Guidance on Evaluation of Reproductive Toxicity Data

Monograph No. 31

ISSN-0773-6347-31

Brussels, February 2002

ECETOC Monograph No. 31

European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Guidance on Evaluation of Reproductive Toxicity Data

CONTENTS

SUMMARY 1

1. INTRODUCTION 3

2. OVERVIEW OF THE REPRODUCTIVE PROCESS 5

2.1 Biology of mammalian reproduction, general aspects 5
2.2 Chemical impairment of normal reproduction and its consequences 6

3. REVIEW OF TEST GUIDELINES 8

3.1 Current test guidelines 8
3.1.1 Prenatal developmental toxicity study 8
3.1.2 Two-generation reproduction toxicity study 10
3.1.3 One-generation reproduction toxicity study 11
3.1.4 Reproduction/developmental toxicity screening test 12
3.1.5 Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test 14
3.2 Emerging issues 15
3.2.1 Endocrine disruption 15
3.2.2 Developmental neurotoxicity 16

4. EFFECTS ON EMBRYO-FOETAL DEVELOPMENT 17

4.1 Purpose 17
4.1.1 General considerations 17
4.2 Definition of terms 18
4.2.1 Abnormality, anomaly and malformation: High level of concern 18
4.2.2 Variation and retardation: Low-to-moderate level of concern 18
4.2.3 Gestational parameters: Moderate-to-high level of concern 19
4.2.4 Post-natal parameters: High level of concern 20
4.3 Guidance on ranking foetal morphological findings for level of concern 20
4.4 Influence of maternal toxicity on study interpretation 28
4.4.1 Background 28
4.4.2 Evaluation of available information 28
4.4.3 Conclusions 30

5. EFFECTS ON FERTILITY AND REPRODUCTION **31**

5.1 Purpose **31**
5.2 Studies **31**
5.2.1 One- and two-generation studies **31**
5.2.2 28-Day and 90-day studies **31**
5.3 Definition of terms **33**
5.3.1 Reproductive performance **33**
5.3.2 Histopathological changes to the reproductive tract **33**
5.3.3 Sperm parameters **34**
5.3.4 Oestrous cycle **35**
5.3.5 Follicular toxicity/oocyte quantitation **35**
5.3.6 Development **36**
5.4 Guidance on ranking of adverse findings on reproduction for level of concern **37**

6. STRUCTURED APPROACH TO EVALUATION OF REPRODUCTIVE TOXICITY DATA **44**

6.1 Worked examples **46**

APPENDIX A: TEST GUIDELINE COMPARISON **57**

APPENDIX B: SUMMARY DATA SETS FOR EXAMPLE SUBSTANCES **106**

BIBLIOGRAPHY **129**

DATABASES CONSULTED **134**

MEMBERS OF THE TASK FORCE **155**

MEMBERS OF THE SCIENTIFIC COMMITTEE **136**

SUMMARY

The reproductive process is complex and there are many stages that are vulnerable to external influences; such influences include chemical exposure. Thus, where there is the likelihood of significant exposure to chemicals, it is important to evaluate their potential to affect adversely reproduction in humans.

The interpretation of data from reproductive and developmental toxicity studies requires expert scientific judgement. Hazard assessment involves the identification of pertinent, treatment-related findings, attributing a level of concern that takes into account data from the study as a whole. The level of concern will depend on the type and incidence of response to treatment, as well as the overall conditions under which the response is elicited. Currently, there is no generally accepted framework that can be applied to perform such an assessment in a consistent and transparent manner.

In this Monograph, guidance is provided in the form of a structured approach for the interpretation of reproductive toxicity data. The approach is illustrated through the use of examples from several fertility and developmental toxicity studies, drawn from the collective experience of the Task Force. This guidance takes into account the possible role of maternal toxicity in the interpretation of the study findings.

Since the publication, in 1992, of ECETOC's previous guidance on this topic, new insights into the manner in which chemicals might impair reproductive function have become available. Some of these have been incorporated into updated versions of international or national test guidelines on the assessment of reproductive toxicity. These advances in testing methodology are also highlighted and discussed in this Monograph. In addition, specific mention is made of emerging issues, such as endocrine activity and developmental neurotoxicity.

1. INTRODUCTION

The reproductive process is fundamental *inter alia* to maintaining the continuity of the species and achieving genetic diversity. It is a complex process involving many stages and is vulnerable to interference from environmental conditions or influences including chemical exposure. Thus, where there is the likelihood of significant exposure to chemicals, it is important to evaluate their potential to affect adversely reproduction in humans.

A range of methods exists to study the possible effects of chemicals on fertility and development. These methods examine effects on a wide range of biological endpoints and the findings require specialist skills in their evaluation. The design and interpretation of such studies is continually evolving with the growth in knowledge of the reproductive process and of the manner in which it can be affected.

Chemicals legislation is based on the intrinsic hazards of chemical substances and on the risks presented to man and the environment. One example of such legislation is the EC 'dangerous substances' Directive (EC, 1967) and subsequent amendments, which requires, *inter alia*, that substances that pose a potential reproductive hazard to humans be so classified.

For reproductive effects, this classification is usually based on data available from animal studies, but can also be based on human experience when available. The quality of human data is variable but where there is clear evidence of an effect, this is taken into account in the classification (Category 1). More usually, classification of chemical substances for reproductive hazard is based on animal data alone that are judged according to the level of concern for man (Categories 2, 3 or unclassified).

Interpretation of the potential hazards for man from data in animals should take account of factors such as dose, route, duration of exposure and likely conditions of use as well as the severity or extent of concern for the effects. This integrated assessment of hazard can then be used for classification as well as for the first stages of a risk assessment.

Guidance on the classification of substances "Toxic to Reproduction" was provided in ECETOC Technical Report No. 47 (ECETOC, 1992). The basic principles advanced in that report continue to be relevant. However, subsequent experience in evaluating chemicals and the scientific developments in this field prompted the establishment of an ECETOC Task Force to formulate further guidance for the evaluation of substances for reproductive effects.

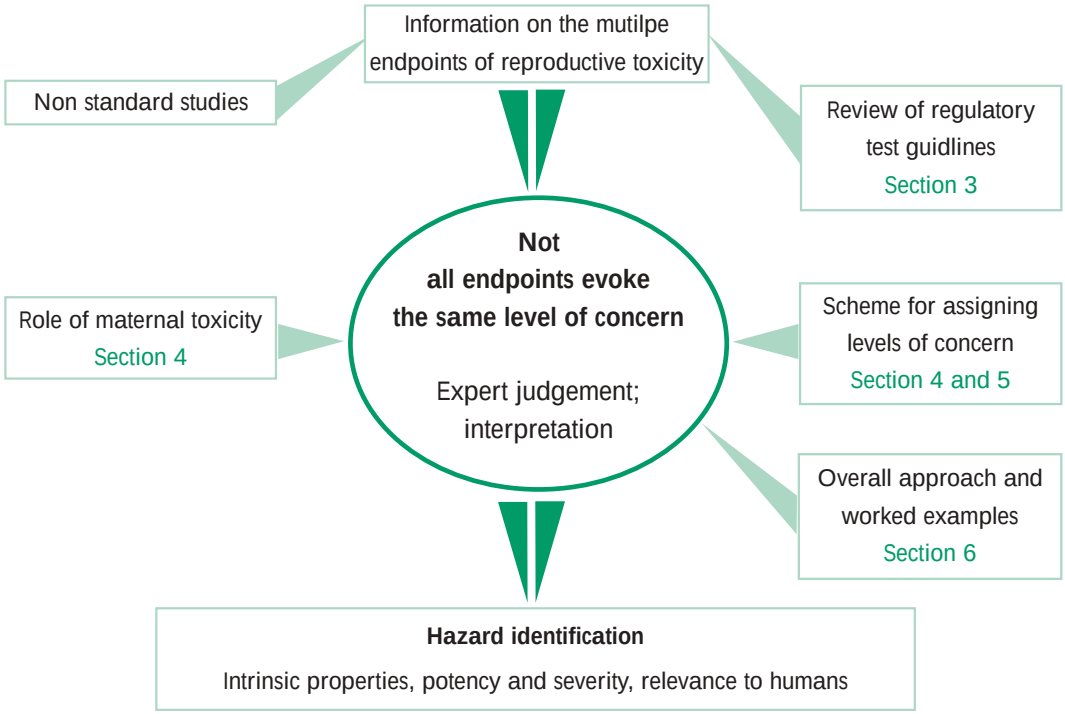
The Terms of Reference for this Task Force included the following:

- Review existing and emerging test guidelines with particular emphasis on new or refined procedures. Comment on their fitness for purpose in hazard and risk assessment schemes.
- Provide support to appropriate ECETOC representation with groups developing such guidelines.

- Develop or identify a concept for ranking effects according to the degree of concern they evoke, taking into consideration their nature, frequency and influences such as parental toxicity.
- Illustrate this concept with examples and comment on its value in hazard assessment and risk characterisation.

In addressing these Terms of Reference the report was prepared with the following structure:

Figure 1: Scheme for an approach to the evaluation of reproductive hazards



This Introduction (Section 1) is followed by an overview of the reproductive process (Section 2). Section 3 provides a review of all major guidelines for testing chemicals for reproductive and developmental toxicity, including those relating to emerging issues such as endocrine disruption and developmental neurotoxicity. However, in view of the present lack of experience in generating and interpreting data in the latter fields, the Task Force considered it premature to formulate guidance on these latter aspects.

A process is proposed for ranking the hazard according to the 'level of concern' for various different endpoints for both developmental and fertility effects (Sections 4 and 5). The influence of maternal toxicity on the interpretation of studies of developmental toxicity is addressed in Section 4.

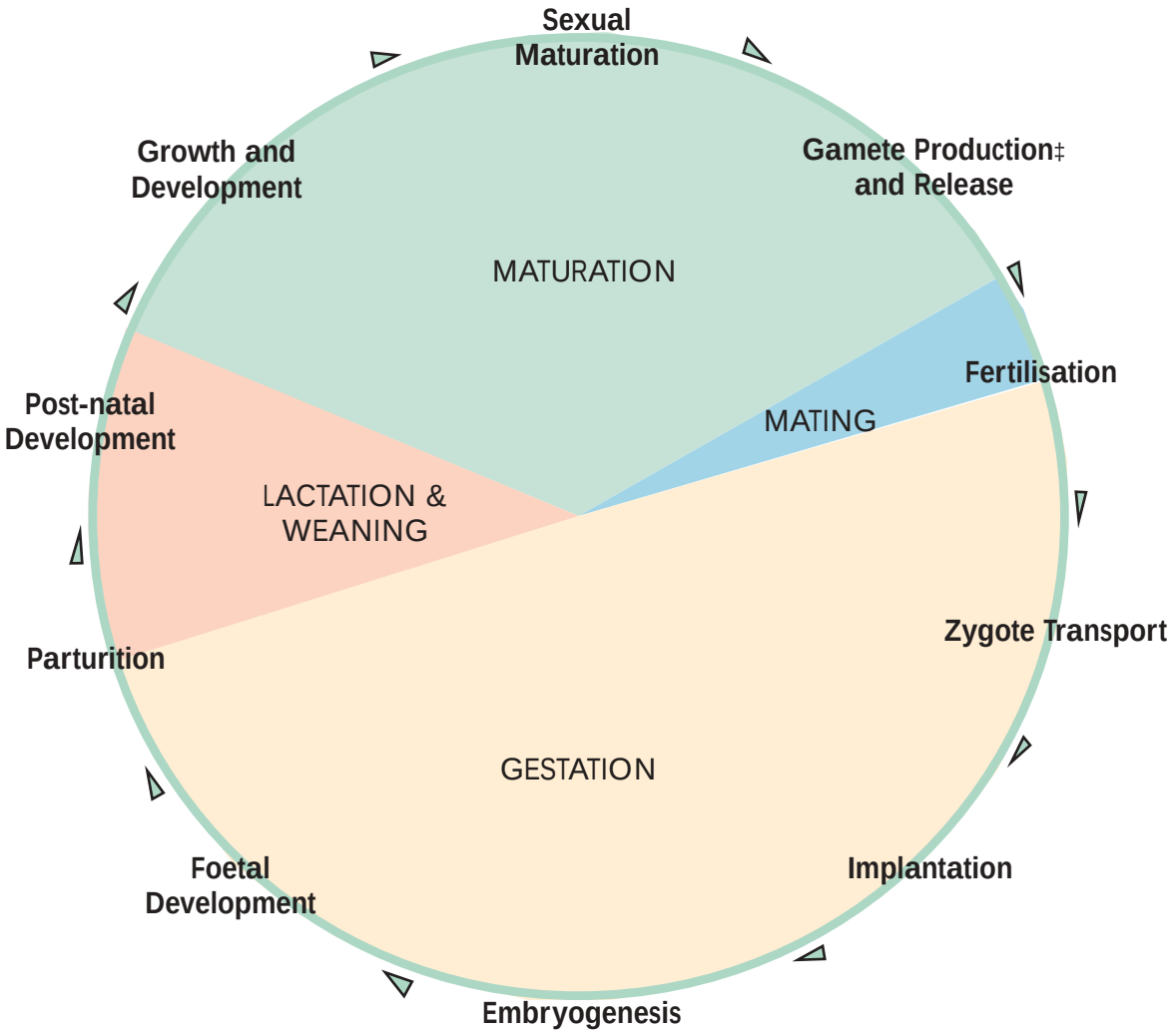
In Section 6, an overall approach for the evaluation of reproductive toxicity is proposed and illustrated for both developmental and reproductive endpoints using data from studies of a wide variety of substances.

2. OVERVIEW OF THE REPRODUCTIVE PROCESS

2.1 Biology of mammalian reproduction, general aspects

Reproduction is a cyclical process which can be broadly divided into four phases, encompassing the various stages of pre- and post-natal development, maturation and mating. The stages of the cycle are temporally similar for both males and females, with the exception of gamete production. The cycle is represented diagrammatically in Figure 2.

Figure 2: Reproductive cycle



‡ Post-natal gamete production occurs only in males. Female gametes are produced during *in utero* development, with meiotic arrest before birth. The primary oocytes undergo final maturation prior to release.

More details of this process can be found in the published literature. Examples include Baker *et al* (1980), Manson and Kang (1994), Zenick *et al* (1994), Kimmel and Buelke-Sam (1994), Witorsch (1995), Hood (1997), Knobil and Neill (1998).

2.2 Chemical impairment of normal reproduction and its consequences

Adverse effects on the reproductive cycle fall into two main categories:

- impairment of male and / or female reproductive ability or capacity;
- effects on development of the progeny (developmental toxicity).

Collectively any such effects resulting from exposure to chemical substances may be defined as 'reproductive toxicity'.

Impairment of fertility (male or female)

This can be defined as impairment of the ability to achieve a normal number of established pregnancies and viable offspring.

It can result from interference with one or more of the stages up to the point of implantation of the embryo in the uterus. Thus reduced fertility can be caused by adverse effects on gametogenesis (sperm or ova), endocrine function, libido, mating behaviour, fertilisation, early development of the fertilised ova, transport and implantation into the uterine endometrium.

