

## Nonclinical Reproductive Toxicity Testing Requirements for Drugs, Pesticides, and Industrial Chemicals in India and China

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### Abstract

India and China have booming chemical, agrochemical, and pharmaceutical industries. Both countries also represent expanding markets for foreign chemical and healthcare companies. All such products require reproductive toxicity testing before marketing. The ICH testing guidelines for medicinal products are not applicable in China and India. Nonetheless, reproductive toxicity studies designed and run to ICH principles are generally acceptable for submission. The Chinese guidelines take into consideration traditional Chinese medicines, which are usually mixtures. Likewise, the specific recommendations of India and China for the reproductive toxicity testing of chemicals and pesticides differ from those of the OECD and the USEPA. Again, studies performed in accordance with internationally recognized principles are usually acceptable for submission in both countries. The Chinese guideline for the reproductive toxicity testing of agrochemicals is currently under revision; the new version is expected to resemble more closely the requirements of the OECD and the USEPA. As a member of the OECD, India has conducted Good Laboratory Practice (GLP) inspection, accreditation, and monitoring activities since 2004. China has made several attempts to join the Council Decisions on Mutual Acceptance of Data in the Assessment of Chemicals since 2005. Currently 47 laboratories in China have been certified by the national GLP authorities. Several laboratories in China have also been recently been certified by OECD member countries as GLP compliant. In India, there are currently 23 GLP-Certified laboratories; about six of these are also AALAC accredited. The specific study designs specified in the guidelines of China and India for reproductive toxicity studies are described in detail in this chapter.

**Key words:** Reproductive toxicity, Regulatory guidelines, India, China

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### 1. Introduction

India and China both have booming economies. This is aided by deeply rooted cultural attitudes which do not block the pace of absorbing and learning modern science and technology. Science and culture in India and China have experienced vicissitudes of inheritance from thousands of years of civilization. At present,

India and China also exhibit nascent power and optimism. It is in this climate that the safety testing requirements of new products are evolving in India and China.

The Indian and Chinese chemical and pesticide industries have been at an advanced state since the 1950s. Indian and Chinese chemists have consistently outperformed other countries in coming up with innovative and cost-effective processes for existing drugs and pesticides. However, recently the Indian/Chinese pharmaceutical industry has been attracting prime attention of the global pharmaceutical companies for research, development, manufacture, and marketing of active pharmaceutical ingredients, intermediates, biologicals, and other drug products.

Beginning in the 1970s, the Indian/Chinese chemical, pesticide, and pharmaceutical industries have been producing generic products without impinging on the patents of innovators. Such products were widely distributed around the world. Those products which needed mandatory safety testing have undergone necessary toxicological testing, including teratogenicity, in reputed toxicological labs in the region.

The pharmaceutical development of New Chemical Entities (NCEs) has not yet developed in India and China to the extent that is prevalent in Western countries. However, in the last decade, several pharmaceutical generic manufacturers in the region have been investing part of their resources in the development of NCEs. Even in the Western pharmaceutical companies, falling productivity is perhaps the greatest challenge facing the industry. A few of the pharmaceutical companies in India and China have developed new molecules for a number of therapeutic areas. However, most of them have been licensed out to major Western pharmaceutical companies for further preclinical and clinical developments. It remains to be seen if any of the pharmaceutical companies from developing countries investing modest sums have any chance in coming up with blockbuster drugs in the foreseeable future.

The focus of this chapter is on reproductive testing guidelines in the region. These test guidelines cover the assessment of fertility and other aspects of reproductive function in adults, prenatal developmental toxicity (including teratogenicity), and postnatal growth and development.

Reproductive safety evaluation of small molecule pharmaceuticals is largely governed through the International Conference on Harmonization (ICH), an international organization with representation from industry and government from three key geographic areas, the USA, Japan, and the EU, along with several others as observers. These deliberations resulted in the production of a document entitled “Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility S5(R2)” (1). This is described in more detail in other chapters. Indian and Chinese government agencies, by and large, accept reproductive data generated according to the global standards of the ICH and OECD.

In China, research on reproductive safety assessment is primarily conducted in state laboratories. In addition, there are contract research laboratories which are accredited to conduct regulatory toxicology studies. India in particular has well established GLP testing facilities.

### **1.1. History of Indian Good Laboratory Practice Program**

Prior to the introduction of Good Laboratory Practice (GLP) in India, the Government of India introduced guidance for the conduct of animal studies under the auspices of the Committee for the Purpose of Control and Supervision of Experiments on Animals (2). These guidelines laid the foundation and boundaries for ethical conduct of animal studies in laboratories in India. CPCSEA mandated registration of all animal testing and research facilities and mandated the formation of Institutional Animal Ethics Committee (IAEC) at animal research facilities. Two members of the IAEC committee are nominated by the Government of India, in addition to strict guidance on the make of the IAEC members.

