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Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)

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IDENTIFICATION AND ASSESSMENT OF THE EFFECTS OF CHEMICALS ON REPRODUCTION AND DEVELOPMENT (Reproductive Toxicology)

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FOREWORD

Over the past few years the European Chemical Industry Ecology and Toxicology Centre (ECETOC) has published a number of Monographs in which it has attempted to clarify, and express its views on, some of the more important, and complex, problems in toxicology. This Monograph is a further addition to the series.

The potential effects of chemicals, natural and man-made, on reproduction in humans is of interest to the chemical industry, the regulatory authorities and the public. To deal effectively with this problem a sound view of its scientific basis is essential. The chemical industry has developed considerable expertise in identifying, assessing and controlling chemicals which could affect reproduction, and this expertise, plus that of a well-recognised university expert, has been drawn upon in producing this document. It represents the continued determination and commitment of the industry to broaden its knowledge in this field in which it has a significant responsibility for protecting its employees and the general public.

It is with pleasure that I present this Monograph, in the hope and belief that it will prove helpful to those in industry and the regulatory authorities who have responsibilities for the control of chemicals.

H.J. Heller
Chairman, ECETOC Board,
Director, Ciba-Geigy.

A. INTRODUCTION

Reproductive toxicology is that part of general toxicology dealing with adverse effects produced by exogenous agents on reproduction *. Various agents have the potential to cause an adverse effect during any of the stages of reproduction in man. Such agents may affect fertility or interfere with prenatal and/or postnatal development. Normal development is determined by interactions of genetic and environmental factors, and despite its maternal protection the developing embryo can suffer impairment by exogenous biological, physical and chemical agents. For example, disturbances in normal human development may lead to malformations easily recognisable at birth, or to developmental defects (e.g. of the metabolic, cardiovascular, urinary and central nervous systems) which are detectable only a few weeks, months or years afterwards. These late effects are often not included in estimates of the frequency of congenital anomalies. In Western Europe the recorded frequency of spontaneous malformations is between 2.5 and 3 per 100 births. However, because of difficulties in defining and detecting abnormalities, considerable variations in these figures appear in the literature. This subject represents an important social and ethical problem.

The causes of adverse effects on reproduction in humans are multiple and not fully understood. Hereditary factors are undoubtedly responsible for a large proportion of these effects and among the remaining causes are exposure to natural and synthetic chemicals, drugs, radiation, alcohol consumption and infections. However, the magnitude of the contribution of each is unknown, for the reasons given above. Nevertheless the limitation and control of exposure to synthetic chemicals can reduce the risk of adverse reproductive effects. Chemicals which may affect reproduction have first to be identified by testing, and ECETOC therefore set up a Task Force to make a critical assessment of the present situation by considering:

a) on what aspects of reproductive toxicology information is required to enable an evaluation of the hazards, and in what order should these aspects be treated;

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* Some definitions are given in Chapter B and Appendix 1.

b) what is the state of the art of testing for these aspects;
c) what is the relevance of the findings to man;
d) does a no-effect level of exposure exist for reproductive toxic effects;
e) what recommendations can be made to improve the evaluation of the hazards.

The work of the Task Force resulted in this monograph which aims to provide guidance for those scientists who are not familiar with this field, for administrators, and for those working at the interface between industry and government.

It is generally accepted that mutagens and clastogens can adversely affect reproduction, but these genetic effects, as well as transplacental carcinogenic effects, are not considered in this report since these topics deserve separate attention because of their complexity.

B. THE REPRODUCTIVE CYCLE IN MAMMALS

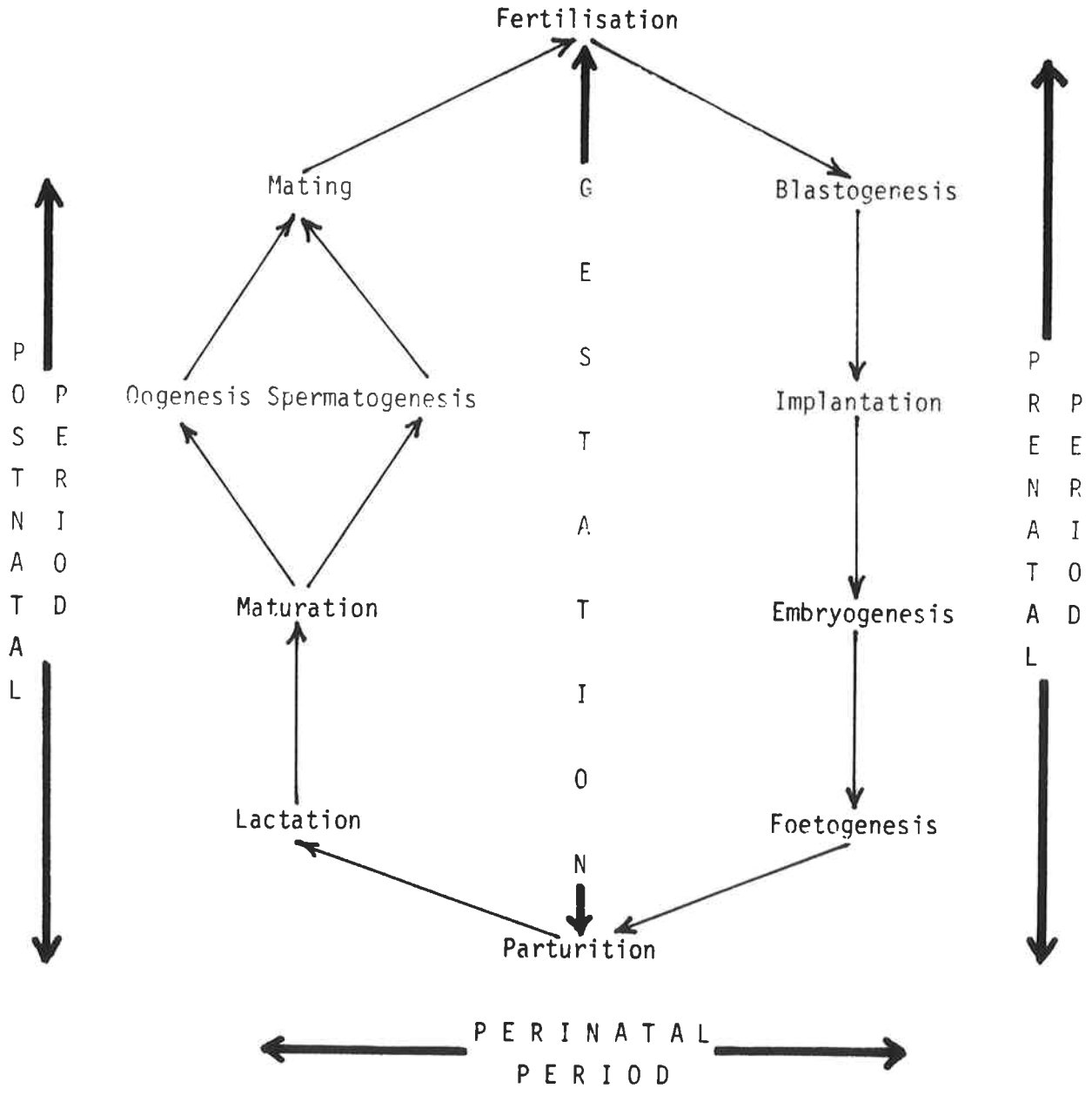
The reproductive cycle in mammals consists of a sequence of inter-related events which are illustrated in Fig.1 .

