase I-IIA clinical studies are preceded by single-dose and limited repeated-dose studies in animals.

Repeated-dose toxicity studies (long-term toxicity tests) and reproduction studies in animals (sections 1.4.3 and 1.4.4) precede Phase IIB studies. Long-term carcinogenicity studies (1.4.6) should begin before starting large-scale clinical (Phase III) and field trials of efficacy and side-effects of the drug. These trials should be allowed to advance only if there is a periodic review of the ongoing animal toxicology studies and if the findings of the reviews continue to be satisfactory (see also section 2.3).

2. CLINICAL STUDIES

2.1 Introduction

In recent years, considerable advances have been made in the study of the pharmacokinetics and pharmacodynamics of drugs in both man and animals. These advances indicate that certain changes should be made in the conduct of clinical trials. The sections below present a brief, general overview and objectives of clinical trials and recommend modified testing procedures.

Unlike the two other parts of the Guidelines (toxicological testing and post-registration surveillance), a historical review of clinical studies of contraceptive steroids is not included here because this subject has been dealt with at length in other publications (8, 24-27), and summaries of clinical trials of hormonal contraceptives can be found in specialized publications (21, 28-30).

The ethical principles guiding the conduct of biomedical research involving human subjects are embodied in the Declaration of Helsinki of the World Medical Association as revised by the 29th World Medical Assembly in Tokyo in 1975 and in the *Proposed international guidelines for biomedical research involving human*

subjects (23). According to these guidelines, all studies should be performed on volunteers who, in the early clinical trials, may receive no therapeutic benefit from the drug administered. The volunteers must give informed consent after being advised of the risks and benefits of the study and the protocol should be reviewed and approved by an independent ethics committee.

2.2 Overview and objectives of clinical trials

Conventionally, the testing of all drugs in the human up to the point of registration (marketing permission or general release) has been divided into three Phases:

- Phase I Studies on the pharmacology of the drug in healthy volunteers
- Phase II A: Studies on the pharmacology of the drug in patients
 B: Definitive dose-finding studies
- Phase III Extended clinical trials in patients for clinical efficacy, acceptability, and assessment of the extent of side-effects.

The following major objectives of early studies (Phase I and Phase II) of drugs for the treatment of disease in the human have been described (15):

- to demonstrate a pharmacological action in man which is likely to be useful in the treatment of specific diseases;
- to characterize the dose (concentration) response curve with special emphasis on inter-individual variability and margins of safety;
- to devise dosage schedules to be used in the continued clinical evaluation of the drug;
- to devise and validate methods for monitoring subjective and objective drug response in these later phases of drug evaluation.

The objective of the clinical studies (Phase III) that follow is to expand the preliminary information generated on the possible mechanism of action and adverse effects and to ascertain the minimum effective dose of the drug. These studies provide the basis for further large-scale trials that precede registration of the product with drug regulatory agencies.

2.3 Recommendations for clinical studies of new contraceptive steroids and steroid-releasing systems

The recommendations outlined in the following section relate to the clinical testing of contraceptive steroids in both men and women. They are applicable to contraceptive steroidal formulations: (a) containing a single steroid or a combination of them; (b) having either a short or a long duration of action; and (c) administered by different routes, including controlled-release delivery systems.

It is anticipated that all new fertility regulating hormonal steroids (including steroid-releasing systems) can be accommodated under these general recommendations provided that the mechanism of action of the drug is known and that that knowledge is used to determine the pharmacodynamic end points, which may vary with different steroids. Examples include: inhibition of ovulation by long-acting injectable progestogens or progestogen-estrogen combinations; inhibition of spermatogenesis by long-acting androgen-progestogen formulations; and interruption of early pregnancy by anti-progestogens.

The stages of testing of new contraceptive steroids and steroid-releasing systems can be classified according to the conventional Phase I-III trial nomenclature used generally in drug development (see section 2.2). As with other drugs, the initial clinical studies of steroidal drugs should be designed to confirm at the outset the prediction of the previous animal toxicity studies (section 1.4.2) that the drug would be non-toxic in man. This is done by assessing various clinical, biochemical and haematological indices. However, since unlike therapeutic drugs steroidal contraceptive drugs are intended for use in healthy people for prolonged periods of their reproductive life (rather than for the treatment of specific diseases), it is desirable to ascertain also the minimum effective dose at the initial stages of clinical testing. In the case of therapeutic drugs, this need not be done in the initial studies.

The minimum effective dose must be derived from the evaluation of pharmacokinetic profiles and pharmacodynamic effects of different doses of the drug. The pharmacokinetic information obtained should also be used in the selection of the dose and animal species to be used in subsequent animal toxicology studies, (i.e., repeated-dose toxicity (section 1.4.3) and reproduction studies (section 1.4.4)).

The studies undertaken to confirm the drug's non-toxicity in man and those to assess the minimum effective dose correspond to the classical studies "Phase I--Volunteer studies" and "Phase IIA—Clinical pharmacology in patients..." as referred to in the *Safety requirements for the first use of new drugs and diagnostic agents in man*(15).