The Government of India has established the National GLP Compliance Monitoring Authority (India GLP), adopting the OECD quality system of the principles of GLP, for inspection, monitoring, and accreditation of test facilities in India for conducting nonclinical and environmental safety studies. Currently, India enjoys the full member status of OECD for GLP. India is a member of the OECD Test Guidelines program.

Currently 23 Indian laboratories have been accredited by the Indian national GLP compliance monitoring authority as per the provisions of the OECD Principles of GLP to conduct nonclinical health and environmental safety studies. The Indian system of GLP inspection, accreditation, and monitoring of test facilities was started in 2004 (India GLP).

### **1.2. Chinese GLP Program**

China has made significant efforts to establish its own GLP inspection program, as well as to increase its competency in conducting toxicological studies. China has made several attempts to join the Council Decisions on Mutual Acceptance of Data in the Assessment of Chemicals since 2005, and has conducted extensive training to make progress in this respect. Currently 47 Chinese laboratories have been certified by the Center for Certification of Drugs of the State Food and Drug Administration (3). There are several regulatory agencies which have established or intended to establish their GLP inspection programs. These include, but are not limited to, Certification and Accreditation Administration of People's Republic of China (CNCA), State Food and Drug Administration (SFDA), Ministry of Agriculture (MOA), Ministry of Environmental Protection (4), and General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ). Over recent years, several laboratories located in China have also been certified by OECD member countries as GLP

compliant. There are over 20 institutes in China that are Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited, which is essential for the institutes to conduct studies that would be accepted worldwide for the safety assessment of chemicals.

### ***1.3. Evolution of Safety Testing Guidelines in India and China***

The test facilities in India and China have evolved over the years and conduct a range of nonclinical safety assessment studies for in-house drug development, and also provide service as contract research organizations (CROs) for sponsors not only from India and China but also from overseas pharmaceutical and agrochemical companies.

The Indian and Chinese regulatory systems for nonclinical safety evaluation of drugs and the safety assessment of pesticides are predominantly similar to the requirements of many other countries. This chapter provides an overview of Indian and Chinese requirements for reproductive testing and relevant quality systems that must be adhered to by organizations involved in drug and pesticide development. Finer aspects of reproductive testing in animals are covered in detail to enable readers to have a comparative understanding of the local requirements with respect to global practices.

The potential reproductive toxicity of chemicals has been studied in both in vitro and in vivo test systems (5). However, in many of these tests a variety of methods were used to assess reproductive toxicity which makes it hard to compare the effects of chemicals from laboratory to laboratory.

Many drugs and pesticides readily cross the placenta to reach significant concentrations in fetal plasma. However, other chemicals cross the placenta less readily and their concentrations are lower in the fetal plasma than maternal plasma (6). In the case of the latter chemicals, the placenta may be exposed to higher concentrations of the chemical than the embryo. Thus, any adverse effects of the drugs on development may result from direct effects to the embryo and fetus or from indirect effects through altered placental function (7).

In order to standardize these tests various government agencies in India and China have come up with their own standards which in general mimic global standards. Due to the time factor and other special considerations involved in promulgating such guidelines, the Indian and Chinese guidelines do differ in some respects with global standards. The Chinese guidelines also took into consideration how to conduct reproductive studies for traditional Chinese medicines (TCM), which are usually mixtures. In this chapter, we provide salient features of regulatory agencies and their guidelines both in India and China. Where possible we will attempt to compare with global standards.

## 2. Safety Assessment of Agricultural and Industrial Chemicals

### 2.1. India

All new pesticides in India must be approved by the Registration Committee constituted under Section 5 of the Insecticide Act, 1968, which empowers the Registration Committee to regulate its own procedure and the conduct of business. The registration of a pesticide is granted after demonstrating its “efficacy and safety to human beings and animals.” Developmental and reproductive toxicity testing are integral to human hazard assessment. The onus of submitting the required scientific data for the registration of the product rests with the importer/manufacturer/formulator.

Due to lack of any formal guidance from the Government of India, the Registration Committee convened a special meeting with the members of Pesticide Association of India (PAI), Pesticide Formulators Association of India (PFAI), and National Alliance of Young Entrepreneurs (NAYE) on 24th June 1977 to discuss the progress and problems faced by the Pesticide Industry in generating the required safety data, including reproductive assessment. Consequently, a committee of experts was formed under the Chairmanship of Dr. B.B. Gaitonde, the then Director of the Haffkine Institute, including representatives from the three associations, to consider and recommend requirements for the registration of pesticides.

The Gaitonde report stated that the conduct of toxicity tests, including reproductive toxicity, should be in accordance with the currently accepted principles of toxicity testing and statistical analysis/procedures laid down by the national and international organizations.