The various events and periods can be defined in the following way :

- Fertilisation is the fusion of the sperm and ovum resulting in a fertilized egg (zygote).
- Blastogenesis is the process of formation of the blastocyst by serial divisions of the zygote.
- Implantation is the attachment of the blastocyst to the epithelial lining of the uterus, its penetration through the epithelium into the uterine wall and the beginning of its major interaction with the mother.
- Embryogenesis is the period in which the embryonic cells differentiate into the various organ systems (organogenesis).
- Foetogenesis is the period of general growth, organ differentiation and formation of the external genitalia and histogenesis of the central nervous system.
- Parturition is the delivery of the young and marks the end of gestation.
- Prenatal period is that between fertilisation and parturition.
- Perinatal period is that shortly before and after parturition.
- Postnatal period is that which follows parturition.
- Lactation period is the first part of the postnatal life from parturition up to weaning of the offspring.

- Maturation is the period of physical and functional development from weaning up to sexual maturity.
- Gametogenesis is the development of male (spermatogenesis) and female (oogenesis) germ cells.
- Mating involves a complex behavioural pattern leading to the transfer of sperm from male to female.

Fig.1 - Reproductive cycle in mammals



C. INFLUENCE OF EXOGENOUS AGENTS ON REPRODUCTION

Exogenous agents include synthetic and naturally-occurring chemicals, biological agents (such as the rubella and cytomegaly viruses), and physical agents (such as x-rays). All may interfere with and influence the reproductive processes. Some examples of chemicals which are known to have a reproductive toxic effect are dibromochloropropane (male sterility), methylmercury, and some other heavy metal compounds (embryotoxic effects). Effects resulting from exposure to exogenous agents differ with the period of the reproductive cycle in which exposure takes place. To facilitate the description of adverse effects the cycle is divided into three periods :

- formation of the gametes
- gestation
- postnatal life

1. Period of Formation of the Gametes

Adverse effects on gametogenesis may result in reduced fertility, or sterility, due to morphological, biochemical or physiological disturbances. Male germ cells may be reduced in number, there may be a change in morphology or motility, or mutation may occur. In the female there may be a failure to ovulate, and changes resulting from mutations of the germ cells may also take place. Mutations may lead to sterility, death of the embryo after fertilization or the occurrence of abnormalities in the offspring. Impairment of fertilization would result in reduced fertility or, ultimately sterility.

2. Gestation Period

This period may be lengthened, shortened or interrupted leading to stillbirth or abortion. The blastocyst may be destroyed, although less severe damage may be compatible with the development of a normal foetus or may lead to its death in the post-implantation period. Implantation of the blastocyst may also be prevented or delayed.

The obvious structural or functional abnormalities are induced mostly during embryogenesis which is the most sensitive period of development. The embryo may also be killed during this period. About 14 days before menstruation, women ovulate and fertilisation usually takes place within one or two days.

Consequently, when the embryo is 20 days old the menstrual period is only one week overdue and women may thus be unaware of the existence of the embryo at the time when it is highly vulnerable to the effects of exogenous agents.

Interference with development during foetogenesis may also result in morphological changes. The most usual consequences are growth retardation and functional disorders. The external genitalia and the central nervous system (CNS) are also susceptible to injury during this period.

The birth process can be affected, resulting in a difficult, delayed, shortened or prolonged parturition which may be deleterious to the offspring and the mother.

3. Postnatal Period

Exogenous agents may influence the maternal behaviour, hormonal balance and/or nutrition which then may affect the survival and the physical and functional development of the newborn. Adverse effects may also result from the presence of chemicals in the mother's milk, or by direct exposure of the young to the chemical. During the maturation period, previously-induced changes may become obvious as various structural or functional abnormalities (e.g. organic, enzymatic, endocrine, immunological). Exposure to exogenous agents during this period may also impair further development and reproductive performance.

D. SOME CHARACTERISTICS OF THE REPRODUCTIVE TOXIC EFFECTS OF CHEMICALS

In order to identify and characterise the reproductive toxic potential of chemicals, their structural features, mode of action and dose-effect relationships should be considered.

1. Structure-activity Relationships

The chemical structure of the substance under consideration should first be examined for similarities to compounds known to have, or be free from, reproductive toxic potential. It is emphasised that with our present knowledge the reliability of structure-activity relationships in predicting

reproductive toxicity is limited. Structural considerations may, however, assist in developing test strategies and in setting priorities.

2. Modes of Action

Exogenous agents may have a direct or indirect action on reproductive processes. Directly-acting agents may affect the normal development of cells, tissues and organs by alteration or inhibition of cell proliferation, migration and differentiation, or by killing the cells. Indirect interferences, by producing hormonal and metabolic disorders or general intoxication of the mother, or by affecting the sexual activity of parents or the function of the placenta, may also seriously affect reproduction, especially embryonic and foetal development. Although extensive investigations have focussed on the mechanisms of reproductive toxic effects, particularly teratogenic effects, few mechanisms are understood. As there are many ways by which adverse effects can be induced, the characterisation of these will require further scientific endeavour.

The potentially most severe effects would result from mutations in the parental germ cells which are heritable and thus cause deaths or abnormalities in subsequent generations.