Developmental toxicity

All non-heritable adverse effects on the further development of the offspring up to attainment of sexual maturity / adult life are included in this category. Such effects may become manifest during embryonic or foetal development, or between parturition and sexual maturation.

More details on the endpoints that are studied in evaluating the effects of chemicals on each of the phases of reproduction are given in Table 1.

Table 1: Milestones and endpoints for each phase of the reproductive cycle

Phase	Milestones and endpoints
Sexual maturation	- Libido - Sexual behaviour and mating (time to mating, vaginal plugs/sperm) - Endocrine function (LH, FSH, testosterone, oestrogen, prolactin)
Gamete production and release	- Post-natal gamete production occurs only in males. Female gametes are produced during *in utero* development, with meiotic arrest before birth. The primary oocytes undergo final maturation prior to release
Fertilisation and early embryonic development (pre-implantation)	- Spermatozoa in the oviduct at 1 h after copulation - Fertilisation in the ampulla of oviduct up to 3 h after ovulation → Cleavage (ca. 25 h, 2-cell stage) → Next 74 h (cleavage mitosis) → Blastomeres, 96 h after fertilisation → Morula (12-16 cells at the oviduct-uterine junction)
Zygote transport	- Zygote → morula → blastocyst on day 5 migration from oviduct to uterus
Implantation	- Blastocyst implants on gestation day 6 - Maternal hormonal state (progesterone, oestrogen) - Placental development - Survival of implants
Embryogenesis	- Survival of embryo - Growth and differentiation - Organ development
Foetal development	- Survival of foetus - Growth and differentiation - Function of organ system(s)
Parturition	- Behaviour - Duration of parturition, dystocia - Ability to nurse
Post-natal development (preweaning, postweaning)	- Survival - Birth weight, growth - Organ system function - Hormone function - Immune function - CNS and peripheral NS function - Anogenital distance - Development (normality of external genitalia, vaginal opening, vaginal smear cytology) - Testis descent, preputial separation, sperm production

3. REVIEW OF TEST GUIDELINES

3.1 Current test guidelines

This section deals with experimental animal studies designed primarily to reveal direct effects on the reproductive process. Such studies are used routinely to determine the reproductive toxicity of chemicals. Reference is made primarily to the test guidelines (TG 414, 415, 416, 421 and 422) issued by the Organisation for Economic Co-operation and Development (OECD). The design and purpose of each study type is described in this section and, where the guidelines have been updated, comment is made on the new features. A detailed comparison of the previous ('old') and current ('new') versions of the guidelines issued by OECD is presented in Appendix A. Also compared in Appendix A are the equivalent guidelines issued by the US Environmental Protection Agency (EPA), the Japanese MAFF, the European Commission and the International Conference on Harmonisation (ICH).

The complex processes involved in fertility and development are dependent *inter alia* on the physiology and biochemistry (and hence on the health) of the mother. It is therefore inevitable that physiological disturbances caused by the toxicity of chemicals will also, in many cases, exert indirect deleterious effects. The increased concern about the potential of certain chemicals to alter the function of various processes under endocrine control, led to distinct modifications and an extended scope of examinations in some of the test guidelines (in particular those methods for studying prenatal developmental and two-generation reproductive toxicity over two generations).

In Figures 3 to 6, the different phases of reproductive cycle covered by the experimental design of the various regulatory studies are illustrated in colour.

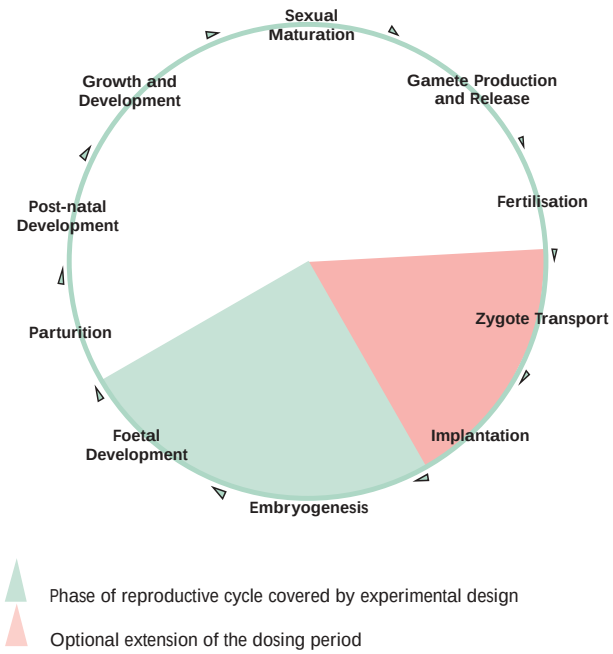
Tests for reproductive toxicity generally involve exposure of sexually mature (adult) animals prior to conception, prenatal development, or post-natally to the time of sexual maturation.

3.1.1 Prenatal developmental toxicity study

The prenatal / developmental toxicity study (TG 414, Figure 3) (OECD, 2001a) is designed to provide:

> "information concerning the effects of exposure on the pregnant test animal and on the developing organism; this may include assessment of maternal effects as well as death, structural abnormalities, or altered growth in the foetus. Functional deficits, although they are of great importance to development, are not a part of this guideline".

Figure 3: Prenatal / developmental toxicity study (OECD TG 414)



According to this guideline, the test substance is administered in graduated doses to pregnant animals from implantation to one day prior to the expected day of parturition. As an alternative, treatment of the dams from fertilisation (day 0) is possible on a case by case basis. The pregnant females are terminated, the uterine contents examined, and the foetuses weighed, sexed, and evaluated for external, visceral, and skeletal alterations. Testing is usually carried out in one rodent (rat / mouse) and one non-rodent (rabbit) species.

The major differences between the new and the old test guidelines lie in the extension of the maternal dosing period, a periodic adjustment of maternal dosage volumes during gestation and an increase in the group size for rabbits. Moreover, concerning the evaluation of the foetal skeletons, in addition to the visualisation and study of the ossified structures, assessment of the cartilage is also recommended. Finally, in order to avoid bias, evaluation of the dams during caesarean section and subsequent foetal analyses, should be conducted blind i.e. preferably without knowledge of the treatment group.

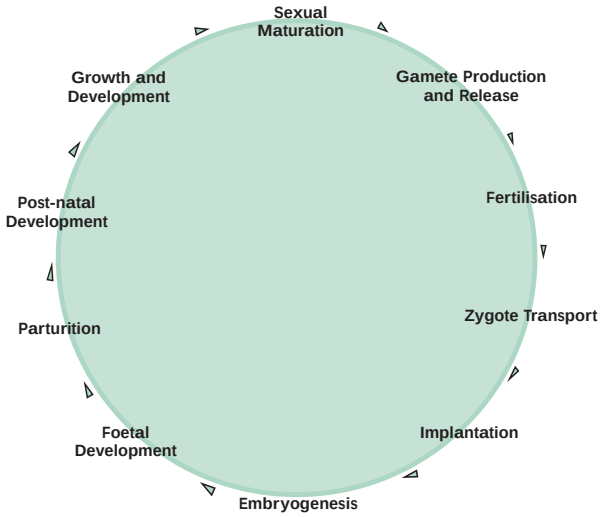
The new test guidelines for prenatal developmental toxicity studies, with expanded requirements (e.g. assessment of the foetal cartilage), better characterise the various types of potential developmental toxicity and together with other studies allow a more precise assessment of the risk for the most susceptible populations.