Among the reproductive tests recommended by the Gaitonde guidance include the following:

1. Effect of pesticides on reproductive processes
  - (a) Effect of pesticides on fertility and general reproductive performance—Segment I
  - (b) Teratology Study in rats—Segment II
  - (c) Effect of pesticides on suckling and lactating dams—Segment III
  - (d) Three-Generation Reproduction Study with Albino Rats

Each of the above studies is described in detail below:

1. *Effect of pesticides on reproductive processes*
  - (a) *Segment I: Effect of pesticides on fertility and general reproductive performance.*

Species	Rats      Weight: Males 250 g, females 200 g	
	No	Duration of medication before mating
Females		
Control	30	14 days
Low dose	30	14 days
High dose	30	14 days
(Maximum tolerated but asymptomatic)		
Males		
Control	10	60 days
Low dose	10	60 days
High dose	10	60 days
(Maximum tolerated but asymptomatic)		

Exposed males and females are mated in a 1:3 ratio and kept together until fertilization is proved by the presence of sperm in the vaginal tract (Day 0). Females are then kept in separate cages and medication continued during gestation and lactation. One-half of the females are sacrificed on gestation Day 13 of their respective pregnancies, and uteri examined. The remaining dams are continued on pesticide and allowed to litter normally. The litter size, number of viable and stillborn fetuses, and gross anomalies are recorded. The growth and survival of the young should be recorded up to weaning (postnatal day 21). The medicated males are to be sacrificed after pregnancy has been established, and the tests are preserved for histopathology.

*(b) Segment II: Teratological study:*

Species	Rats
Number of animals/group	20
Route	Oral
Duration of treatment	Day 6 through Day 15 of pregnancy
Autopsy	Day 20 of gestation
Dosage	Control: Treated with Vehicle
Low dose	Low dose not likely to produce toxicity
High dose	Maximum tolerated asymptomatic dose on subacute rat studies

Rats are sacrificed on gestation day 20. Fetuses are removed by cesarean section after noting the number of resorptions, implantations, and normal fetuses. The size, weight, and any abnormality of each fetus are noted. Two-thirds of the fetuses are eviscerated and then preserved in absolute alcohol for staining with Alizarin Red S for skeletal assessment. The other one-third of the fetuses is fixed in Allen's modification of Bouin's fluid for slicing with a razor blade (Wilson's Technique) to evaluate visceral anomalies.

(c) *Segment III: Effect of pesticide on suckling and lactating dam*

Species	Rats
Number of animals/group	20
Duration	Day 15 of gestation through Day 21 (weaning 3 weeks post partum)
Route	Oral
Observation	Weight gain and feed intake of dams. Onset of labor and delivery, Dystocia, prolonged and delayed labor, litter size pup weight, survival rate at birth and on 4, 14, and 21 days post partum

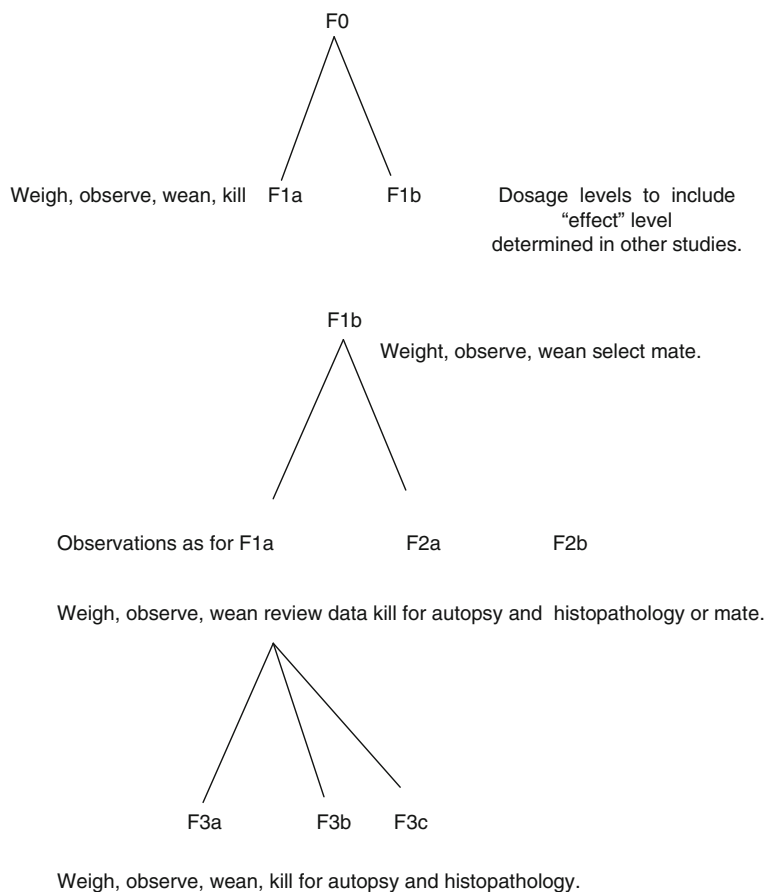
(d) *Three-Generation Reproduction Study with Albino Rats:*

Generation	Gr. No.	Number of animals		Dietary level (ppm)
		M	F	
F <sub>0</sub>	1	8	16	Control
	2	8	16	Low
	3	8	16	Medium
	4	8	16	High
F1 b	Same as F0			
F2 b	Same as F0			
Age	100 days old			
Mating	1 M:2 F			

Observations on mating and offspring same as Seg.1. Rep. studies.