3. Dose-effect Relationships and No-effect Levels

In establishing a causal relationship between an agent and observed effects it is essential to demonstrate that there is a corresponding progression between dose and effect. Increasing concentrations or dose levels of a test material generally elicit a corresponding increase in the severity of the effect, and/or the incidence of the effect, and/or the variety of effects. These three types of increase in effect are exemplified by reproductive toxicity studies on salicylates in rats. With an increase in dose an increase in the number of extra ribs and the frequency of their occurrence is seen. At higher dose levels other abnormalities such as omphalocele and/or cranio-rachischisis appear. Likewise, the type of effect may become more severe, e.g. growth retardation may be found at low exposure, and malformation and intra-uterine death at higher exposure levels.

In some cases it may be impossible to establish a clear dose-effect relationship because it is masked by other toxic events. Alternatively, the curve expressing the dose-effect relationship may be steep, so that at lower

levels the effect cannot be detected and at higher levels lethal effects occur, in which case a graduated dose-response relationship cannot be readily established.

An important aim of establishing the dose-effect relationship is to determine the "no-effect level" i.e. that exposure level at which no morphological, physiological or functional modification at any reproductive stage in a given species is detectable by the present methods.

E. CURRENT STATUS OF TESTING FOR REPRODUCTIVE TOXICITY

Various test procedures have been developed for evaluating the effects of chemicals. Some are considered by regulatory and other agencies to be standard and are described in various guideline documents. Other methods have been proposed in the literature but have not been incorporated into current guidelines.

1. Tests in Current Guidelines

The aim of the procedures described in these guidelines is to assess the effects of chemicals on reproduction using lower mammals as a model. In formulating tests it was recognised that it is not practicable to examine the effects of a chemical in each separate stage of the reproductive cycle.

Various legislatory and international organisations have published guidelines for reproductive toxicity investigations (Appendices 2 and 3). The first, which was for testing pharmaceuticals (Fig. 2), was published by the FDA in 1966. . This obviously led to the elaboration of the subsequent guidelines. The first guidelines for testing pesticides and other chemicals were published by the EPA in 1978. The OECD has also published guidelines for industrial chemicals in 1981 and 1983.

The following are considered in more detail (see Appendix 3) :

- the FDA (US), CSM (UK) and MHW (Japan) test guidelines for pharmaceuticals.
- the EPA (US), PSPS (UK) and MAFF (Japan) test guidelines for pesticides.
- the EPA (US) and OECD test guidelines for industrial chemicals.

1.1. Principles

The guidelines have a number of common requirements. They all require chemicals to be tested for their effect on general reproductive performance as well as embryonic development. The preferred species are the rat, mouse and rabbit. Other species may be used when differences in metabolism or toxicokinetics between the preferred species and man have been recognised, or when it is necessary to clarify ambiguous results. The number of animals in each study should be sufficient to permit a statistical evaluation of the results. The test compound should be administered by a route closely equivalent to the typical route of human exposure. Generally, chemicals have to be administered at several dose levels (usually three). The lowest dose should produce no observable adverse effects. An appropriate intermediate dose should also be selected. As far as is possible, the highest dose level chosen should be that which has a marginally toxic effect either on the parent animal or the conceptus, whichever of these doses is the lowest. This will ensure the generation of an adequate number of foetuses for assessing the toxic effects. From the results obtained at these three doses, effects on the offspring which arise from intoxication of the mother may be distinguished from direct effects on the offspring. The OECD recommends an initial limit test with a single dose level of at least 1000 mg/kgbw when a compound has proved to be of low toxicity in other studies. All guidelines require the use of concurrent control groups.

The test procedures can be subdivided into two groups according to the period of the exposure (see Figure 2).

1.2. Exposure during the whole of the reproductive cycle

These studies have been described as "fertility and general reproductive performance" (FDA), "multigeneration" (EPA) or "one and two generation" (OECD) tests. The chemical is administered over at least one spermatogenic cycle and the last stages of oocyte maturation before the parent (P or F_0) generation animals are mated. Exposure of the females is continued after mating but ends at weaning : FDA(US), CSM(UK), EPA (US), PSPS (UK), and MAFF (Japan). It is recommended that some of the females are examined by caesarian section before parturition, either at mid-pregnancy (FDA) or just before term (CSM;

Application Periods in Reproductive Toxicology Experiments for the Rat.