3.1.2 Two-generation reproduction toxicity study

The most commonly used comprehensive test of reproductive toxicity is the OECD two-generation study (TG 416, Figure 4) (OECD, 2001b). The purpose of TG 416 is to provide:

> "general information concerning the effects of a test substance on the integrity and performance of the male and female reproductive systems, including gonadal function, the oestrous cycle, mating behaviour, conception, gestation, parturition, lactation, and weaning, and on the growth and development of the offspring through the production of one litter in each generation. The study may also provide information about the effects of the test substance on neonatal morbidity, mortality, target organs in the offspring, and preliminary data on prenatal and postnatal developmental toxicity and serve as a guide for subsequent tests. Additionally, since the study design includes in utero as well as postnatal exposure, this study provides the opportunity to examine the susceptibility of the immature / neonatal animal".

Figure 4: Two-generation study (OECD TG 416)



In this study the test substance is usually administered at three dose levels to parental (P) animals (rat as preferred species), prior to and during their mating, during the resultant pregnancies, and through the weaning of their F_1 offspring. The substance is then administered to selected F_1 offspring during their growth into adulthood, mating, and production of an F_2 pup generation, until the F_2 pups are weaned.

The major differences between the old and the new test guidelines lie in the additional requirements for an extension of the number of organs to be weighed at necropsy in the parental animals, a more thorough histopathological examination on a broader spectrum of organs (particularly on organs of the male and female reproductive tract), and additional evaluations of different sperm parameters and of the oestrous cyclicity in the

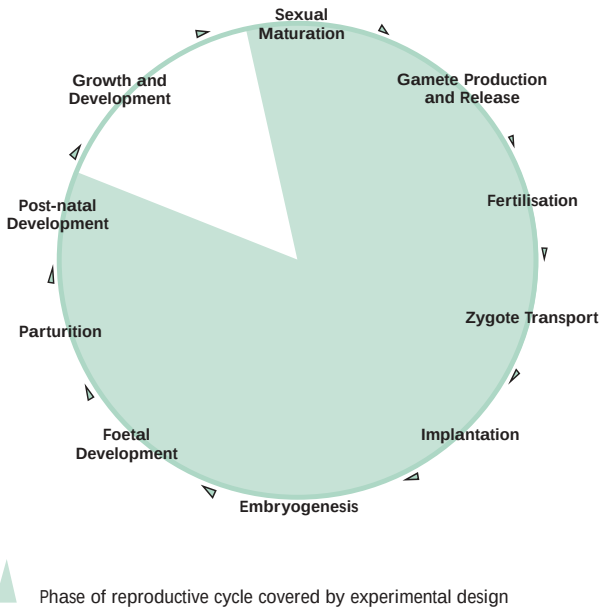
adult animals. Moreover F_1 and F_2 weanlings are examined for sexual maturation, the weights of certain pup organs are determined, and histopathology is performed on selected pups of each litter. Finally, there is a new requirement to measure the anogenital distance of the F_2 pups on post-natal day 0 if this is triggered by alterations in the sex ratio of the F_1 pups or timing of their sexual maturation.

The new guideline for two-generation studies characterises and defines endpoints for male and female toxicity more completely, and provides the basis for the correlation between quantitative measures and functional endpoints (e.g. sperm count, oestrous cycle and fertility) or the analysis of the relationship between reproductive toxicity and other toxicity. The expanded list of endpoints, together with the broader evaluation of functions and dose-response relationships allows a more comprehensive assessment of the risk for the most susceptible populations.

3.1.3 One-generation reproduction toxicity study

The one-generation reproduction toxicity study (TG 415, Figure 5) (OECD, 1983a) is designed to provide "information on the effects of a substance on male and female reproductive performance" over one generation, and addresses several of the endpoints described before for the two-generation reproduction toxicity study. Usually, the test substance is administered at three dose levels to groups of male and female rats. Male parents are dosed while still growing and for at least the duration of spermatogenesis plus epididymal transit time (approximately 56 days in the mouse and 70 days in the rat) in order to elicit any adverse effects on spermatogenesis. Female parents are dosed for at least two weeks (i.e. during two complete oestrous cycles) in order to elicit any adverse effects on oestrous. The test substance is administered to both sexes during mating and to females during pregnancy and for the duration of the nursing period until the F_1 generation is weaned, usually day 21 *post partum*.

Figure 5: One-generation reproduction toxicity study (OECD TG 415)



At the present time, no revised version of this guideline, originally issued in 1983, is available. Unlike the one-generation study, the two-generation study evaluates reproduction in parental animals exposed pre- and post-natally. Only the two-generation reproductive toxicity protocol provides information on the growth and development of the offspring post-weaning, including the integrity and performance of the male and female reproductive systems of the F_1 generation.

With regard to possible refinements to the one-generation study, recent updates of the two-generation reproduction study guidelines (EPA, 1998b; OECD, 2001b) include a variety of new endpoints to assess endocrine-mediated toxicity. These include measurement of sperm, oestrous cycle, organ weights including accessory sex tissues, histology of reproductive organs in parents and offspring, and additional endpoints on development of individual live pups.

Some of the endpoints mentioned above may be incorporated into the one-generation study protocol without extending its duration. However, in order to assess fully sexual maturation and function of the offspring, it would be necessary to increase the observation period for the F_1 generation at least to the age of sexual maturity, and possibly up to the time of mating. Even so, this would not address all of the limitations of the one-generation study design, as experience shows that two-generation studies are capable of detecting qualitative and quantitative differences between successive generations. There is some evidence that the second-generation offspring may be more sensitive than those of the first generation.

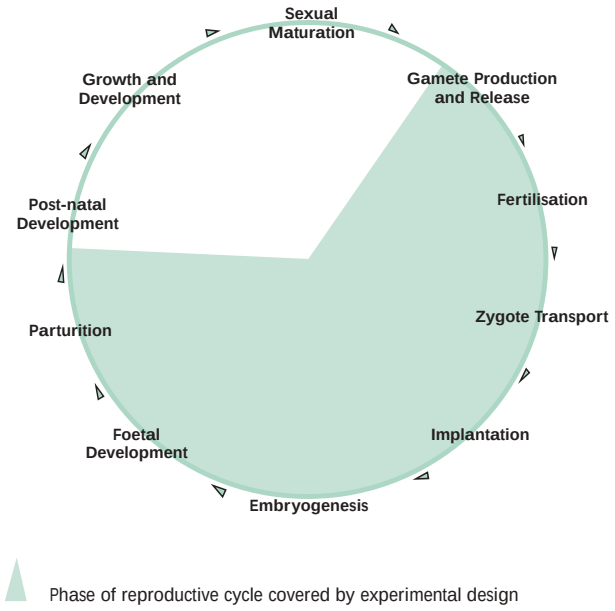
3.1.4 Reproduction / developmental toxicity screening test

The reproduction / developmental toxicity screening test (TG 421, Figure 6) (OECD, 1995a) is designed:

> "to generate limited information concerning the effects of a test substance on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing Test Guidelines 414, 415 and 416".
>
> "This Screening Test Guideline can be used to provide initial information on possible effects on reproduction and / or development, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of concern. It can also be used as part of a set of initial screening tests for existing chemicals for which little or no toxicological information is available, as a dose range finding study for more extensive reproduction / developmental studies, or when otherwise considered relevant".
>
> "This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting post-natal manifestations of pre-natal exposure, or effects that may be induced during post-natal exposure. Due (amongst other reasons) to the relatively small numbers of animals in the dose groups, the selectivity of the endpoints, and the short duration

of the study, this method will not provide evidence for definite claims of no effects. Although, as a consequence, negative data do not indicate absolute safety with respect to reproduction and development, this information may provide some reassurance if actual exposures were clearly less than the dose related to the No-Observed-Adverse Effect Level (NOAEL). Moreover, in the absence of data from other reproduction / developmental toxicity tests, positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing."