On completion of repeated-dose toxicity studies (long-term toxicity tests) (section 1.4.3) and reproduction studies in animals (section 1.4.4), Phase IIB and Phase III clinical trials can be undertaken. In the case of contraceptive steroids, Phase IIB clinical trials are commonly undertaken in 50–100 subjects to assess the efficacy and side-effects of the dosage determined in earlier trials (Phase I–IIA); the objective at this stage is to confirm the previously determined dosage rather than to carry out definitive dose-finding studies which, in the case of other drugs, are usually conducted at this stage of testing. Phase III clinical trials of contraceptive steroids are also carried out with the objective of reconfirming the dosage, but on a much larger scale. At the same time, separate in-depth studies are carried out, whenever necessary, on particular biochemical variables (e.g., lipid and lipoprotein metabolism, coagulation, fibrinolysis and platelet function) or other physiological events such as vaginal blood loss.

The recommended timing of clinical testing in relation to animal testing (see also section 1.4.12) is as follows:

- initial toxicity studies in rodents; pharmacokinetic studies in the most suitable animal species, e.g., a sub-human primate;
- Phase I and IIA clinical testing; studies on drug metabolism;
- repeated-dose toxicity and reproduction studies in animals;
- Phase IIB clinical testing;
- initiation of long-term carcinogenicity studies;
- Phase III clinical testing;
- specific studies on biochemical variables and physiological events.

2.3.1 *Assessment of pharmacokinetic profiles and pharmacodynamic effects of different dose levels (Phase I–IIA)*

2.3.1.1 Overall study design

The initial clinical trials should assess the pharmacological action of the drug, identifying any possible toxic reactions. The design and

conduct of these studies should, whenever possible, be based on the knowledge of the mechanism(s) of action of the drug since this (these) will determine the end points in the assessment of pharmacodynamic effects. It is necessary to undertake a series of sequential studies using different dose levels with the objective of ascertaining the minimum effective dose. The studies should begin in groups of 10-20 fully informed volunteers for each dose level in a single centre and must be conducted in a hospital-based clinic or a special facility such as a metabolic ward.

In the case of contraceptive steroids that inhibit ovulation, early human studies should comprise one or more control cycles and a treatment period, the length of which will vary with the duration of action of the steroid, or its release characteristics from an implant, vaginal ring, or another delivery system. The subjects should be followed for a minimum of one month after the end of the pharmacological activity of the drug or until normal ovarian function is re-established.

Studies for the assessment of contraceptive steroids that do not inhibit ovulation, but are used because of other antifertility effects, should be carried out in a similar manner except that they should include appropriate end points, such as sperm-cervical mucus penetration tests (31).

Steroids having an abortifacient action can be tested only in pregnant women. Following the confirmation of pregnancy, the drug should be administered and the subject followed until abortion occurs or until clinical intervention is deemed necessary. The clinical outcome (i.e., abortion rate) determines the minimum effective dose. Follow-up should be continued until the return of ovulation.

Male antifertility agents should be studied in volunteers who act as their own control in that "normal" testicular function must be established prior to the administration of contraceptive steroids. The control period is followed by a treatment period and a subsequent period of post-treatment observation during which the return of normal testicular function must be monitored.

Since pharmacokinetic and pharmacodynamic responses to contraceptive steroids may vary between ethnic groups with, for example, aqueous microcrystalline suspensions, because of factors such as body size and fat distribution, pharmacokinetic and pharmacodynamic studies with different dose levels need to be conducted in different populations (32).

2.3.1.2 Selection of dose

The dose used in the first clinical study is calculated on the basis of the results of the pharmacokinetic and pharmacodynamic studies carried out in a suitable animal species, such as a non-human primate. The amount of the drug used represents the best estimate of the dose expected to exert the required pharmacological effect. In this regard, it is important to note that while it has been usual to extrapolate the human dose (from the animal dose) on a milligram-per-kilogram-body-weight basis, this approach has limitations since the pharmacological activity of different steroids varies with the species-specific characteristics of each compound, such as absorption, distribution, metabolism, and elimination (see section 1.3.2).

Upon the completion of the first study (the objective of which is to assess the pharmacological action of the drug and its possible toxic effects), further studies are conducted to establish the minimum effective dose and to obtain a dose-response relationship. These studies involve the use of multiples of 2 or 3 of the initial dose, sequentially. Whether a factor of 2 or 3 is used is determined by the range of the dosages that gives rise to the desired pharmacological activity. If no pharmacodynamic effect is seen with the first dose, then the dose is increased, but if an effect is observed, the dose is reduced until the minimum effective dose is found (32).

2.3.1.3 Pharmacokinetics

Drug levels in biological fluids should be measured by specific assays which fulfil recognized criteria of assay reliability (33). The preferred method for most steroids is immunoassay.

The study should be designed to ascertain the pharmacokinetics of the drug, for example, by the estimation of: (a) the maximum plasma concentration of the steroid after dosing, (C_{max}); (b) the time of C_{max} , (T_{max}); (c) the terminal elimination half-life, ($T_{1/2}$), or plasma clearance rate; and (d) the total area under plasma concentration/time curve (AUC) (11, 34). The frequency of blood sampling for the measurement of a synthetic steroid depends upon the route of administration of the drug, on its expected half-life, and on its duration of action.

2.3.1.4 Other measurements to be undertaken

Certain routine examinations should be carried out on all subjects. These include a medical history, a physical examination, and the measurement of weight, blood pressure and certain

biochemical and haematological variables. Blood samples should be taken prior to the administration of the drug, during the course of "treatment" (the exact time in this case will depend on the duration of action of the steroid), and at the end of the treatment period for measurement of a number of biochemical and haematological indices which may include:

- haemoglobin and haematocrit;
- white cell, platelet and differential cell counts;
- renal function tests;
- liver function tests;
- HDL-cholesterol and other selected lipid fractions;
- fasting glucose;
- sex hormone binding globulin;
- total and free thyroxine.