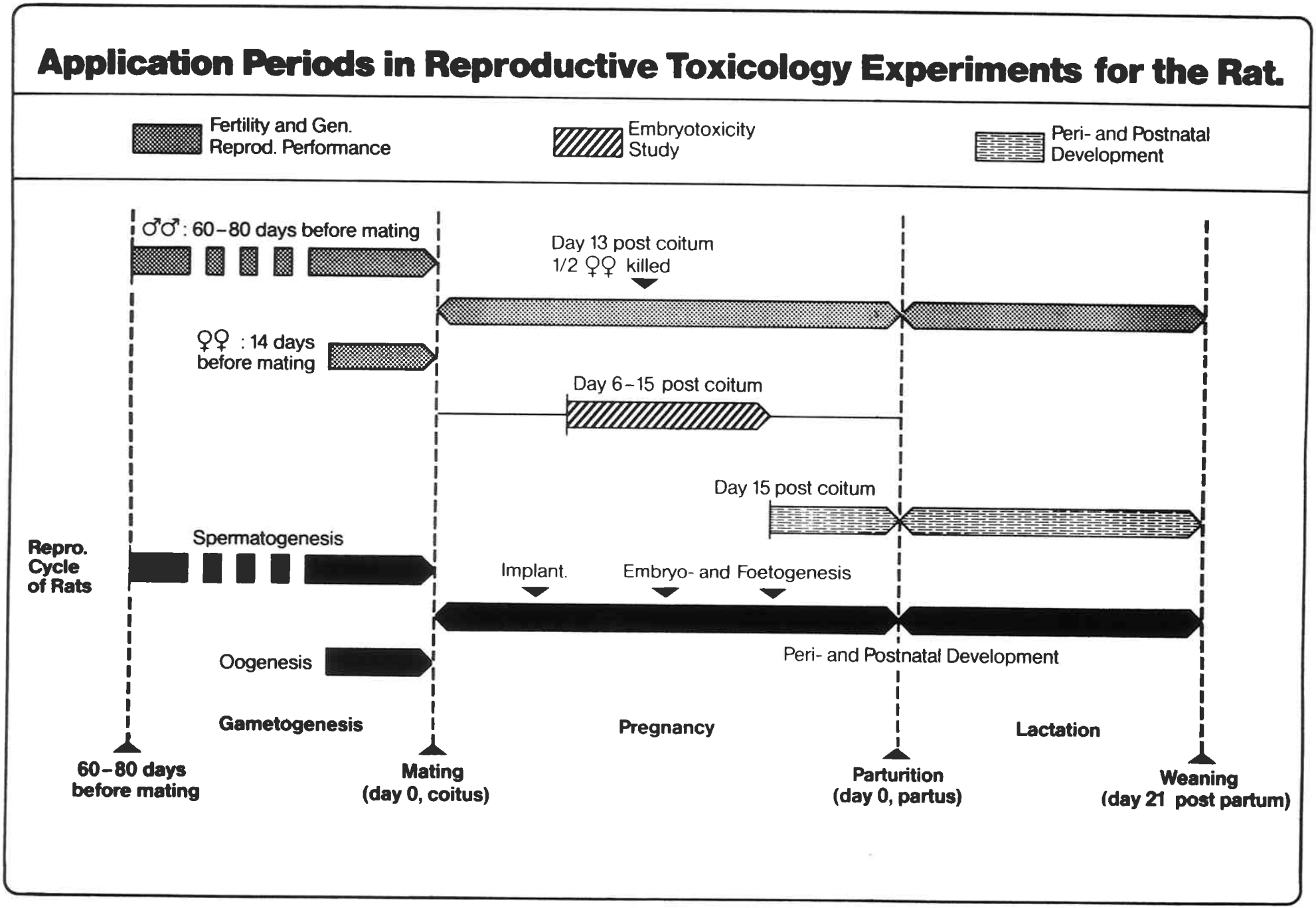


Fig. 2

MAFF). The EPA and OECD require extension of the exposure of the first (F_1) generation until weaning of the second (F_2) generation. The CSM recommend testing the reproductive capacity of the F_1 generation without further treatment. The preferred species are rats or mice. Although not covering the entire reproductive cycle, the fertility study required by the Japanese MHW for pharmaceuticals should be mentioned here. In this study, exposure of the parental animals ends after implantation, all dams being examined by caesarean section just before parturition.

All these studies permit the detection of adverse effects on the fertility and reproductive capacity of more than one generation.

1.3. Exposure during parts of the reproductive cycle.

Conventional studies in this category are usually called "teratogenicity" and "perinatal and postnatal" studies. All guidelines require studies with exposure of the dams to the agent during organogenesis. In teratogenicity studies, pregnant animals are caesarean-sectioned before the expected delivery date. The outcome of pregnancy and the incidence of morphological alterations in the foetuses are recorded. The Japanese guidelines require an additional group which is reared up to weaning. Perinatal and postnatal studies in which the dams are exposed to the chemical during the last third of pregnancy (period of foetogenesis), through parturition and during lactation, are recommended for pharmaceuticals by the FDA (US), MHW (Japan) and CSM (UK). Teratogenicity (MHW,Japan) and perinatal and postnatal studies (MHW; CSM) have to be extended to allow the reproductive ability of the F_1 generation to be assessed.

The preferred test animals include rodents (e.g. mice, rats) and non-rodents (e.g. rabbits). Studies in which the dams are allowed to litter and rear their young are usually carried out with rodents.

The above procedures permit the detection of embryotoxic and foetotoxic effects, e.g. growth retardation, anatomical variations, teratogenicity and lethality. In addition, a rearing group will give information about survival and postnatal development, including functional abnormalities. The development of the central nervous system (CNS) and the differentiation of the urogenital system can be assessed in a perinatal

and postnatal study, as can the survival and physical and functional maturation of the offspring and the effects on labour, delivery, and lactation and behaviour of the dams.

2. Other Techniques

A variety of *in vitro* and *in vivo* techniques are under evaluation, or have been described, but are not incorporated in guidelines. Some examples, the aims of which are to define the adverse reproductive toxic effects with the use of fewer experimental animals and in a shorter time, are given below.

2.1. Male fertility

The analysis of sperm from exposed males for number, morphology, motility of spermatozoa, and viscosity and pH of the ejaculate (seminal fluid) may provide valuable information. The sperm head abnormality test (SHA-test) in rats, mice, and man enables the detection of morphological changes which may impair fertility.

The hamster ova test is an assay for investigating the fertilising ability of spermatozoa from rodents and man.

Chemical interference with the Sertoli cell-spermatogonia interaction may be studied by the use of primary testicular cultures.

Data from other toxicological studies on the weights and histopathology of the reproductive organs can also provide valuable information.

2.2. Female fertility

Observation of the oestrous or menstrual cycles, and the determination of blood hormone levels may give additional information relevant to the interference of chemicals with female fertility.

Examination of vaginal smears can enable changes in the sequence of oestrous/menstrual cycles to be detected.

Ova can be flushed out of the oviduct to check for ovulation and viability.

Data from other toxicological studies, for example on the weights and histopathology of the reproductive and endocrine organs, can provide additional information.

2.3. Embryotoxicity

A number of techniques for detecting adverse effects on the embryonic development of mammals and sub-mammalian species have been described. The chick embryo system has been used mainly to study developmental mechanisms and attempts have also been made to use it as a routine test procedure. Other sub-mammalian test systems, such as the Hydra test and the use of embryonic stages of amphibians and sea-urchins, have also been suggested.

More recently, mammalian screening test systems have been developed. The rodent whole-embryo culture technique permits the cultivation of embryos *in vitro* during early organogenesis. Similarly, the use of embryonic organs or tissue cultures has been suggested.

3. General Comments

The methods recommended in the various guidelines usually permit the successful detection of the effects of chemicals on fertility, and intra-uterine and postnatal development of the test species, including effects on gametogenesis, mating, fertilisation, blastogenesis, implantation, embryogenesis, foetogenesis, parturition, lactation and maturation. It should be emphasised that, even when a chemical has been tested by the most appropriate and sensitive methods a possibility remains that reproductive toxic potential exists but has not been revealed.