Figure 6: Reproduction / developmental toxicity screening tests (OECD TG 421 and TG 422)



In TG 421, the test substance is administered in (usually three) graduated doses to several groups of males and females. Males and females are dosed for two weeks prior to mating and during mating. Exposure of the males is continued for about 2 weeks post-mating until necropsy, while the females are dosed after mating for the duration of pregnancy and at least 4 days after delivery. It is not known whether there are any plans for this test guideline to be revised in the near future. However, it is possible to extend the study by adding some of the endpoints included in the current version of a two-generation reproduction toxicity study (e.g. sperm evaluation, cyclicity determination, raising the pups to some stage of sexual maturation) to examine additional aspects e.g. endocrine disruption.

Equivalent guidelines for this test design have also been published by the EPA (EPA, 2000a).

3.1.5 Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test

The combined repeated dose toxicity study with the reproduction / developmental toxicity screening test (TG 422, Figure 6) (OECD, 1996) provides information on possible health hazards likely to arise from repeated exposure over a relatively limited period of time. As this test further comprises several endpoints of reproductive / developmental toxicity, it can also be used to "provide initial information on possible effects on male and female reproductive performances and on development of the conceptus and parturition".

As is the case for TG 421, this test does not provide complete information on all aspects of fertility and development. The general design of this study is similar to that of TG 421, but is supplemented by more sophisticated clinical observations, additional clinical pathology and an extended scope of histopathological evaluations.

The method comprises the basic repeated dose toxicity study that may be used for chemicals for which a 90-day study is not warranted (e.g. when the production volume does not exceed certain limits), or preliminary to a long-term study. It further comprises a reproduction and developmental toxicity screening test. The test can also be used as part of a set of initial screening tests for existing chemicals for which little or no toxicological information is available, as a dose-range finding study for more extensive reproduction / developmental studies, or when otherwise considered relevant. The limitations recognised for TG 421 also apply to this test protocol. In addition, the selection of dose is critical in this test; if it is too high then reproductive and developmental effects may be obtained which are consequential to general systemic toxicity; on the other hand, if it is too low it may fail to detect all forms of general toxicity.

Equivalent guidelines for this test design have also been published by the EPA (EPA, 2000b).

It is not known whether there are any plans for this test guideline to be revised in the near future. However, it is possible to extend the study by adding some of the endpoints included in the current version of a two-generation reproduction toxicity study (e.g. sperm evaluation, cyclicity determination, raising the pups to some stage of sexual maturation) to examine additional aspects e.g. endocrine disruption.

3.2 Emerging issues

Test guidelines reflect the state of the science at the time of their development. However, as our understanding of the processes and control of reproduction develops, so too does the requirement to ensure that such processes are not perturbed by chemical insult. Two issues are currently emerging for which test guidelines are not yet fully established, or for which there is not a great deal of practical experience. These are endocrine disruption and developmental neurotoxicity.

3.2.1 Endocrine disruption

The normal development and function of the reproductive system in males and females is under the control of the endocrine system, and strict hormonal balance is essential for the integrity of all stages of reproduction. Xenobiotics may impair reproduction through a number of mechanisms, including disruption of endocrine homeostasis (e.g. agonist / antagonist interaction at hormone receptors, disturbance of hormone distribution or metabolism). It is not the intention of this document to give a definitive review of assays for endocrine disruption, which include a number of novel and specific *in vivo* and *in vitro* methods. The reader is directed to other sources for such guidance (e.g. ECETOC, 1996).

However, endocrine disruption can be detected in a number of suitably adapted 'routine' reproductive toxicity assays. This is achieved by the incorporation of endpoints that are under hormonal control, and thus sensitive to disturbance by endocrine disrupters, and some amendments to the overall protocol where appropriate. Such endpoints include the age of sexual maturation of offspring (e.g. preputial separation in males, vaginal opening in females), disturbance of sexual differentiation (e.g. anogenital distance), sperm parameters (e.g. number, morphology, motility), circulating hormone levels and regularity and duration of the oestrous cyclicity, as well as more conventional endpoints such as histopathology and weights of organs of the reproductive tract. However, it must be borne in mind that many of these endpoints are not specifically indicative of an endocrine-mediated mechanism of toxicity *per se*, but may also be influenced by overall growth and health status of the animal, or by toxicants that influence homeostasis through other mechanisms. For example, the oestrous cycle is perturbed in cases of bodyweight reduction, and reduced sperm counts may be indicative of a male germ cell cytotoxicant. Consequently, these endpoints must be interpreted with reference to other endocrine-sensitive endpoints and to additional observations on growth and histopathology. They are most useful as a measure of effect rather than a mechanism of toxicity.

Further experience with the use of these new approaches is necessary before more specific guidance can be given in respect of their impact on the reproductive hazard assessment of chemicals. In due course it would be appropriate to undertake a more in-depth review by a future ECETOC Task Force.

3.2.2 Developmental neurotoxicity

Developmental neurotoxicity (DNT) studies, have been designed to collect data on the potential functional and morphological effects on the nervous system which may arise in the offspring from exposure of the animal to chemicals during pregnancy and lactation (Kaufmann, 2000).

A new, finalised version of a DNT test guideline has been issued (EPA, 1998c) and a further proposal published by OECD (OECD, 1999). There are considerable differences between these two test guidelines in respect of the key requirements (e.g. treatment period of the dams, number of pups for neurobehavioural tests, time and scope of pup post-mortem examinations).

Moreover, since there is only limited experience in the interpretation of results from DNT studies and their relevance to humans, the Task Force decided not to elaborate further in this report on the critical appraisal of the test protocols. When more experience with these or more advanced DNT protocols has been gathered, a critical evaluation of the corresponding test methods would be appropriate.

4. EFFECTS ON EMBRYO-FOETAL DEVELOPMENT

4.1 Purpose

The ultimate objective of developmental toxicity studies in laboratory animals is hazard assessment for the developing human embryo and foetus. To achieve this goal, developmental toxicity studies should detect any effect that is related to the process of embryo-foetal development. The major manifestations of developmental toxicity include death, structural abnormalities, growth alterations and functional deficiency. A biologically significant change in any of these four parameters is considered indicative of a potential on the part of a test substance to affect normal development (OECD, 2001a). The evaluation of offspring for structural abnormalities comprises external, visceral and skeletal examinations. No harmonised system exists to assign a concern level for structural findings and to categorise findings into malformations and variations / retardations. Harmonisation of the type of changes in each category and the different degrees of severity of these changes is currently being developed (Chahoud *et al*, 1999a, BGVV, 2000).

This Section provides a basis from which a study investigator can develop a strategy for the evaluation of developmental toxicity studies according to available data. The definitions for gestational and embryo-foetal parameters and the terms of foetal findings with their suggested 'levels of concern' in Tables 2 to 4 form the basis for an approach to an overall assessment of hazard.

4.1.1 General considerations

Terminology describing observations on individual findings of morphological foetal examinations is still used inconsistently (Black and Marks, 1986). However, considerable progress in the attempt to establish a harmonised glossary of foetal morphological findings was made with the publication of a Terminology of Developmental Abnormalities in Common Laboratory Mammals (Version 1) by the International Federation of Teratology Societies (IFTS) (Wise *et al*, 1997). The terms for foetal morphological findings listed in Tables 2 to 4 are based on this published terminology.