The objective of these measurements is to establish that there is no toxic effect of the drug at the dosages chosen for the human studies.

In the case of implantable devices or injectable preparations, inspection of the implant/injection site should be undertaken to assess any local effect, such as skin irritation. Both the degree and duration of the effect and the distance from the incision or injection site should be recorded.

2.3.2 *Studies on drug metabolism*

In the case of a new steroid or a derivative of a steroid with a modifying group or side chain, studies of drug metabolism should be performed. These studies should determine whether there is a prodrug-product reaction in target tissues by estimating the steroid bound to nuclear receptors. This can be done in animals or on human cells *in vitro*. With certain compounds, it may be necessary to determine whether there are significant unconjugated steroid metabolites in blood which may be biologically active or may interfere with appropriate assays of the parent compound (34).

2.3.3 *Assessment of efficacy and side-effects (Phase IIB-III)*

2.3.3.1 Initial assessment (Phase IIB)

Once the minimum effective dose has been established in the early human studies (see section 2.3.1) and repeated-dose toxicity and

reproduction studies completed in animals, larger-scale studies of efficacy can be initiated. These studies begin with the assessment of efficacy and side-effects in a relatively small group of subjects (50–100). With contraceptive steroids, this is usually considered part of Phase IIB testing. The study protocol should provide indices for the early termination of the study should an unacceptably high rate of pregnancies or adverse reactions be observed. Depending on the event, it may be necessary either to abandon further development of the product or to reformulate the drug and/or to re-evaluate it at a different dose level.

2.3.3.2 Phase III clinical trial

Large-scale clinical trials should be randomized, where feasible, between the test drug and a comparable established method of fertility regulation. The studies should be designed to have a sufficient statistical power to demonstrate a clinically important difference between the treatment groups, either with regard to efficacy or side-effects. For example, if the objective of the study is to differentiate between pregnancy rates of 4% and 2% at one year between two groups with a two-sided significance level at $p = 0.05$ and a power of 50%, 560 subjects per group are required, while for a power of 80%, 1140 subjects per group are required. However, if it is necessary to differentiate between pregnancy rates of 0.5% and of 0.25%, 4600 and 9400 subjects are required for the powers of 50% and 80%, respectively. These calculations assume that no subjects are lost during follow-up or discontinue for other reasons. In practical terms, however, it has been demonstrated (35–36) that the study of up to 1000 subjects, with a minimum of 10 000 months of exposure to a new drug or device in comparison with an established method in a randomized trial is sufficient to allow an adequate assessment of safety and efficacy prior to registration and wider usage. As such large groups of subjects are difficult to recruit at a single centre, it will usually be necessary to involve several centres.

Population differences may affect the results of a large clinical trial with regard to efficacy and side-effects and must be allowed for in the design and subsequent analysis of the study. Hence, it is necessary to undertake Phase III clinical trials on a multi-centre basis in several representative populations.

An appropriate indicator of efficacy must be selected for Phase IIB and Phase III trials. For a contraceptive steroid this will be the pregnancy rate and for an abortifacient steroid, the abortion rate.

For a male method, a direct measurement such as impairment of spermatogenesis and sperm function could be used, although the pregnancy rate for the couple is a better indicator of efficacy.

Adverse effects and complaints should be elicited at each follow-up visit, first by open-ended questioning followed by specific direct questions. For many drugs, certain events, such as changes in vaginal bleeding patterns need to be specifically assessed. Vaginal bleeding records should be completed by the volunteers in the study to allow bleeding analysis in terms of reported duration, frequency and amount. Data analysis should include reasons for discontinuation of the method and complaints related to vaginal bleeding in order to assess the subject's perception and acceptance of the bleeding patterns.

Subjects should visit the clinic at regular intervals during the duration of the study, which in the case of oral and injectable contraceptives, should be at least one year. Long-term methods such as steroid releasing implants and IUD's must be studied for the duration of their active life (37-38). In addition, for implantable methods, the implant site must be examined at each clinic visit.

In the past a component often missing from studies of efficacy and side-effects has been the return of fertility following discontinuation of the method. The study design must address this issue at the outset. Studies should involve a large number of subjects, enough to allow a meaningful interpretation of data from the sub-group of volunteers who discontinue the use of the method because they wish to become pregnant following the study. Although the probability of restoration of fertility can be gauged from the return of ovulation or normal spermatogenesis, more precise information can be obtained only from the larger-scale Phase III trials.

In addition, in these larger-scale clinical trials the opportunity should be taken to make a preliminary assessment of the acceptability of the method, to develop and test user and provider information, and to investigate user-provider relationships.

2.3.4 *Specific studies on biochemical or other pharmacological variables*

Information obtained in Phase I and IIA clinical trials may point to the need to undertake specific studies on a particular metabolic process or pharmacological event. Furthermore, with steroidal contraceptives, drug regulatory agencies often require information

on their effect on lipid and lipoprotein metabolism, or on coagulation, fibrinolysis and platelet function as markers of potential cardiovascular problems. Other separate studies may also need to be undertaken: (a) to quantify vaginal blood loss and the assessment of indicators of body iron stores; and (b) to assess the effects of the steroid on carbohydrate metabolism, or on the function of the hypothalamo-pituitary-gonadal axis and of other endocrine organs, e.g., pituitary, thyroid and the adrenal gland.