Studies in which the dams are exposed to a chemical during the organogenesis phase of embryonic development are often referred to as "teratogenicity studies". These are more correctly designated "embryotoxicity studies" because teratogenic effects are one part of the total spectrum of the embryotoxic effects to be studied. The Task Force considers that in these studies the exposure should be restricted to the period from implantation up to the end of organogenesis. This would prevent adaptations of the maternal organism to the test compound (e.g. enzyme induction) and would avoid the confusion caused by excessive pre-implantation losses. The use of rearing

groups as requested in the Japanese guidelines can give additional information, particularly about functional anomalies.

The main purpose of studies in which pre-mating administration is adopted is to determine effects on gametogenesis, and the treatment can therefore be restricted to one spermatogenic cycle and the last stages of oocyte maturation (60 and 14 days, respectively, in rats and mice). If it is required to detect effects on mating, gestation and lactation, the treatment should be continued up to weaning, which also allows any influence on the prenatal, perinatal and postnatal development of the offspring to be observed. It may be useful to carry out preliminary studies for selecting appropriate dose levels.

To enable the examination of more chemicals with limited resources, the development of short-term tests is essential. However, when evaluating embryotoxicity most of these systems suffer from the limitation that they exclude one essential factor, the maternal-placental-foetal relationship. Thus, for example, a substance administered to a pregnant animal may exert its toxic action on the embryo directly or through a metabolite, and may also act by modifying the maternal metabolism. Another serious shortcoming, especially in organ and cell-culture techniques, is that the compound reaches the target directly, which does not reflect the situation in the whole animal. These considerations therefore make it unlikely that whole-animal testing will be entirely replaced by other approaches.

4. Epidemiology

Observations on people exposed to chemicals give the only unequivocal evidence of a reproductive toxic effect in man. Information may come from random clinical observations or from epidemiological studies (case-control studies or cohort studies). Cohort studies may be retrospective (historical) or prospective. The former are aimed at establishing a relationship between the occurrence of adverse reproductive effects in people and their past exposure to a defined chemical under defined conditions. In a prospective study, a cohort exposed to a chemical is compared with a control group in order to detect any reproductive toxic effect.

Case-studies and retrospective studies have permitted the detection of the teratogenic effects of some exogenous agents such as X-rays, viruses (rubella) and drugs (thalidomide), and of other effects on reproduction

caused by compounds of lead and mercury, and by dibromochloropropane. Epidemiological studies are at present being used to investigate the possible effects on the fertility of male and female workers associated with exposure to certain chemicals.

There are two possible sources of data. The first is medical records on infertility, menstrual history, spontaneous abortion, late foetal death, neonatal death and developmental defects in industrial populations. The reliability of these data is doubtful as they are often incomplete, unstandardised and lacking in adequate information on exposure. When questionnaires are used, specific targets should be set for the collection of information under these headings. The limited categories of information required should be carefully defined before the start of a study according to the objectives of that particular study. Failure to do this leads to the generation of a plethora of useless and confusing data.

The second source is laboratory investigations on the number, morphology and motility of sperm, the biochemistry of semen, vaginal smears, endocrinology, and cytogenetics. It is emphasised that, apart from purely scientific difficulties, such investigations may be limited by the inability to establish the exact exposure at a critical time.

The data on reproductive impairment or developmental defects generated in the study population should be compared with the background incidence in the general population. This may present a serious problem as the existing data on such effects in the general population is often insufficient or unreliable.

For obvious reasons, epidemiological studies cannot be used to assess the potential of "new" chemicals to affect reproduction, and with such substances the only means of identifying a hazard is the use of animal experiments. Prospective epidemiological studies are an important approach for ensuring the adequacy of protective measures taken against a suspected reproductive toxic hazard.

F. SIGNIFICANCE OF EXPERIMENTAL ANIMAL DATA AND THEIR RELEVANCE TO MAN

There are only few instances (e.g. for some pharmaceuticals) where case histories or epidemiological data have permitted the direct assessment of the reproductive toxic potential of a chemical in man. The existence of such data implies that the chemical concerned has already been used for a significant time. For many chemicals, only animal experiments are available for this assessment. Although the present animal test methods appear satisfactory for hazard evaluation in most cases, the extrapolation of experimental results to man can be difficult for reasons discussed in this chapter.

1. Species and Strain Differences

Species and strain specificities resulting from structural and functional dissimilarities, and differences in susceptibility, need to be considered with particular care when assessing the relevance to man of findings in animals. The confidence in these assessments may be increased if information from several species is available.

The differences between various animal species and man in response to a toxic chemical affecting reproduction depend on physiological differences (e.g. metabolism, toxicokinetics, transplacental passage) and anatomical differences (e.g. kind of placenta, uterus, gonads). Experimental factors such as husbandry (diet, stress, etc.), and the health of the parents, are also important. Differences between strains of a particular species are most likely to be due to genetic factors.

There is some evidence that the results obtained in embryotoxicity studies on rodents and lagomorphs (e.g. rabbits) are applicable in many cases to man. Based on experience with drugs, the predictive value of experiments carried out in these species seems to be adequate. In some areas of reproductive toxicity (e.g. male and female fertility) experience is limited, and the information obtained from rodent studies may not be adequate to permit extrapolation to humans with as much confidence.

The parallelism in the reaction of primates, rabbits, cats and man to the teratogen thalidomide is not general but probably incidental, as is the similarity between rodents and humans in their reaction to aminopterin. Nevertheless, taking into account i) the present knowledge about animal

models commonly used, and ii) the insufficiency of data on the reproductive physiology of other species and their response to toxic chemicals affecting reproduction, it is difficult to recommend alternative species to those used at present. Sub-human primates are sometimes used as a third species, especially when doubtful results are obtained from rats or rabbits in embryotoxicity studies. The great similarity in the reproductive physiology (e.g. sexual physiology, hormonal control of pregnancy, placentation) in women and primates makes results from the latter species more reliable when these factors are of importance. Their use will, however, always be limited for economic and ethical reasons.

2. Exposure Conditions

It is desirable to administer a chemical to experimental animals by the route expected or known to correspond to that of human exposure. In special cases other routes of administration, such as intravenous and subcutaneous applications, may be used. The time of treatment (before mating, during pregnancy, etc.) and the duration of treatment should also be as close as possible to that typical of human exposure. The response of animals may differ according to the exposure conditions, depending on the rate and extent of absorption, and the kinetics and metabolism, of the compound. It is preferable that the animal model represents the human situation and that the chemical, after absorption, has the same metabolic fate in the animal as in man. This requirement, however, cannot always be readily fulfilled because there are usually no data available on humans at the time of animal testing.