F1a litters will be Weaned at 21 days post partum and killed. The females will be given 10 days rest and again mated; the above procedure is repeated to obtain F1b weanlings.

The procedures followed for the second and third generations will be identical to those described for the first generation except that the parental animals will be selected from F1b weanlings. Parents for F3 generation will be selected from F2b litters.

*Observations:*

Body weight and food consumption recorded weekly.

*Pathology:*

(a) Parental animals:

F2b and F3b—Histopathology on all tissues as in 90 days subacute oral toxicity.

Many universities and scientific institutes operated by the Government of India offer educational, training, and research opportunities in basic and applied sciences. The foremost among them are the Central Drug Research Institute (CDRI) and Indian Institute of Toxicological Research (IITR). In addition, several institutes of the Indian Council of Medical Research (ICMR), e.g., the National Institute of Nutrition, offer training and research opportunities. These institutes focus on the fields of drug research

and development, including safety evaluation/toxicity testing. The scientific personnel of these institutes in the fields of chemistry, biology, and veterinary sciences formed the backbone of 15 GLP-accredited facilities for the safety evaluation and testing of chemicals, pharmaceuticals, veterinary drugs, agrochemicals, etc. in the country (India GLP) (<http://indiaglp.gov.in/TestFacilities.html>).

## 2.2. China

Similarly, in China, all pesticides have to be registered prior to manufacturing activities and use. The competent authority is the Inspection and Control of Agrochemicals, Ministry of Agriculture, known as “ICAMA.” Data requirements for registering a pesticide active ingredient or formulated product are both described and published on the “China Pesticide Information Network” Web site (<http://www.chinapesticide.gov.cn/>, in Chinese language). Reproductive and developmental toxicology information is needed for registrations of new active ingredients. One major difference between reproductive toxicity studies for pesticides and pharmaceuticals is that a more comprehensive two-generation reproductive toxicity is required for pesticides versus one-generation, or segment I, II, and III repro-tox studies required for pharmaceuticals. Study guidelines are available for the safety evaluation of pesticides in China (GB15670-1995), published in August 1995, entitled “Toxicological Test Methods of Pesticides for Registration” (8). This document issued by the National Technology Supervision Bureau became effective from Jan 1st of 1996 and comprised a national standard composed by area experts, led by the Ministry of Agriculture and Ministry of Health. The guideline GB15670-1995 is still in use and serves as the main reference for toxicological studies for pesticide registration in China. This guideline differs from other international guidelines, e.g., OECD, in several respects and has been under revision since 2007. A new version is expected to be published by the Ministry of Agriculture and National Standardization Management Bureau upon approval and will include more up-to-date developmental and reproductive study guidelines, similar to those of the current OECD and USEPA guidelines.

In both GB15670-1995 and the upcoming new national standard, two study guidelines are most relevant to developmental and reproductive toxicology studies: the teratology study guideline (GB15670-1995.15 or GB15670.23, first revision), and the two-generation reproductive toxicology study guideline (GB15670-1995.16 or GB15670.24, first revision). Notably, the current guideline differs somewhat from the OECD and USEPA guidelines with respect to the requirements for test animals, dose selection, animal grouping, pathological observations, etc. However, we will not attempt to describe the current guideline in detail since it is due to be replaced shortly.

All new chemical substances to be imported, manufactured, or used in China will be subject to the New Chemical Substance Notification (NCSN) administered by the Ministry of Environmental Protection, China (MEP). The former MEP, State Environmental Protection Agency (SEPA), issued SEPA Order 17, “New Chemical Substances Environmental Management Measures,” which became effective in 2003. SEPA Order 17 was revised, and was replaced by MEP Order 7, on 15th October 2010 (9). Under the regulatory framework described above, safety assessment of new chemical substances needs to be made prior to importation, manufacturing, and use inside China. Detailed data requirements for NCSN are described in the “New Chemical Substance Notification Guideline” and amendments (NCSN Guideline). According to the guideline, a reproductive screening test is required for notification level 10–100 ton/annum, and two-generation reproductive toxicity and teratology tests are required for notification levels greater than 100 ton/annum. “The Guidelines for the Testing of Chemicals” (10) published in Chinese by MEP include study designs for reproductive toxicity tests which are similar to those of the OECD and USEPA and will not be discussed further.