Foetal observations should be divided into external, skeletal and visceral (or soft tissue) findings (EPA, 1998d). However, there is no common agreement on how to categorise or grade foetal morphological findings from developmental toxicity studies with rats, mice and rabbits. Frequently the observed effects are categorised as major malformations, minor malformations, variants and anomalies. Such categorisation and the various types of structural anomalies included under different categories frequently overlap (IPCS / OECD, 1994). It is left to the laboratory performing the study to decide whether a foetal finding is categorised into major or minor malformations, an abnormality, a variation, a deviation or a retardation (EPA, 1998d). The laboratory should, however, define what is meant by the terms used such as malformation, abnormality and variation.

Foetal morphological findings span a spectrum of severity, based upon their adverse impact upon the viability and survivability of the offspring. However, there has often been disparity in the categorisation of such findings, with variations in terminology, categorisation and ranking of effects (Black and Marks, 1986; IPCS/OECD, 1994).

A report of a workshop held on categorisation terms in developmental toxicology proposed a scheme of categorisation for foetal abnormalities that consists of only two categories, namely malformation and variation (Chahoud *et al*, 1999a). The definition of the two categories is given below. The agreed definitions of malformations and variations of terms for skeletal findings were applied as listed in the IFTS glossary at a workshop on terminology in developmental toxicology organised by the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BGVV, 2000).

The following definition of terms is an attempt to simplify and categorise the terminology frequently used to describe malformations, anomalies/abnormalities, variations, retardations and deviations, and to assign a 'level of concern' for each. It is not intended to be a definitive harmonisation of the diverse systems used in interpreting and categorising foetal morphological findings, but is offered as general guidance. In applying this scheme, scientific judgement is required for the reliable interpretation of the study observations. The final categorisation of the observations rests with the user.

4.2 Definition of terms

4.2.1 Abnormality, anomaly, malformation: High level of concern

These are persistent structural or functional deviations outside the normal biological variation, and are considered to have a significant adverse effect on the foetus, either with or without fatal consequence (ECETOC, 1983; EPA, 1992). A malformation can also be defined as a permanent structural change that is likely to affect adversely the survival or health of the species under investigation (Chahoud *et al*, 1999a)

4.2.2 Variation, retardation: Low-to-moderate level of concern

A *variation* is a divergence within the normal range of structural or functional qualities of an organism, whereas a *retardation* is a delay in growth or morphogenesis which has followed an otherwise normal pattern of development (Khera, 1981). Both variations and retardations are considered to be of moderate or low concern, depending on the species in which the observation was made and the gestational day of examination. A variation can also be defined as a change that occurs within the normal population under investigation and is unlikely to affect adversely survival or health. This might include a delay in growth or morphogenesis that has otherwise followed a normal pattern of development (Chahoud *et al*, 1999a).

4.2.3 Gestational parameters: Moderate-to-high level of concern

These are numerical parameters such as the numbers of pregnant and non-pregnant females, *corpora lutea*, alive and dead implantations and foetuses, or abortions. From these parameters indices such as the pre- and the post-implantation loss, can be calculated. Gestational parameters provide additional important information for the interpretation of foetal morphological findings with regard to their concern. Further, the maternal health condition during gestation has also to be considered since it can affect gestational parameters as well as embryo-foetal findings (see Section 6).

Although embryo- and foeto-lethality is, in a strict sense, an embryo / foetal parameter it is usually summarised together with gestational parameters. Embryolethality means that conceptuses (embryos) died during organogenesis; foeto-lethality means that conceptuses (foetuses) died after the end of organogenesis.

A conceptus can die *in utero* as a result of a primary toxic effect or as a consequence of structural defects (malformations) due to teratogenic properties of a chemical. In rodents and rabbits the dead conceptus undergoes resorption or may be aborted in rabbits. Thus, in regular developmental toxicity studies, inspection of the uterus at the end of gestation does not permit elucidation of whether the conceptus died spontaneously, as a result of malformations or as a result of a primary toxic effect. A substance which produces marked embryo- / foeto-lethality and possibly a few malformed foetuses at a given dose level is, therefore, suspected to be a teratogen. The few malformed foetuses surviving to term and observed at caesarean section may represent a majority of foetuses which already died *in utero* due to their malformations, and were consequently resorbed. Results from lower dosages at narrow intervals are necessary to interpret such findings and to evaluate fully the teratogenic potential of a substance. Sometimes further elucidation of, for example the dose / effect and mechanism, can only be achieved by applying modified treatment schedules, specific foetal examination methods and, eventually, studies in other species.

In developmental toxicity studies, most known teratogens have been demonstrated to have a threshold dose below which there is no effect on embryo development. The threshold is not always clear cut, however and can be manifested as a subtle increase of variations or minor malformations. With increasing dose, increasing incidences of more serious malformations and embryonic death are seen. Further increase in dose results in an increase of embryolethality, although teratogenicity appears to decrease. This is because increasing numbers of conceptuses die before term and are only seen as resorptions at uterine examination or are aborted. This pattern of effects varies with the exposure and susceptibility of the dam and the embryo to the teratogen. Thus the same dose may result in maternal toxicity as well as embryo / foetolethality and foetal structural abnormalities.

4.2.4 Post-natal parameters: High level of concern

The exposure of the embryo and foetus *in utero* to chemicals may be manifested not only as embryo-foetal death and foetal morphological alterations, but also in the form of functional deficits which become evident only during post-natal life. The classical prenatal developmental (teratogenicity) studies focus on the examination of gestational and foetal-morphological parameters, whereas post-natal evaluations of the F_1-generation are examined in one- and two-generation reproduction toxicity studies. Post-natal parameters and their meanings are discussed in Section 5.

4.3 Guidance on ranking foetal morphological findings for level of concern

The level of concern indicated for findings listed in Tables 2-4 is intended as a guide to the inherent character of the observations and should not be used in isolation as the only criterion for assessment. Rather, levels of concern for any given endpoint will be defined by the magnitude of the effect detected (the degree of deviation from the concurrent and historical control), the dose at which the effect is seen and whether the effect is part of a dose response. In general, major changes that compromise the ultimate development are of greatest concern; of lower concern are those changes that reflect an alteration in the rate of development or any measures that do not directly affect normal development.

When data from foetal morphological findings are interpreted and ranked for their level of concern several criteria should be considered which may alter the final assessment:

- The level of concern is raised if a finding occurs more frequently together with other findings (clusters, syndromes).
- The level of concern is raised if the finding occurs in isolated foetuses from several litters than if all foetuses of only one litter is affected (i.e. the litter as a whole is the experimental unit).
- The level of concern is raised if the finding occurs at maternally non-toxic doses.
- The level of concern for variations at low doses is greatest when malformations of the same structure occur at higher doses. Sometimes these malformations may be incompatible with embryo viability, and then only an increase in embryonic deaths would be observed at the higher dosage.

The level of concern for a given finding can change depending on the species and even the strain.