3. General Considerations

Evaluation of the reproductive toxic hazard of a chemical to man can be made only by those well experienced in this field. Conditions of absolute safety for man cannot be deduced solely from a consideration of animal data on biological effects, in view of the morphological and physiological differences between animal models and man. However, when a chemical has shown no reproductive toxic effects in the most appropriate animal models used, in tests conducted under conditions relevant to human exposure, it can be assumed that the chemical should not pose a hazard to humans.

When reproductive toxic effects are observed in animals exposed to a chemical, the relevance to man should be assessed by taking into consideration the circumstances under which the effects occur, the frequency of the anomalies, the dose-effect relationship and the number of species in which the effects are found. No-effect levels for the species in question should be measured if possible, or deduced. Evidence about the biological mechanism responsible for the development of the reproductive abnormality should be sought.

A critical assessment of all the above evidence, together with that on other aspects of the toxic action of the chemical, by a group experienced in appropriate disciplines is necessary for the establishment of suitable preventative measures, including health-based permissible exposure limits.

G. AREAS DESERVING FURTHER INVESTIGATION

In preparing this report the Task Force identified some areas in which further investigations should be considered.

The efficient protection of humans during the whole reproductive cycle requires a better understanding of the modes of action of those chemicals which adversely affect reproduction. Research in this area would involve collaboration between experts in various disciplines within the scientific community. Research in the following areas should be considered:

1. Adequate systems of data-gathering should be established in exposed and general populations to enable appropriate epidemiological evaluations to be made.
2. Further knowledge of normal male and female reproductive physiology (e.g. hormonal balance, transport phenomena of sperm and eggs, sexual behaviour, etc.) is required to determine the adequacy of the existing test models and, where necessary, to improve them.
3. The mechanisms of action of chemicals producing a reproductive toxic effect should be further investigated, e.g.:

   a) The reasons for species and strain differences in the reproductive toxic response to chemicals, a knowledge of which would assist in selecting the most appropriate test species.

b) The role of parental and foetal enzyme functions and metabolic activities.

c) The role of the placental structure and metabolic abilities in the development of adverse effects in response to chemicals, in the early stages of development corresponding to the maximum susceptibility of the embryo.

d) The relationship between chemical structure and reproductive toxic effects.

e) Mechanisms by which the regulation of gametogenesis, the oestrous and menstrual cycles and sexual behaviour are affected by chemicals.

4. More effort should be devoted to develop and validate rapid short-term tests for predicting toxic effects on reproduction (cf. section E.2).
5. Test guidelines should be internationally harmonised by organisations such as the OECD.

H. SUMMARY AND CONCLUSIONS

Most studies for determining the reproductive toxicity of a chemical have to be conducted with whole animals. Test procedures used to investigate parts or the whole of the reproductive cycle are described in current guidelines. Other techniques, such as *in vitro* methods, and those for investigating specific events in the cycle, are under development. Epidemiological studies can give valuable information, although they are difficult to perform and interpret in practice. There is a need for more epidemiological studies of exposed populations and for recording and quantifying the concentrations of chemicals to which they are exposed.

The Task Force considers that animal experiments should be programmed in a stepwise manner, and should take into account effects seen in previous toxicity studies. The programme of tests for determining reproductive toxic potential should be established on a case-by-case basis, since many factors will influence the choice of studies and the sequence in which they should be performed. The following reasons support such an approach :

a) the conditions of human exposure vary from chemical to chemical;

b) the structure of a chemical may indicate the likelihood of a specific effect;
c) the mode of action may vary from chemical to chemical;
d) there is a diversity of response between species and strains;
e) any one species may be particularly representative of the human situation for a particular chemical;
f) the implementation of a rigid, standardised or obligatory sequence of steps may lead in some cases to the performance of inappropriate studies, while in other cases key studies would not be undertaken.

The Task Force believes that the current test guidelines provide a good framework for testing chemicals for their reproductive toxicity. Other test methods may be useful for defining specific effects revealed by the guideline tests. The results of such studies can be used in assessing the reproductive toxic hazard of a chemical provided that the limitations of the test systems are taken into account. There are differences between certain of the guidelines for which there is no obvious scientific justification and an attempt should be made to resolve these differences.

I. APPENDICES

APPENDIX 1: GLOSSARY OF TERMS

Abnormalities (syn. anomalies, malformations) : persistent structural or functional deviations outside the normal biological variation.

Blastocyst : the embryo at the stage at which implantation occurs.

Dam : a pregnant or lactating female animal.

Dose-effect relationship : the relation between dose and the magnitude of a graded effect, either in an individual or in a population.

Dose-response relationship : the relation between dose and the proportion of individuals responding with a quantal effect.

Epithelium : a cellular tissue lining which covers free surfaces.

Embryo : in mammals, the offspring in the early stages of intra-uterine development (see p.3) (in humans, until the end of seventh or eight week of pregnancy).

Embryo- and Foetotoxicity : toxic effects on the developing embryo and foetus.

Endogenous : originating within the organism.

Exogenous : originating from outside the organism.

Foetus : in mammals, the offspring in the post-embryonic period of intra-uterine development (in humans, after the seventh or eight week of pregnancy).

Gametes : in mammals, the mature gametes are spermatozoa and ova.

General reproductive performance : the ability of male and female partners to generate offspring and to rear them.

Labour : the physical activities involved in parturition.

Menstrual cycle : in higher primates, the periodic preparation of the uterus to receive a fertilised ovum.

No-effect level : the dose at which a toxic effect is not detected in a study.

Oestrous : the recurrent period of sexual receptivity in most female mammals.

Oogenesis : the process of formation of the female gametes (ova).

Organogenesis : the differentiation and formation of the organ systems during embryonic development.

Placenta : the organ by which the developing conceptus is attached to, and communicates with, the mother.

Rearing group : a group of dams allowed to litter and rear their young.

Retardation : delay of the structural or functional maturation of an organism.

Spermatogenesis : the process of formation of the male gametes.

Teratogenicity : a part of foeto- and embryo-toxicity which deals with the specific interference of exogenous agents with intra-uterine development and growth. A chemical substance is defined a teratogen when, after maternal exposure to it, detectable irreversible structural and/or functional abnormalities are caused in the progeny.*

Variation : a divergence within the normal range of structural or functional qualities of an organism.

Zygote : the fertilized ovum.