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### 3. Nonclinical Safety Assessment of Pharmaceuticals

#### 3.1. India

The Central Drugs Standard Control Organization (CDSCO), under the Directorate General of Health Services, Ministry of Health and Family Welfare, also called the Drug Controller General of India (DCGI), has the statutory responsibility of approving new drugs. Prior to any clinical trials or for marketing, DCGI reviews all preclinical data, which includes testing on reproduction and teratogenicity. Several advisory committees, like the Drug Technical Advisory Board, Drugs Consultative Committee, and Toxicology Panel, assist the Drug Controller. The ICMR ([www.icmr.nic.in](http://www.icmr.nic.in)) and Department of Biotechnology ([www.dbtindia.nic.in](http://www.dbtindia.nic.in)) Web sites provide information about additional guidelines and links to various medical research institutes.

The drug development with respect to reproductive risk assessment process in India is principally no different from that in other countries.

The Indian system is taking all necessary initiatives to strengthen the office of the DCGI to support new drug discovery and development. The Indian regulatory system is also working closely with other regulatory agencies, such as the US Food and Drug Administration (FDA) for strengthening diverse areas of drug development, manufacture, and marketing. Also, the US FDA opened India Offices of International Programs in New Delhi and Mumbai in 2008 and 2009. The India Office of the US

FDA engages proactively and consistently with Indian regulatory counterparts and industry representatives to better accomplish the FDA's domestic mission to assure the safety, efficacy, and quality of FDA-regulated products.

Reproductive testing of drugs is covered under Schedule Y of the Indian Drugs and Cosmetics Act and its amendments on the general principles for animal (nonclinical) toxicology studies (9). Schedule Y provides regulatory requirements and guidelines for toxicity studies, which should comply with GLP. Key requirements of Schedule Y are the following: (i) such studies should be performed by suitably trained and qualified staff; (ii) they should employ properly calibrated and standardized equipment of adequate size and capacity; (iii) studies should be done as per written protocols with modifications (if any) verifiable retrospectively; (iv) SOPs should be followed for all managerial and laboratory tasks related to the studies; (v) test substances and test systems (in vitro or in vivo) should be properly characterized and standardized; and (vi) all documents belonging to each study, including its approved protocol, raw data, draft report, final report, histology slides, and paraffin tissue blocks, should be preserved for a minimum of 5 years after marketing of the drug. Approval for recombinant products is granted by the Biotechnology Department and its advisory committees, like the Institutional Biosafety Committee (IBSC—<http://dbtbiosafety.nic.in/>) on Genetic Manipulation (GM) and Genetic Engineering Approval Committee—GEAC.

The recent amendment in November 2008 to the drugs and cosmetic rules (11) includes a new Schedule L-1, which covers the “Good Laboratory Practices and Requirements of Premises and Equipments,” primarily for laboratories that support manufacture. Despite such specific emphasis of its applicability, the details of GLP requirements are in general alignment with those of the OECD, USFDA, USEPA, etc.

The Schedule Y (12) of nonclinical safety evaluation also describes application of GLP for the animal studies to be conducted in an accredited laboratory.

#### Animal Toxicity Studies as mandated by Clinical Phases

The Indian regulatory system requires submission of reproductive toxicity study data in animals in support of Phase II and Phase III clinical trials. Table 1 provides all preclinical data that are required for the various phases of clinical studies.

In addition, certain special toxicity studies (Table 2) are mandated by the Indian regulatory system as part of application submission to the DCGI for review and approval of each of the clinical phases.

Animal toxicity data generated in other countries may be accepted in India, and may not need to be repeated in India, depending upon the quality of data and the accreditations of the laboratories where the data were generated.

**Table 1**  
**Nonclinical toxicity testing and safety evaluation data of an IND needed for the conduct of different phases of clinical trials**