Such studies should run concurrently with the study on efficacy and side-effects but they must be designed as separate studies. Sub-groups of the larger efficacy and side-effects study should not be used since the sub-group will immediately become a population subjected to abnormal assessment and, as such, will have different reasons and rates of discontinuation from the remainder of subjects in the main clinical trial. Groups of up to 40 subjects should be investigated in these studies and compared in a randomized trial with a similar number of subjects using a comparable established method.

For a drug or device that will be used continuously, the specific studies should be undertaken for 6 to 9 months or for the entire duration of its effect, if it is longer than 9 months. For a drug used on a non-continuous basis, such as an antiprogestogen, these specific studies can be undertaken only during and following one treatment regimen.

2.3.5 *Introductory trials (field trials)*

On completion of Phase I-III trials, the new method is ready for introduction into family planning programmes. This poses a number of service-related and procedural questions. For example, how can the acceptability and effectiveness of the method be evaluated in specific populations? What measures should be adopted by family planning programmes to ensure user satisfaction and compliance with the use of the method? How can this knowledge be obtained so that it will be useful for programme planners and policy-makers?

Field studies are the first stage at which a new method is assessed in the context of family planning services. These studies are conducted in a specific programme setting, and are designed to confirm the acceptability, use effectiveness, continuation of use, side-effects (tolerance and management), and to evaluate specific service-related needs (e.g., provider training, client counselling and logistics) (38-41).

The main purpose of field studies is to assess, through a limited initial cohort of users, any method problems as well as various needs of users so that service and counselling procedures can be established in a national programme.

It is recognized, however, that in order to make a new method become freely available in family planning programmes, certain countries may require expanded Phase III-type clinical trials in a network of 5–10 centres, in which an acceptability component would be incorporated. Such studies might involve 1000–5000 subjects.

On the other hand, with some methods, such as a new injectable preparation, populations may exist in which there is an already defined acceptability of the available products. In these situations, larger-scale field trials of the new drug could be considered with up to 20 000 subjects receiving the preparation within a relatively short time span (1–2 years). These would provide additional acceptability information, simultaneously elucidating any service requirements at the clinic, provincial and national levels. Limited data would still be collected in a manner similar to that undertaken in the more restricted field studies.

3. POST-REGISTRATION SURVEILLANCE

3.1 Introduction

Many of the adverse effects of drugs in humans can be discovered only after the drug has been in widespread use (42–43) and this recognition has led to the concept of “post-marketing”, or, “post-registration” surveillance of drugs. Post-registration surveillance is necessary because studies in laboratory animals may not predict human toxicity and Phase III clinical trials in human subjects can detect only relatively common side-effects that occur over a short period during or after the use of a drug. In addition, clinical trials generally enrol selected groups of subjects who are closely followed and whose use of steroids would be terminated at the first sign of adverse effects; there may occur effects of a steroid in the general population that were not detected previously.

In this part of the Guidelines, the general strategies currently used in the post-registration surveillance of drugs are briefly described. For more detailed information the reader is referred to references

42-45. The first section below reviews the strategies currently used in post-registration surveillance of drugs. The next presents a historical review of post-registration surveillance of contraceptive steroids, identifying the limitations of past approaches. Finally, recommendations are made for the post-registration surveillance of new contraceptive steroids.

3.2 Strategies currently used in post-registration surveillance of drugs

Five strategies are used in the post-registration surveillance of drugs. These are: (a) the collection and use of reports of adverse reactions; (b) large-scale experimental studies; (c) formal epidemiological studies (cross-sectional, case-control and cohort); (d) indirect correlational studies; and (e) investigations of outbreaks.

These methods are complementary, with each having its strengths and limitations. All have potential uses in a comprehensive post-registration surveillance scheme.

3.2.1 Reports of adverse reactions

Side-effects of a drug are noted in the usual practice of medicine and may be reported to a central agency or the manufacturer, or they may be published. Regardless of the method of reporting, such reports are called "adverse drug reactions". In some countries, these reports are collected by a central agency, where they form the basis for a monitoring system. In many countries, especially in the developing world, there is no system for the collection of reports of adverse drug reactions, and publication is the most likely method by which reactions might be brought to light.

Individual reports of suspected adverse drug reactions have been a fundamental part of post-registration surveillance of drugs (42-43). This method of reporting has been the most useful of the five post-registration surveillance strategies mentioned above for first drawing attention to unsuspected adverse drug reactions. Evidence from reports of adverse reactions is sometimes sufficient to elicit regulatory action. Most often, however, the notification of a reaction is followed by formal epidemiological (see section 3.2.3) or indirect correlational studies (see section 3.2.4).

The strengths and limitations of the reporting of suspected adverse reactions as a method of drug surveillance have been

discussed in detail elsewhere (42-45). The most serious problem with this method is the difficulty of obtaining accurate data on the incidence of adverse reaction. The reports rarely establish cause and effect, and selective reporting of an effect among users can lead to an exaggeration of the magnitude of the problem. On the other hand, spontaneous reports of adverse drug reactions often provide the first evidence of an association of drug use with a disease, and in many cases constitute the only evidence for an association between an uncommonly used drug and a rare event. In addition, the cost of reporting is low and the reports provide the opportunity for detailed exploration of individual cases.