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* If the doses are so high that toxic effects occur in the mother, the distinction between a direct teratogenic effect and abnormalities in the progeny caused by intoxication of the mother should be taken into account when assessing the hazard to health posed by a chemical (see page 8, line 18).

APPENDIX 2 - LEADING GUIDELINES FOR REPRODUCTIVE TOXICITY TESTING : STAGES OF REPRODUCTIVE CYCLE COVERED

(* see legend)

Guidelines (References see Appendix 4)	Test	Gameto-genesis	Mating	Fertili-sation	Blasto-genesis	Implan-tation	Embryo-genesis	Foeto-genesis	Parturi-tion	Gesta-tion	Lacta-tion	Matu-ration
FDA (1966) USA	Repro.	+	+	+	+	+	+	+	+	+	+	(+)
	Embryo.					(+)	+	(+)				
	Peri/Post							+	+	+	+	(+)
EPA (1979;1982) USA	Repro.	+	+	+	+	+	+	+	+	+	+	+
	Embryo.					(+)	+	(+)				
CSM (1974) UK	Repro.	+	+	+	+	+	+	+	+	+	+	(+)
	Embryo.						+	+				
	Peri/Post							+	+	+	+	(+)
PSPS (1982) UK	Repro I,II	+	+	+	+	+	+	+	+	+	+	+
	Embryo				((+))	((+))	+	((+))				
MAFF (1973) Japan	Repro	+	+	+	+	+	+	+	+	+	+	+
MHW (1975;1982), Japan	Fertility	+	+	+	+	+	(+)	(+)				
	Embryo					(+)	+	(+)	(+)	(+)	(+)	(+)
	Peri/Post							+	+	+	+	+
WHO (1967)	Embryo					(+)	+	(+)				
OECD (1981;1983)	Repro I, II	+	+	+	+	+	+	+	+	+	+	+
	Embryo					(+)	+	(+)				
EEC, draft (1978)	Repro.	+	+	+	+	+	+	+	+	+	(+)	
	Embryo.					(+)	+	(+)				
	Peri/Post							+	+	+	(+)	

* Legend :

Repro	- General Reproductive Performance Study (I,II: one and two generation studies)
Embryo	- Embryotoxicity Study
Peri/post	- Perinatal and postnatal Study
+	- covered
(+)	- partially covered (stage not, or not entirely, treated)
blank	- not covered
((+))	- treatment is through whole pregnancy period

APPENDIX 3 : DETAILED RECOMMENDATIONS OF SOME LEADING GUIDELINES FOR REPRODUCTIVE TOXICITY TESTING

1. FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE (References see Appendix 4)

	MHW (1982)	CSM (1974)	FDA (1966)	EPA (1979,1982)	OECD (1981;1983)
ANIMAL SPECIES	At least 1 (mouse, rat, etc.)	At least 1 (mouse, rat)	Rat	Rat preferred. Can be same as in teratogenicity	Mouse or rat
TOTAL ANIMALS PER DOSE GROUP	At least 20 M and 20 F for mating, if rats or mice	Enough to provide reliable data; at least 24 M and 24 F	Min.: 10 M and 20 F	Rodents: min.20 F pregnant and 10 M	20 M min. 20 F pregnant
NUMBER OF DOSAGES	At least 3 + control. Highest: some toxicity Lowest: no effect Middle: geom.mean	3 + control	Min. 2 + control. Highest dose : maternally subtoxic with no overt effects in dams	Min. 3 + control. Highest dose : maternally subtoxic	3 + control. Highest dose : maternally subtoxic
TYPE OF TEST	Fertility	Fertility	Fertility	Multigeneration test	Multigeneration test
METHOD OF APPLICATION	As clinical application. Forced admin. is superior to feed admix.	As clinical application	As clinical application	Equivalent to typical route of human exposure if possible	As clinical application

M : males
F : females

APPENDIX 3 : DETAILED RECOMMENDATIONS OF SOME LEADING GUIDELINES FOR REPRODUCTIVE TOXICITY TESTING
1. FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE (References see Appendix 4) (continued)

	MHW (1982)	CSM (1974)	FDA (1966)	EPA (1979,1982)	OECD (1981;1983)
DURATION OF TREATMENT	M (min. 40 d. old): 60 d. prior to mating through copulation F(sexually mature) : 14 d. prior to mating to : 6 d. pc (mouse, rabbit), 7 d. pc. (rat). Day 0 = day of successful copulation.	M and F: sufficient time before mating to reveal effects on gametogenesis, and through mating. F: 50% dosing to end gestation. F: 50% dosing to end of lactation.	M (min. 40 d. old): 60-80 d. prior to mating through copulation. F(sexually mature): 14 d. prior to mating and continue to a) d. 13 pc and b) through gest., lact. and weaning	M (min. 40 d. old): 100 d. prior to mating through copulation. F(min. 40 d. old): 100 d. before mating and continue weaning of F1. F1 for 120 d. post-weaning to weaning of F2.	P-M: 8 wks before and and through the mating period P-F: 8 wks before, through mating, gestation, and weaning of pups. F1-M: after weaning through mating period, gestation, and until F2 are weaned.
EVALUATIONS	Hysterectomy of all dams at end of gestation; foetal examination for external and internal anomalies. If mating not successful after 2 wks : treated M + untreated F and untreated M + treated F. Examinations : no. of corpora lutea, pregnancy, foetus mortality, foetus sex., skelet. and viscera. Autopsy of M and F without succ. copul. Parameters in P-animal : BW, food cons., water intake. if necessary: copulation rate, fertility rate.	a) hysterectomy of 50% dams shortly before term. Foetal examination (skeletal and visceral). b) 50% of dams bear and rear pups until weaning; autopsy P-F at weaning. Postnatal development of foetuses assessed (incl. visual, auditory and behaviour). Fertility of F1 determined by producing a F2-gen. M and F from each litter used.	a) Hysterectomy of 50% on d. 13 pc. with examination of uterine contents. b) 50% of dams bear and rear pups until weaning with postnatal evaluation on pups. Fertility of F1 checked by a limited number of pups mated to produce F2. P- and F1 pups not used in mating, are necropsied at end of lactation.	M: P-, F1, F2 measure of spermatogen, and/or histpath. exam. on those used for mating. F: P-dams, litter pups examined and litter parameters recorded. F1: from animals mated to produce F2, 10M and 25 F/ group selected for necropsy and histopath. at end of dosing. F1 and F2: M + F weaning pups/group and histopath. exam. to include organs of reproduction.	F: All dams give birth. F1: 1M and 1F from each litter/group selected to produce F2. F1 animals not selected and P-dams have full necropsy exam. at weaning. F1 dams and F2 offspring necropsied at weaning; physical and behavioural abnormalities recorded, all groups. M: always sacrificed after mating period with full necropsy, especially organs of reproduction.