Clinical phase	Requirement of safety assessment studies
I	<ul style="list-style-type: none"> <li>i. Single-dose toxicity studies</li> <li>ii. Dose ranging studies</li> <li>iii. Repeat-dose systemic toxicity studies of appropriate duration to support the duration to support the duration of proposed human exposure</li> <li>iv. Male fertility study</li> <li>v. In vitro genotoxicity tests</li> <li>vi. Relevant local toxicity studies with proposed route of clinical application (duration depending on proposed length of clinical exposure)</li> <li>vii. Allergenicity/hypersensitivity tests (when there is a cause for concern or for parenteral drugs, including dermal application)</li> <li>viii. Photo-allergy or dermal phototoxicity test (if the drug or a metabolite is related to an agent causing photosensitivity or the nature of action suggests such a potential)</li> </ul>
II	<ul style="list-style-type: none"> <li>i. Provide a summary of all the nonclinical safety data (listed above) already submitted while obtaining the permissions for Phase I trial, with appropriate references  In case of an application for directly starting a Phase II trial—complete details of the nonclinical safety data needed for obtaining the permission for Phase I trial, as per the list provided above, must be submitted</li> <li>ii. Repeat-dose systemic toxicity studies of appropriate duration to support the duration of proposed human exposure</li> <li>iii. In vivo genotoxicity tests</li> <li>iv. Segment II reproductive/developmental toxicity study (if female patients of child-bearing age are going to be included)</li> </ul>
III	<ul style="list-style-type: none"> <li>i. Provide a summary of all the nonclinical safety data (listed above) already submitted while obtaining the permissions for Phase I and II trials, with appropriate references.  In case of an application for directly initiating a Phase III trial—complete details of the nonclinical safety data needed for obtaining the permissions for Phase I and II trials, as per the list provided above, must be provided</li> <li>ii. Repeat-dose systemic toxicity studies of appropriate duration to support the duration of proposed human exposure</li> <li>iii. Reproductive/developmental toxicity studies</li> <li>iv. Segment I (if female patients of child-bearing age are going to be included)</li> <li>v. Segment III (for drugs to be given to pregnant or nursing mothers or where there are indications of possible adverse effects on fetal development)</li> <li>vi. Carcinogenicity studies (when there is a cause for concern or when the drug is to be used in humans for more than 6 months)</li> </ul>
IV	<ul style="list-style-type: none"> <li>i. Provide a summary of all the nonclinical safety data (listed above) already submitted while obtaining the permissions for Phase I, II, and III trials, with appropriate references  In case an application is made for initiating the Phase IV trial, complete details of the nonclinical safety data needed for obtaining the permissions for Phase I, II, and III trials, as per the list provided above, must be submitted</li> </ul>

**Table 2**  
**Special toxicity studies required for clinical trials in different phases**

Toxicity study	Human clinical trial phase	Specific requirements
Male fertility study	I, II, III in male volunteers/patients	
Female reproduction and developmental toxicity studies	II, III involving female patients of child-bearing age	Segment II studies in 2 species
	III involving female patients of child-bearing age	Segment I study
	III for drugs to be given to pregnant or nursing mothers for long periods or where there are indications of possible adverse effects on fetal development	Segment III study

- 3.1.1. Male Fertility Study

One rodent species (preferably rat) should be used. Dose selection should be based on the results of the previous 14- or 28-day toxicity study in rat. Three dose groups, the highest one showing minimal toxicity in systemic studies, and a control group should be included. Each group should consist of six adult male animals. Animals should be treated with the test substance by the intended route of clinical use for minimum 28 days and maximum 70 days before they are paired with female animals of proven fertility in a ratio of 1:2 for mating.  
Drug treatment of the male animals should continue during pairing. Pairing should be continued until the detection of a vaginal plug or 10 days, whichever is earlier. The number of females becoming pregnant should be noted after day 13 of gestation and fertility indices should be calculated. All the male animals should be sacrificed at the end of the study. Weights of each testis and epididymis should be separately recorded. Sperm from one epididymis should be examined for their motility and morphology. The other epididymis and both testes should be examined for their histology.
- 3.1.2. Female Reproduction and Developmental Toxicity Studies

These studies need to be carried out for all drugs proposed to be studied or used in women of child-bearing age. Segment I, II, and III studies (see below) are performed in albino mice or rats, and segment II studies should also include albino rabbits as a second test species.

(i) *Female Fertility Study (Segment I)*: The study should be done in one rodent species (rat preferred). The drug should be administered to both males and females, beginning a sufficient number of days before mating (28 days for males and 14 days for females). Drug treatment should continue during mating and, subsequently, during the gestation period. Three graded doses should be used; the highest dose (usually the maximum tolerated dose determined from previous toxicity studies)

should not affect the general health of the parent animals. At least 15 males and 15 females should be used per dose group. The control and treated groups should be of similar size. The route of administration should be the same as that intended for therapeutic use.

Dams should be allowed to litter and treatment should continue up to weaning of the pups. Observations on body weight, food intake, clinical signs of intoxication, mating behavior, progress of gestation/parturition, length of gestation, parturition, postpartum health, and gross pathology (and histopathology of affected organs) of dams should be recorded. The pups from both treated and control groups should be observed for general signs of intoxication. The pup sex ratio, body weight, growth parameters, and survival rates should be recorded. A gross examination is performed of the pups at autopsy. Histopathology should be performed of any organs of abnormal appearance.

- (ii) *Teratogenicity Study (Segment II)*: One rodent (preferably rat) and one non-rodent (rabbit) species are to be used. The drug should be administered throughout the period of organogenesis, using three dose levels as described for segment I. The highest dose should cause minimum maternal toxicity and the lowest one should be proportional to the proposed dose for clinical use in humans or a multiple of it. The route of administration should be the same as intended for human therapeutic use.

The control and the treated groups should consist of at least 20 pregnant rats (or mice) and 12 rabbits, at each dose level. All fetuses should be subjected to gross examination; one-half of the fetuses should be examined for skeletal abnormalities and the other half for visceral abnormalities. The following should be recorded: signs of intoxication of the dams, body weight, and food intake. The uterus, ovaries, and uterine contents are examined. The numbers of corpora lutea, implantation sites, resorptions, and live fetuses are recorded. Fetal sex, body length, weight, and any gross visceral and/or skeletal abnormalities are noted.