Table 2: Foetal morphological findings - External

Abnormalities and malformations[1] - Level of concern: High		
General	**Trunk**	**Cranium**
Anasarca (generalised oedema)	Anal atresia (imperforate)	Acephaly
Runt (small foetus < half the size of litter mates)	Craniorachischisis	Anencephaly
	Gastroschisis	Cranioschisis
Conjoined twins	Anogenital distance[2]	Domed Head
Cutis aplasia	Omphalocele	Exencephaly
Hemorrhage (haematoma)	Spina bifida	Macrocephaly
Oligo-/Poly-hydramnios	Umbilical hernia	Meningoencephalocele
	Scoliosis	Microcephaly
Ear	**Eye**	**Nose**
Anotia (agenesis)	Ablepharia (eye open)	Arhinia (agenesis)
Macrotia (enlarged)	Anophthalmia (eye bulge absent)	Malpositioned
Microtia (small)		Misshapen (malformed, abnormal)
Misshapen pinna	Exophthalmos	
Malpositioned	Macrophthalmia (eye bulge enlarged)	Single naris
		Naris atresia (imperforate)
	Microphthalmia (eye bulge small)	
Mouth and jaw	**Limb, paw and digit**	**Tail**
Agnathia (agenesis)	Adactyly	Acaudate (agenesis, anury)
Micrognathia (brachygnathia)	Amelia(agenesis)	
	Brachydactyly	Curled
Cleft lip	Ectrodactyly (oligodactyly)	Kinked
Cleft palate	Phocomelia	Narrowed (constricted)
Protruding tongue (in fresh specimen only)	Polydactyly	Brachyury (short tail)
	Talipes (malrotated paw)	

1 Terms in parenthesis are synonyms or explanations

2 Increased or decreased

Table 3: Foetal morphological findings - Skeletal

Variations and retardations[1] **Level of concern: Low to moderate[2]**	**Abnormalities and malformations[1]** **Level of concern: High**
Skull	**Skull**
Hyoid bone	*Hyoid bone*
Incomplete ossification (reduced ossification)	Absent (agenesis) Misshapen (malformed, abnormal)
Skull bones	*Skull bones*
Small (hypoplastic, rudimentary, reduced) Incomplete ossification (reduced ossification)	Agenesis (absent) Misshapen (malformed, abnormal) Fused
Fontanelle	*Fontanelle*
Enlarged	Fused (closed)
Vertebral column	**Vertebral column**
Atlas, axis	*Atlas, axis*
Small (hypoplastic, rudimentary, reduced) Incomplete ossification (reduced ossification)	Agenesis (absent) Fused Misaligned Misshapen (malformed, abnormal) Split (cleaved)
Vertebral alteration of centra[3]	*Vertebral alteration of centra*[3]
Bipartite (two ossification centres) Dumb-bell shaped Incomplete ossification (reduced ossification) Supernumerary (additional)	Agenesis (absent) Fused Hemicentric (asymmetric, unilateral ossification centre) Misaligned Misshapen (malformed, abnormal) Split (cleaved)
Vertebral alteration of arch	*Vertebral alteration of arch*
Small (hypoplastic, rudimentary, reduced) Incomplete ossification (reduced ossification) Supernumerary (additional)	Agenesis (absent) Fused Misaligned Misshapen (malformed, abnormal)

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

3 Cervical, thoracic, lumbar, caudal

Table 3 (cont.): Foetal morphological findings - Skeletal

Variations and retardations[1] Level of concern: Low to moderate[2]	Abnormalities and malformations[1] Level of concern: High
Rib	**Rib**
Bent	Agenesis (absent)
Cervical rib	Branched (bifurcated, forked, split)
Discontinuous (incomplete)	Fused
Short (rudimentary)	Intercostal (flying)
Incomplete ossification (reduced ossification)	Misaligned
Knobbly	Misshapen (malformed, abnormal)
Supernumerary (additional, extra thoraco-lumbar)	
Thickened	
Wavy	
Sternebra	**Sternebra**
Bipartite ossification	Agenesis (absent)
Extra ossification	Malpositioned
Incomplete ossification (reduced ossification)	Misaligned (severe)
Unossified	Misshapen (malformed, abnormal)
	Sternoschisis (cleft sternum)
	Fused
Clavicle, scapula	**Clavicle, scapula**
Bent (angulated)	Agenesis (absent)
Small	Misshapen (malformed, abnormal)
Incomplete ossification (reduced ossification)	Unossified
Thickened	
Ilium, ischium, pubis	**Ilium, ischium, pubis**
Small (hypoplastic, rudimentary, reduced)	Agenesis (absent)
Incomplete ossification (reduced ossification)	Misaligned (ilium, ischium)
Thickened	Misshapen (malformed, abnormal)
Unossified (pubis)	
Misaligned	
Extremities[4]	**Extremities[4]**
Incomplete ossification (reduced ossification)	Agenesis (absent)
Unossified (if confined to distal bones of phalanges)	Bent
	Fused
	Malpositioned
	Misshapen (malformed, abnormal)
	Supernumerary
	Thickened

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

4 Alteration of individual bones

Table 4: Foetal morphological findings - Visceral

Variations and retardations[1] **Level of concern: Low to moderate[2]**	**Abnormalities and malformations[1]** **Level of concern: High**
General	**General**
Dilated	Agenesis (absent)
Enlarged	Aneurism
Haemorrhagic	Fluid filled abdomen
Small	(watery = ascites, blood = haemorrhage)
Discoloured (mottled)	Congestion (if excessive and generalised)
Shortened (unless shorter than 50% of normal)	Malposition
	Fistula
	Situs inversus
	Stenosis
Coronal sections (Head)	**Coronal sections (Head)**
Alteration of brain	*Alteration of brain*
Cerebral ventricular enlargement	Asymmetric
(mild dilation, < 2-fold of normal)	Small (hypoplastic, rudimentary, reduced)
	Misshapen (malformed, abnormal)
	Hydrocephaly (internal, external)
Alteration of nasal cavity	**Alteration of nasal cavity**
Enlarged (only if mild dilation, < 2-fold of	Small
normal; not visible from exterior)	Agenesis (absence of nasal septum and /
	or conchae)
	Malpositoned nasal septum and / or conchae
Eye	**Eye**
Haemorrhagic eye	Anophthalmia
	Macrophthalmia (megalophthalmia)
	Malpositioned
	Microphthalmia (hypoplastic, rudimentary)
	Lens alteration
	Aphakia (agenesis)
	Cataract (opacity)
	Malpositioned
	Misshapen (malformed, abnormal)
	Small (hypoplastic, rudimentary, reduced)
	Retina alteration
	Fold (only if dose related; may be due to
	processing artifact)
Thymus	**Thymus**
Remnants	Agenesis (absent)
Asymmetric	Split (bipartite)
	Malpositioned
	Misshapen (malformed, abnormal)
	Enlarged (larger than double the normal)
	Small (smaller than half normal)

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

Table 4 (cont.): Foetal morphological findings - Visceral

Variations and retardations[1] **Level of concern: Low to moderate[2]**	**Abnormalities and malformations[1]** **Level of concern: High**
Lung and trachea	**Lung and trachea**
Alteration of lung	*Alteration of lung*
Enlarged lobe(s)	Agenesis (absent)
Abnormal lobation (fused lobes if <3 lobes affected)	Haemorrhagic
	Small
Discoloured (mottled)	Misshapen (malformed, abnormal)
Pale	Unilobular
Supernumerary lobe(s) (if <2)	*Alteration of trachea*
	Malpositioned
	Narrowed (stenosis, coarctation)
Heart	**Heart**
Ostium enlarged	Acardia
	Atrial septal defect
	Atrium-ventriclular canal persistent
	Septal defect
	Cardiomegaly
	Dextrocardia
	Malpositioned
	Globular shaped
	Hydropericardium
	Valvular alteration
	Agenesis (absent)
	Enlarged
	Hypoplastic
	Misshapen (malformed, abnormal)
	Supernumerary
	Ventricular alteration
	Enlarged
	Small
	Ventricular septal defect
Blood vessels[3]	**Blood vessels[3]**
Branching variation	Agenesis (absent)
Dilated, elongated (< 2-fold of normal)	Dilated (diameter (>50% of normal)
	Double
Short (in length)	Hypoplastic
Supernumerary (if small vessels affected)	Interrupted
	Malpositioned
	Narrowed (stenosis, coarctation)
	Patent (persistent) ductus arteriosus (normal in foetal period only)
	Aneurysm
	Aorticopulmonary septal defect