pc : post coitum, after mating.
P : parent
F1 : first generation
F2 : second generation

APPENDIX 3 : DETAILED RECOMMENDATIONS OF SOME LEADING GUIDELINES FOR REPRODUCTIVE TOXICITY TESTING

2. EMBRYOTOXICITY AND TERATOGENICITY (References see Appendix 4)

	MHW (1982)	CSM (1974)	FDA (1966)	EPA (1979,1982)	OECD (1981;1983)
ANIMAL SPECIES	Min.: 1 rodent (mouse, rat, etc.) and 1 non-rodent (rabbit etc.).	Min.: 2, one being non-rodent; if experiment is to be repeated, use non-rodent and non-lagomorph.	Most often 2, using combination of mouse rat, rabbit.	Min.: 2 mammalian species: rat, mouse, hamster, rabbit. One should be the same as used in the Fertility Study.	Rat, mouse, hamster, or rabbit. Rat and rabbit preferred.
TOTAL ANIMALS PER DOSE GROUP	Mouse, rat : 30 F pregnant rabbit: 12 F pregnant.	Rodent : 12 F pregnant, non-rodent : 10 F pregnant.	Mouse, rat : min. of 10 F pregnant. Rabbit: min. 10 F pregnant.	Rat, mouse, hamster : min. 20 F pregnant. Rabbit: min. 12 F pregnant.	Rat, mouse, hamster : min. 20 F pregnant. Rabbit: min. 12 F pregnant.
NUMBER OF DOSAGES	At least 3 + control Highest: some toxicity. Lowest: no effect. Middle: geom. mean.	3 + control. (highest dose should produce maternal toxicity)	Min. 2 + control (highest dose should be subtoxic and produce no overt symptoms in dams).	Min. 3 + control (highest dose should produce slight maternal toxicity).	Min. 3 + control (highest dose should produce slight maternal toxicity).
METHODS OF APPLICATION	As clinical application. Forced admin. is superior to feed admin.	As clinical application.	As clinical application.	Equivalent to typical route of human exposure, if possible.	Usually orally, by gavage.
DURATION OF TREATMENT	Mouse: d. 6 - 15 pc rat: d. 7 - 17 pc rabbit:d. 6 - 18 pc. Day 0 = day of successful copulation.	Period of embryogenesis.	Mouse, rat: d. 6 - 15 pc rabbit: d. 6 - 18 pc. Day 0 = day of successful copulation.	Implantation thru organogen. to 1 d. before term.	Rat, mouse: d. 6 - 15 pc hamster: d. 6 - 14 pc rabbit : d. 6 - 18 pc. Day 0 = day of successful copulation.
EXAMINATIONS	Rat, mouse: hysterectomy of 2/3 F at end of gest. Rabbit: hysterectomy of all F at end of gestation. Evaluation of foetuses for skeletal and visceral anomalies. Rat, mouse: remaining 1/3 litter and pups examined for postnatal devel. incl. sensory, behaviour, learning. Fertility eval. of F1 by producing F2.	Hysterectomy at end of gest..Examine all foetuses for skeletal and visceral anomalies.	Hysterectomy 1 d. before term. Examine foetuses for skeletal and visceral anomalies. Rat, mouse: 1/3 for visceral, 2/3 skeletal evaluation. Rabbit: all foetuses external inspection, autopsy and skeletal eval.. Also a 24-hr survival test is desirable before examination.	Hysterectomy 1 d. before term, and examine for ext. and int. anomalies. 1/2 - 2/3 of litter skeletal. 1/2 - 1/3 of litter visceral.	Hysterectomy at end of gest. and examine for ext. anomalies. Hamster, rat, mouse, 1/3 - 1/2 of litter: skeletal. Remaining 2/3 - 1/2: visceral. Rabbit: visceral by dissection and skeletal eval..

APPENDIX 3 : DETAILED RECOMMENDATIONS OF SOME LEADING GUIDELINES FOR REPRODUCTIVE TOXICITY TESTING

3. PERINATAL AND POSTNATAL DEVELOPMENT (References see Appendix 4)

	MHW (1982)	CSM (1974)	FDA (1966)	EPA (1979;1982)	OECD (1981;1983)
ANIMAL SPECIES	Min. 1 (mouse, rat, rabbit) as species in embryotoxicity and teratogenicity.	rat	rat	NO PERI- POSTNATAL DEVELOPMENT	NO PERI- POSTNATAL DEVELOPMENT
TOTAL ANIMALS PER DOSE GROUP	Mouse, rat: 20 F pregnant. Rabbit: 10 F pregnant.	Min. 12 F pregnant.	Min. 10 F pregnant.		
NUMBER OF DOSAGES	At least 3 + control. Highest:some toxicity. Lowest: no effect. Middle: geom. mean.	3 + control (highest dose should produce maternal toxicity).	Min. 2 + control (highest dose should be subtoxic and produce no overt symptoms in dams).		
METHOD OF APPLICATION	As clinical application. Forced admin. is superior to feed admix.	As clinical application.	As clinical application.		
DURATION OF TREATMENT	Mouse: d. 15 pc - d. 21 pp Rat : d. 17 pc - d. 21 pp Rabbit:d. 18 pc - d. 30 pp Day 0 = day of successful copulation.	For period of gest. not included in embryotoxic and teratogenic study, and continue thru lact. to weaning.	For the last 1/3 of gest., and thru lact..		
EXAMINATIONS	Birth and rearing for all groups. Postnatal growth and devel. assessed incl. sensory function, learning, behaviour; as the embryotoxic and teratogenic study, produce F2 to evaluate fertility of F1.	Birth and rearing for all groups with postnatal eval. of growth and devel. of offspring; possibly the fertility of F1 offspring evaluated to produce F2.	Birth and rearing for all groups with postnatal eval. of growth and devel. of offspring; possibly the fertility of F1 offspring eval. to produce F2.		

pp : post partum

Appendix 4

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