- (iii) *Perinatal Study (Segment III)*: This study is specially recommended if the drug is to be given to pregnant or nursing mothers for long periods or where there are indications of possible adverse effects on fetal development. One rodent species (preferably rat) is used. Dosing at levels comparable to multiples of human dose should be done by the intended clinical route. At least 4 groups (including control), each consisting of 15 dams, should be used. The drug is administered throughout the fetal period of pregnancy (from day 15 of gestation). The dose that causes low fetal loss should be continued throughout lactation and weaning. Dams should then be sacrificed and examined as described below.

One male and one female pup from each litter of the F1 generation (total 15 males and 15 females in each group) should be selected at weaning and treated with the vehicle or test substance (at the dose levels described above) throughout their periods of growth to sexual maturity and during pairing, gestation, parturition, and lactation. Mating performance and fertility of the F1 generation should thus be evaluated to obtain the F2 generation, whose growth parameters should be monitored up to weaning.

The regulatory guidelines adopted for nonclinical safety assessment during drug development are primarily those of the ICH (1). The Indian regulatory system accepts any animal toxicity data generated in other countries as well.

### **3.2. Nonclinical Safety Assessment of Pharmaceuticals, China**

All drugs entering the Chinese market are approved by the state FDA (13). The nonclinical safety assessment process of pharmaceuticals in China is very similar to that of other countries. Most up-to-date data requirements for the approval of new drugs are available on the Web site of Center for Drug Evaluation (CDE) of the SFDA (<http://www.sfda.gov.cn/>).

Reproductive toxicity evaluation in animals is a key content of nonclinical safety evaluation of drugs. Reproductive toxicity is also closely related to acute, chronic, genetic, and other toxicological studies. Before any drug enters into clinical trials, reproductive safety must be evaluated. For drugs to be used in humans, consideration should be given on the most relevant route of administration and desirable effects before designing the reproductive toxicity study. In the drug discovery process, reproductive studies in animals are designed to understand the effects of the test substance on the reproduction and development of the animals. In 1993, China published the original reproductive toxicity test guidance document for drugs. The 2006 version includes the most recent update on reproductive toxicity testing, and was designed based on the many years of practical experiences on reproductive toxicity research and testing in China, and to reach a more internationally harmonized approach. On 19th October 2005, the task force met in the Beijing Science and Technology Meeting Hall to discuss the first draft on “Guidance on the Research and Evaluation of Reproductive Toxicity of Drugs.” Several follow-up meetings were conducted to replenish the first draft and produce a final guidance. In November of 2006, “Guidance on the Research and Evaluation of Reproductive Toxicity of Drugs” was published on SFDA Web site (SFDA Repro-tox Guidance, <http://www.sfda.gov.cn/>). This guidance document became available to guide applicants on how to conduct reproductive toxicity of TCM, natural medicines, and chemical medicines. Taking into consideration the scientific frameworks of ICH, FDA, Japan, and OECD reproductive study guidelines and related documents, the guidance also included

guidance on aspects specific to China. For example, the guidance provides suggestions on overcoming the difficulties in conducting reproductive toxicity studies for TCMs, most of which are mixtures. The guidance document also encourages flexibility in the application of the guidelines; testing laboratories and sponsors are instructed to apply scientific principles and judgment on a case-by-case basis rather than dogmatically following the guidelines to the letter.

*3.2.1. Key Points of the  
“Guidance on the Research  
and Evaluation of  
Reproductive Toxicity of  
Drugs,” China*

- The guidance outlines the common principles of reproductive toxicity testing for TCM, natural medicines, and chemical medicines, but gives advice in separate chapters where necessary.
- Footnotes are adopted and appear in the appendix, to reduce bulky text in the guideline and to be more user-friendly. This is consistent with the ICH guideline.
- The guidance proposes a three-segment strategy, consistent with the ICH guideline applied in other countries.
- The selection of animals is described in detail in the new guidance. For example, two species should be used, primarily rats and rabbits. Reasons should be given if other species are preferred. The minimum number of animals in each dose level is also specified, referring to the ICH notes.
- Integration of toxicokinetic investigations into the reproductive studies is encouraged to better evaluate dose–response relationships, although this is not a mandatory requirement.
- To evaluate the results of reproductive studies, secondary effects due to maternal toxicity should be evaluated, based on evaluation together with other related toxicity studies, e.g., chronic studies. If the reproductive toxicity study shows any positive findings, risk assessment should be carried out regarding the developmental and reproductive risk in humans, based on animal studies and/or clinical trials.
- In China, the commonly recognized three reproductive toxicity segments were called “General Reproductive Toxicity Test,” “Teratology Sensitive Phase Toxicity Test,” and “Pre-natal Toxicity Test” (Segment I, II, and III). The internationally applied ICH guideline uses the names: “Study of Fertility and Early Embryonic Development to Implantation,” “Study for Effects on Pre- and Postnatal Development,” and “Study for Effects on Embryo-Fetal Development.” Previous regional guideline used the Segment I, II, and II nomenclature, which has persisted until today, even though it is not officially used by the ICH. The latest Chinese guidance uses the following names: “Fertility and Early Embryo Developmental Toxicity Test,” “Embryo-fetal Developmental Toxicity Test,” and “Perinatal Toxicity Test,” and also refers to the Segment I, II, and III nomenclature.