3.2.2 *Large-scale experimental studies*

In experimental studies, subjects are allocated randomly to a treatment or a comparison group, and the occurrence of disease or adverse reactions after allocation to the two groups is determined. Experimental prospective studies provide the best means for the evaluation of adverse reactions because randomization and the use of controls permits the measurement of both known and unexpected differences between the treated and the comparison group. However, large-scale controlled trials are costly and often difficult to conduct. Even when very large, they may lack the statistical power to detect important but rare adverse reactions. They are seldom continued for a sufficient period of time to study the effect of the drug on carcinogenesis, especially when the issue of latency is considered. Also, for many drugs, it is an ethical impossibility to randomize patients to a "no-treatment" group. When there is randomization to an alternative treatment, the absence of an increase in the risk of adverse reactions may be due to an increase in the risk of the same condition in those who receive the alternative treatment. A finding of an increase in the risk of a condition may be due to a beneficial effect of the alternative treatment.

3.2.3 *Formal epidemiological studies*

Formal epidemiological studies, which comprise an array of techniques including cross-sectional, cohort, and case-control studies, are summarized below. Further details on the conduct of such studies can be found in various publications (46-54).

3.2.3.1 Cross-sectional studies

Cross-sectional studies examine the relationship between a disease and one or more characteristics in a representative sample of the population of interest at a given point in time. When cross-sectional studies are used for the post-registration surveillance of drugs, groups of individuals are asked about their use of a drug and their health status, which may be assessed by the measurement of appropriate physiological variables. The relationship between use of the drug and the disease or the altered physiological measurement is then determined.

Cross-sectional studies can be conducted easily and cheaply. However, they normally do not provide information on the temporal relationship between use of the drug and an association of the drug with a disease or an alteration of physiology. They are most useful for studying physiological changes (e.g., blood pressure).

3.2.3.2 Case-control studies

Case-control studies compare the history of drug use of persons with a newly diagnosed disease with that of a comparable group of non-diseased persons. With case-control studies it is possible to study even rare diseases (e.g., hepatocellular adenoma). These studies may be the only suitable method for studying the risk of disease where there may be a long latent period between exposure and disease. Case-control studies are usually less costly than other types of formal epidemiological study. On the other hand, it is often difficult to identify a comparison group that everyone considers suitable, which often renders the results of case-control studies controversial. Unless they are population-based, information on general incidence cannot be estimated from them, and, in the absence of information on incidence rates, it is difficult to place the estimated risk from them in perspective. Case-control studies are of limited use when only a small percentage of the population has been exposed. When ascertainment of drug exposure is dependent on interview, there is a serious possibility of bias due to over-reporting of prior drug exposure among the subjects who have the disease or adverse reaction.

3.2.3.3 Cohort studies

Cohort studies are carried out by first identifying a group of individuals who have been exposed to a drug, and usually, but not always, a comparison group of persons not exposed to the drug.

Then the development of the disease over a period of time after exposure is determined, and the incidence of the disease in the exposed and unexposed groups is compared. Cohort studies provide information on the incidence of disease, and they are not subject to recall or information bias. Also, a large variety of diseases can be studied simultaneously in the exposed and unexposed groups. On the other hand, unless based on a linkage of already computerized data sets, these studies are expensive. Differences between the exposed and unexposed groups with respect to factors known to be associated with the incidence of the diseases (e.g., smoking, socio-economic status, level of education, level of nutritional status and occupation) may make the interpretation of differences in disease rates difficult.

3.2.4 *Indirect correlational studies*

Indirect correlational studies involve the evaluation in a given population of trends in incidence, mortality, and/or hospitalization in relationship to the prevalence of use of a drug. When the use of a drug has become widespread, indirect correlational studies can either provide an early alert about the possibility of an adverse reaction, or, more commonly, provide an estimate of the magnitude of a problem. These studies usually use data collected for other purposes, such as death certificates, and they are inexpensive for this reason. However, cause and effect or lack of cause and effect cannot be inferred from such studies. Moreover, results from such studies can be misleading when, for example, increasingly widespread use of a drug is superimposed on changes in incidence or mortality from a disease for reasons other than drug use.

3.2.5 *Investigations of outbreaks*

The investigation of outbreaks involves the detailed study of events that are clustered in time and geographical location. This method has been used mainly in the study of infectious diseases and congenital malformations. Applied to post-registration surveillance of drugs, it involves the examination of clusters of adverse reactions in users of a drug. Investigations of outbreaks can also provide an insight into factors that modify adverse drug reactions. Such studies can be undertaken cheaply. However, from these studies it is not possible to establish a cause-and-effect relationship with certainty.

3.3 Historical review of post-registration surveillance of contraceptive steroids

Contraceptive steroids, and, in particular combined oral contraceptives, are without doubt the most extensively studied of all human drug exposures. In nearly three decades since steroids were first used for contraception on a widespread basis, there have been thousands of reports and studies about their effects in humans. These have permitted the evaluation of the predictive value of animal studies for human toxicity. Furthermore, a review of the large body of information and literature on post-registration surveillance of oral contraceptives has allowed conclusions to be drawn about the strategies for post-registration surveillance that have been most successful and about the limitations of these methods as they have been applied in the past. This experience provides a framework for making recommendations about the post-registration surveillance of new contraceptive steroids.

Table 3 lists the effects in humans of combined oral contraceptives that are known with considerable certainty, including the method by which each condition's association with oral contraceptive use was brought to light initially, and the surveillance methods that contributed most prominently to the establishment of an association or lack of it. The next table (Table 4) lists conditions associated with the use of combined oral contraceptives that have been extensively investigated epidemiologically but remain controversial and reported associations that have been inadequately studied epidemiologically.