- Takayama et al. conducted an extensive literature search (14) to look at the influence of the duration of dosing before mating of male rats, and found that dosing 2–10 weeks prior to mating had consistent toxicity findings. Longer dosing period did not provide more findings on reproductive toxicity. The study also found that mating with females was a relatively insensitive indicator of male reproductive abilities. In contrast, adequate pathological and histological examinations were found to be the most sensitive methods for identifying adverse effects on male fertility. Based on the above findings, the ICH issued an amendment in 2005 (1) to the S5 guideline to allow shortening of the premating period to 2 weeks for both females and males provided that the available general toxicity data does not indicate the cause for concern. Considering the situations in China, and ICH principles, the task force for the guidance suggested a 4–10-week dosing period before mating for male animals, depending on the results found after 1 month of dosing.
- Segment II studies seem to be more important in the risk assessment for human safety. Usually Segment II studies in two species are required to be completed before Phase II clinical trials. Based on situations in China, and to ensure the safety of clinical trials, the guidance divided new chemical drugs into two categories for the different segments of reproductive toxicity studies. Case-by-case considerations will also be given to drugs intended for treatment of special diseases and TCMs.

The authors will not attempt to translate the detailed step-by-step guideline on how to conduct reproductive toxicity studies for drug registration in China, described in the “Technical Guiding Principles on the Reproductive Toxicity of Drugs” in this Book-Chapter, considering that the guidance document is essentially similar to the ICH and other internationally recognized guidelines.

## References

1. ICH (2005) ICH harmonised tripartite guideline detection of toxicity to reproduction for medicinal products & toxicity to male fertility S5(R2). [http://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Safety/S5\\_R2/Step4/S5\\_R2\\_Guideline.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5_R2/Step4/S5_R2_Guideline.pdf). Accessed 30 Jan 2012
2. CPCSEA (1998) Committee for the purpose of control, supervision of experiments on animals, Animal Welfare Division, Ministry of Environment and Forests, Government of India. [http://moef.gov.in/divisions/awd/awd\\_overview.html](http://moef.gov.in/divisions/awd/awd_overview.html). Accessed 29 Jan 2012
3. Dong L, Huang W (2010) Overview of drug evaluation system in China. *Scientific Res Essays* 5:514–518
4. Ministry of Environmental Protection of the People's Republic of China (2010) Ministry of Environmental Protection Order 7—environmental measures on new chemical substances. MEP website <http://www.mep.gov.cn/>. Accessed 29 Jan 2012
5. Golden R, Gandy J, Vollmer G (2005) A review of the endocrine activity of parabens and implications for potential risks to human health. *Crit Rev Toxicol* 35:435–458
6. Pacifici GM, Nattoli R (1995) Placental transfer of drugs administered to the mother. *Clin Pharmacokinet* 28:235–269
7. Beckman DA et al (1990) Experimental manipulation of the rodent visceral yolk sac. *Teratology* 41:395–404

8. National Standard of the People's Republic of China (1995) Toxicological test methods of pesticides for registration, GB 15670-1995. National Technology Supervision Bureau
9. Ministry of Health, Govt. of India (2010) The Drugs and Cosmetics Rules, 1945 (As amended vide GSR 780(E), dated 10 November 2008, w.e.f. 1 Nov 2010)
10. Ministry of Environmental Protection of the People's Republic of China (2003) The guidelines for the testing of chemicals. China Environmental Science Publisher
11. Ministry of Health, Govt. of India (2010) The Drugs and Cosmetics Rules, 1945 (As amended vide GSR 780(E), dated 10 November 2008, w.e.f. 1 November 2010)
12. CDSCO (2005) Central Drugs Standard Control Organization, Ministry of Health, Govt. of India. Schedule—Y, Amendment version 2005, Drugs and Cosmetics Rules, 1945
13. State Food and Drug Administration of the People's Republic of China (2006) Technical guiding principles for the reproductive toxicity of drugs. SFDA website <http://www.sfda.gov.cn/>. Accessed 30 Jan 2012
14. Takayama S et al (1995) A collaborative study in Japan on optimal treatment period and parameters for detection of male fertility disorders in rats induced by medical drugs. *J Am Coll Toxicol* 14:266–292



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