Several important conclusions can be drawn from Table 3. First, reports of adverse drug reactions have been the starting point for the identification of almost all of the adverse effects of combined oral contraceptives. For example, case reports of venous thromboembolism, stroke and acute myocardial infarction in women taking oral contraceptives were published within several months of marketing of these drugs (55). Likewise, the association of oral contraceptives with gallbladder disease and with benign hepatocellular adenoma were noted first in reports to drug regulatory agencies or in publications (56). On the other hand, not all reports of adverse reactions in oral contraceptive users were confirmed in further studies (e.g., reports of Down's syndrome in pregnancies conceived after the cessation of oral contraceptive use (57)). This

emphasizes the importance of careful confirmation of putative adverse reactions.

Second, Table 3 also shows that the use of combined oral contraceptives has important beneficial effects in humans, in addition to their effect in preventing pregnancy. The use of combined oral contraceptives has been shown to decrease the risk of benign breast tumours (58,59) (and benign uterine tumours (60), see Table 4), ovarian and endometrial cancer (61-64) and pelvic inflammatory disease (65-67). In general, the beneficial effects of combined oral contraceptives have been identified in the search for increased risk of certain conditions (e.g., of benign breast tumours) or by observations in cohort studies (e.g., of benign uterine tumours, see Table 4). Information on benefits of the use of combined oral contraceptives in humans has allowed the risks to be placed in perspective.

Third, Table 3 shows that the case-control approach has been instrumental in confirming and refuting reports of adverse effects of combined oral contraceptives and in establishing associations with decreased risks. The use of this method has made a unique contribution to the identification of sub-groups of users of combined oral contraceptives who are at high risk of adverse effects (e.g., myocardial infarction in heavy cigarette smokers) (68), and to the investigation of questions about increases in the risk of common diseases in numerically important sub-groups of users (e.g., breast cancer in women who use oral contraceptives before their first pregnancy) (69).

In addition to studies of the association of oral contraceptives with an increase or decrease in the risk of specific conditions, post-registration surveillance has been used to evaluate the risks of contraceptives as a function of estrogen and progestogen dose, effecting reformulation of combined oral contraceptives to make them safer. For example, Inman et al. used information on the estrogen dose and progestogen type and dose among cases of cardiovascular disease in oral contraceptive users reported to the United Kingdom Committee on the Safety of Medicines, in conjunction with data on market share of various oral contraceptive preparations to show a relationship between high doses of estrogen and a high risk of venous thromboembolism (70). Soon afterwards, oral contraceptives containing more than 50 micrograms of estrogen were no longer recommended for use in the United Kingdom and elsewhere, and this led to a documented decline in the

Table 3. Effects of combined oral contraceptives (OCs) in humans known with considerable certainty

Type of association	Condition	Surveillance strategy(ies) leading to initial interest in possible association with OC use	Main surveillance method(s) used in confirming association or lack of it
Increased risk	Venous thromboembolism	ADR*	case-control studies
	Thrombotic stroke	ADR	case-control studies
	Acute myocardial infarction	ADR	case-control studies
	Haemorrhagic stroke	ADR, cohort studies	case-control studies
	Benign liver tumours	ADR	case-control studies
	Malignant liver tumours	ADR	case-control studies
	Impaired lactation	ADR	cohort studies
	Delayed return of fertility	<i>a priori</i> interest	cohort studies
Physiological alteration dependent on formulation	Glucose tolerance	<i>a priori</i> interest	cross-section studies, cohort studies
	Lipids	<i>a priori</i> interest	cross-section studies, cohort studies
	Blood pressure	ADR	cross-section studies, cohort studies
No increase or decrease in risk	Breast cancer (overall)	<i>a priori</i> interest	case-control studies
	Pituitary tumours	ADR studies, correlation studies	case-control studies
	Birth defects (overall)	ADR, <i>a priori</i> interest	cohort studies, case-control studies
	Down's syndrome	ADR	case-control studies, cohort studies
	Diabetes mellitus	cross-sectional studies	cohort studies
Decreased risk	Benign breast disease	observation in clinical trials	case-control studies
	Benign functional ovarian cysts	cohort studies, <i>a priori</i> interest	case-control studies
	Ovarian cancer	<i>a priori</i> interest	case-control studies
	Endometrial cancer	<i>a priori</i> interest	case-control studies
	Pelvic Inflammatory disease (PID)	observation in case-control studies of IUDs and PID	cohort studies
	Iron deficiency anaemia	cohort studies	cross-sectional studies
	Dysmemorrhoea/ menorrhagia	<i>a priori</i> interest	cross-sectional studies, cohort studies

*ADR - Adverse drug reaction

Table 4. Conditions reported in epidemiological studies to be associated with the use of combined oral contraceptives

1.	Effects that have been extensively studied epidemiologically, but still remain controversial.	
	Breast cancer in sub-groups	
	Cervical cancer	
	Rheumatoid arthritis	
	Malignant melanoma	
	Gallbladder disease	
	Depression	
2.	Putative effects that have not been adequately investigated through epidemiological studies:	
	<i>Adverse effects</i>	<i>Beneficial effects</i>
	Haemolytic uraemic syndrome	Thyroid disease
	Malignant hypertension	Peptic ulcer disease
	Mesenteric vein thrombosis	Pre-menstrual syndrome
	Renal vein thrombosis	Benign uterine tumours
	Retinal vein thrombosis	
	Budd-Chiari syndrome	
	Crohn's disease	

rate of venous thromboembolism in users of combined oral contraceptives (71).

In another case, post-registration surveillance of sequential oral contraceptives resulted in their being withdrawn from the market in the USA and elsewhere. In sequential oral contraceptives, estrogen is given for a period of 15 days, followed by 5 days of combined estrogen/progestogen, followed by 7 days of tablets not containing any hormone. This hormone regimen contrasts with that used in combined oral contraceptives, in which an estrogen and a progestogen are taken in combination for 21 days, followed by a 7-day hormone-free interval after which the cycle is repeated. The effectiveness of the sequential oral contraceptive was shown in large-scale trials to be similar to that of combined oral contraceptives. These preparations underwent extensive safety testing in animals, and in neither animals nor women who participated in the Phase I-III clinical trials was there any suggestion of an increased risk of any condition. Their use was approved by the FDA, and they were marketed in the United States beginning in 1965. In about 1972, there were isolated reports of endometrial cancer in women using sequential oral contraceptives (72). An epidemiological case-control study of endometrial cancer, published in 1980, found a 4-fold increase in the risk of the disease in users of sequential, but not of combined oral contraceptives (61).

The experience with sequential oral contraceptives has taught three lessons. First, it shows once again that animal studies cannot be relied upon entirely to predict risks in the human. Second, it shows that the use of contraceptive steroids can alter the risk of cancer in a short period of time following their introduction and widespread use. Third, and most importantly, in the presence of high risks and a reasonably workable system for collecting and following up on reports of adverse drug reactions, important associations of drug use and side-effects can be found.

Past experience with post-registration surveillance of contraceptive steroids, however, has not been a complete success. Case reports and results of inadequately designed studies have sometimes been widely publicized and have caused anxiety about the possibility of excessive risks of oral contraception. Case-control studies have been used extensively to estimate the risks of oral contraceptives, but they have been used at times in such a way that the risk estimates derived from them have failed to provide information on the absolute incidence of the adverse effects studied. The outcome has been that adverse effects of little real public health consequence have sometimes appeared to be of great concern. For example, the relative risk of benign hepatocellular adenoma in long-term users of high-dose combined oral contraceptives was estimated in one case-control study to be over 500 (73). The absolute probability of a long-term oral contraceptive user suffering from this particular condition as a consequence of using the drug is about 3 per 100 000 users, a fact not evident from the estimate of risk derived from the case-control study.

In contrast to combined oral contraceptives and despite its widespread use in several countries for a number of years, the possible adverse effects of depot medroxyprogesterone acetate (DMPA) have only recently begun to be studied adequately. The deficiency of high-quality information about risks and benefits of DMPA use in humans has led in some countries to a reliance on the results of toxicology studies in animals for the purposes of policy decisions about the use of DMPA for fertility regulation in humans.

3.4 Recommendations for post-registration surveillance of new contraceptive steroids

The most important general recommendation that can be made about post-registration surveillance of new contraceptive steroids is

that an explicit plan for carrying out such surveillance should be made in advance of their widespread use so that timely information on their effects in women will be available to individuals and to policy-makers. Manufacturers, government agencies, and international organizations should share the burden of responsibility for funding and initiating these activities. Post-registration surveillance should be an integral part of plans for the introduction of new contraceptive steroids in settings where the infrastructure to carry out such surveillance exists.

In planning a strategy for the post-registration surveillance of a new contraceptive steroid, the following general points must be considered. First, since contraceptive steroids are likely to be used by a large number of healthy people, any change in the risk of diseases of high incidence resulting from these drugs will be of great significance in public health terms. Thus, studies must be designed such that even small changes in risk are detected. Second, as hormonal steroids are thought to be related to a risk of neoplasia in the main target organs, the planned studies should address this concern. Third, past epidemiological studies have shown combined oral contraceptives to be associated with the risk of a variety of conditions (e.g., cardiovascular disease). Plans must, therefore, include studies of a possible association with the previously documented conditions. Fourth, post-registration surveillance of new contraceptive steroids should aim to ascertain the benefits as well as the risks of use. Finally, epidemiological studies that permit the estimation of absolute risks, and not just relative risks, should be carried out.

Within this general framework, post-registration surveillance of contraceptive steroids should continue to use an array of complementary strategies, including the collection and use of adverse reaction reports, formal epidemiological studies, and indirect correlational studies. It is recommended that all the strategies described in the following paragraphs be pursued, although it is acknowledged that in many countries only some of these will be feasible.

The first post-registration surveillance method that becomes applicable once a drug is licensed is adverse reaction reporting. Owing to the high cost and difficulty of assigning women and men to different contraceptive regimens chosen at random, it seems unlikely that large-scale randomized studies of the effects of new contraceptive steroids will be undertaken. Cohort studies can also be

started at an early stage, especially if drug use is concentrated in certain institutions or a particular locality. Prevalence studies, case-control studies, and correlational studies, which relate changes in the prevalence of drug use to vital statistical trends, become feasible only when a drug is being used by an appreciable proportion of the population.

3.4.1 *Spontaneous reporting of adverse reactions*

Spontaneous reports will continue to play a role in the identification of suspected adverse reactions to new contraceptive steroids. Their usefulness can be improved if: (a) there is more standardization of the forms on which reports are made; (b) all reports contain a small core of information about the reaction; and (c) there is a freer exchange of information on adverse reactions. It would be advantageous if the mechanism of adverse reactions could be determined and susceptible individuals identified.

Even in countries that do not have the infrastructure for monitoring adverse reactions to drugs in general, it might be possible to set up a scheme that would monitor adverse reactions to a new contraceptive steroid. Efforts should be made to facilitate the development of limited and perhaps short-term adverse reaction monitoring schemes in countries where a new contraceptive steroid is to be used on a widespread basis.

When training health personnel in the use of new contraceptive methods (e.g., insertion of a contraceptive implant system) the training sessions should be used to educate health care providers about how to report suspected adverse reactions. For example, standardized forms for reporting adverse reactions could be distributed along with instructions on how and where to report reactions.

3.4.2 *Formal epidemiological studies*

3.4.2.1 Cohort studies

The identification and follow-up of cohorts of drug users is generally difficult. Questions have been raised about the possibility of conducting cohort studies successfully in developing countries. Furthermore, these studies are expensive except when they involve computerized record linkage.

In spite of these problems, cohort studies of users of new contraceptive steroids will probably be of interest, and the feasibility of conducting them should be explored for all new contraceptive steroids. Such studies should aim to rule out the possibility of very high risks of rare diseases in users of the new contraceptive steroid and to test specific hypotheses about increases in the risk of diseases of intermediate incidence which are of importance (e.g., benign breast disease). They should be used to determine the incidence of certain diseases as a reference point for assessing risks even when the risks cannot be measured precisely (e.g., the risks of cardiovascular disease). They should seek to develop an overall picture of the risks and benefits of the new contraceptive steroid. It should be noted, however, that cohort studies should be done only if good follow-up is possible.

The conduct of studies should be preceded by pilot studies to document the possibility of obtaining reasonably complete follow-up of subjects. Commitments to adequate long-term funding of cohort studies should be secured in advance of their initiation.

In addition, opportunities to take advantage of record linkage using existing data as a method for assembling a cohort exposed to a new steroid contraceptive and following them for mortality and morbidity should be exploited. Whenever possible, data bases should be created that can be interlinked by computer and that can be used for the identification and follow-up of cohorts exposed to the drug. Also, whenever a cohort of drug users is identified, information on unique identifiers (e.g., social security number) should be collected with care ensuring that the same unique identifiers can be used in future studies. Adequate safeguards for the protection of confidentiality must be an integral part of these studies.

In cohort studies of new contraceptive steroids, a suitable comparison group not using the new contraceptive steroid should as a general rule be identified. Information on factors that may differ between the contraceptive steroid user and the comparison group and which affect risk of disease (e.g., smoking, age at first pregnancy, parity and socio-economic status) should be collected so that differences in disease rates between the users and the comparison group can be properly interpreted.

Plans to ascertain method discontinuation and "crossovers" (non-users becoming users of a contraceptive steroid and vice versa) should be made. An approach to handling discontinuation and crossovers in the analysis of data should be formulated prior to the

initiation of the study. The number of subjects to be recruited for cohort studies of contraceptive steroids should be decided upon using calculations of the sample size in which differences of a specified magnitude can be detected with reasonable power. It is likely that ten to twenty thousand subjects will be required in each group.

3.4.2.2 Case-control studies

The importance of the case-control approach as an effective method for studying disease risk in drug-exposed subjects has been emphasized above. Case-control studies will play an important role in the post-registration surveillance of new contraceptive steroids once they have been used by an appreciable proportion of the population.

A serious limitation of case-control studies has been their inability to derive estimates of incidence. If the studies are conducted in settings where all the cases in a defined population are included (i.e., population-based studies), estimates of disease incidence and attributable risk can be derived.

Case-control studies have been generally conducted singly. In other words, each has determined the association of one disease with the use of contraceptives. Therefore, a series of separate studies has been required for each disease. The case-control approach would be more efficient if several diseases were studied at the same time by the same methodology.

The timeliness of information on new contraceptive steroids should be improved by anticipating the need to conduct case-control studies of cervical, endometrial, breast and ovarian cancer five to ten years or more after the introduction of the new contraceptive steroid. Based on the anticipated need to conduct case-control studies, work to develop ties with, for example, a tumour registry or hospitals in which patients with these diseases are treated should begin soon after the introduction of a new contraceptive steroid.

Case-control studies can be done quickly and cheaply, but are often difficult to do well. Since proper application of the case-control methodology is so important, epidemiological training programmes in developing countries should focus on the design, conduct, and analysis of data from case-control studies. Every effort should be made to train scientists to conduct properly designed case-control studies of relevance to their own country.

3.4.3 *Indirect correlational studies*

Indirect correlational studies have had limited use in the monitoring of the effects of oral contraceptives. In the context of introduction of new contraceptive steroids, carefully planned studies of trends and diseases known to be of concern, from prior epidemiological studies of oral contraceptives, should be used more extensively. The establishment of national or regional hospital discharge registries and cancer registries, where these do not exist as yet, is encouraged. Such registries would also facilitate the conduct of case-control studies. In the absence of registries, it might be possible to set up a scheme to collect information on all hospitalizations for, say, acute myocardial infarction, venous thromboembolism, and thrombotic stroke in a large urban area the year before and for several years after the introduction of a new contraceptive steroid. If the use of a contraceptive steroid is becoming widespread, then a finding of no change in the rates of hospitalization for these conditions would be reassuring. As with spontaneous reports of possible adverse reactions, indirect correlational studies should be considered only as a rough means of testing and generation of hypotheses, and they should not be expected to substitute for high quality epidemiological studies of the cohort or case-control type.

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