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

3 Alterations of aorta, aortic arch, azygos vein, carotid, ductus arteriosus, innominate, pulmonary, subclavian, vena cava, other

Table 4 (cont.): Foetal morphological findings. Visceral

Variations and retardations[1] Level of concern: low to moderate[2]	Abnormalities and malformations[1] Level of concern: High
Diaphragm	**Diaphragm**
-	Agenesis (absent) , partly ("holes") Diaphragmatic hernia
Liver	**Liver**
Enlarged lobe(s)	Agenesis (absent)
Fused lobes (<3)	Haemorrhagic
Discoloured (mottled)	Hepatomegaly
Pale	Infarctation
Supernumerary lobe	Malpositioned
	Misshapen (malformed, abnormal)
	Small
Gall Bladder	**Gall Bladder**
Bile duct alteration	*Bile duct alteration*
Elongated	Agenesis (absent)
Shortened	Misshapen (malformed, abnormal)
Gall bladder alteration	*Gall bladder alteration*
Absent (agenesis, aplastic) (rabbit only)	Misshapen (malformed, abnormal)
Enlarged	Supernumerary
Small (hypoplastic)	
Digestive Tract	**Digestive Tract [4]**
-	Agenesis (absent)
	Atresia (imperforate)
	Diverticulum
	Enlarged
	Fistula
	Malpositioned
	Narrowed
	Short
Spleen	**Spleen**
Discoloured	Absent (asplenia, agenesis)
Supernumerary (in rabbits)	Misshapen (malformed, abnormal)
	Malpositioned
	Small
	Splenomegaly
Adrenal	**Adrenal**
Enlarged	Agenesis (absent)
Malpositioned	Fused

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

4 Alterations of tongue, oesophagus, stomach, intesties, pancreas, rectum

Table 4 (cont.): Foetal morphological findings. Visceral

Variations and retardations[1] **Level of concern: Low to moderate[2]**	**Abnormalities and malformations[1]** **Level of concern: High**
Kidney *Kidney alteration* *Renal pelvic alteration* Dilated (if mild dilation and papilla still visible) Small Papilla	**Kidney** *Kidney alteration* Agenesis (absent) Enlarged Fused Hydronephrosis Malpositioned Misshapen (malformed, abnormal) Small Supernumerary *Renal pelvic alteration* Dilated (if papilla not visible; see also hydronephrosis) Misshapen (malformed, abnormal)
Ureter and Bladder *Ureter alteration* Convoluted Dilated (if not by distal obstruction)	**Ureter and Bladder** *Ureter alteration* Agenesis (absent) Doubled Hydroureter (dilated) *Urinary bladder alterations* Absent (acystia) Distended Small
Sexual Organs -	**Sexual Organs[5]** Agenesis (absent) Enlarged Haemorrhagic Malpositioned (displaced, ectopic) Misshapen (malformed, abnormal) Small Supernumerary

1 Terms in parenthesis are synonyms or explanations

2 The following findings may also evoke high concern depending on the affected organ system and frequency

5 Ovary, oviduct, uterine horn, testis, epididymis, vas deferens

4.4 Influence of maternal toxicity on study interpretation

4.4.1 Background

The relationship between maternal toxicity and effects on the developing foetus are important in interpreting of the outcome of developmental toxicity studies. In evaluating findings in the foetus, the level of concern may be reduced when there is evidence of maternal toxicity at the same treatment level. The basis for taking into considering maternal homeostasis in the evaluation and interpretation of developmental toxicity studies is reviewed below.

As the foetus is intimately reliant upon the dam for its development, it is reasonable to assume that an imbalance in maternal health status may, as a consequence, adversely affect normal foetal development. The possibility that maternal toxicity influences developmental toxicity is acknowledged in regulatory guidelines to the extent that guidance on dose level selection sets an upper limit based on maternal toxicity. Other expert opinion reinforces the care with which upper dose levels should be selected, and recognises the importance of maternal toxicity in determining the conditions for the conduct of a study, such that interpretation is not compromised (ECETOC, 1992).

The inter-relationship between maternal and developmental toxicities, and its influence on hazard assessments, should be judged on a case-by-case basis.

4.4.2 Evaluation of available information

It is biologically plausible that developmental toxicity may be mediated by maternal toxicity. A number of investigators have attempted to correlate specific maternal and developmental toxic effects, and to quantify these relationships. Generally such analyses have focussed on mechanistic studies into the aetiology of developmental toxicity and, for a number of developmental toxicants, toxicity appears to be related, at least in part, to disruption of maternal homeostasis (Daston, 1994; Carney, 1997).

Disturbances such as uterine hypoxia, hypercapnia, metabolic acidosis and alkalosis, ion imbalance and poor nutrition have all been implicated (Millicovsky and Johnston, 1981; Robertson *et al*, 1981; Watkinson and Millicovsky, 1983; Clark *et al*, 1984, 1986; Weaver and Scott, 1984a,b; Danielsson *et al*, 1989, 1990; Brent, 1990; Nakatsuka *et al*, 1993). In many cases the type and severity of effects can be replicated by direct physiological manipulation to mimic the suspected mechanism, such as uterine clamping for uterine hypoxia or food deprivation for reduced nutritional intake.

Environmental factors, such as physical stress, are also implicated in the induction of developmental toxicity and exacerbation of the effects of developmental toxicants (Beyer and Chernoff, 1986; Chernoff and Golden, 1988; Chernoff *et al*, 1988; Nelson *et al*, 1991; Harding and Edwards, 1993; Rasco and Hood, 1994a,b, 1995a,b). In these specific investigations, non-standard endpoints of maternal condition have been evaluated in order to understand more fully the changes in maternal homeostasis.

In the majority of studies however, evidence of a casual relationship is lacking. This is because the reporting of maternal toxicity in guideline developmental toxicity studies is limited, and usually founded on less sensitive endpoints than those studied in the assessment of developmental toxicity. For example, whereas the foetuses undergo intense examination, often including gross morphology, visceral and skeletal examinations, maternal toxicity assessment is usually limited to relatively crude estimates, such as survival and bodyweight gain (Hood and Miller, 1997). In view of these differences in the level of observations between the dams and the foetuses, it is not surprising that reviews attempting to demonstrate a relationship between maternal and foetal toxicity have been inconclusive or provide conflicting information.

Two reviews of 476 developmental toxicity studies in rodent and non-rodent species (Khera, 1984, 1985) concluded that maternal toxicity could be linked to characteristic patterns of adverse effects on the foetus. In particular, doses of test substances that induced maternal toxicity (indicated by decreased bodyweight, clinical signs of toxicity, or increased mortality) also corresponded with reduced foetal bodyweight, increased resorptions and foetal death. A consistent pattern of foetal malformations was also identified at maternal toxic doses. This consisted principally of exencephaly, open eyes and various malformations of ribs, sternebrae and spine in mice; fused ribs, exencephaly, encephalocele, micro- or anophthalmia in hamsters; and malformations of ribs, sternebrae and spine in rats and rabbits. The rarity of these malformations in the absence of maternal toxicity, and an apparent relationship between degree and severity of maternal and foetal lesions, led to the conclusion that toxicity to the maternal animal played a role in their aetiology. However, this conclusion has been questioned, since it is considered to have been favourably influenced by the fact that the data examined were confined to those from studies that had shown foetal effects in the presence of maternal toxicity.

In a study of ten diverse developmental toxicants in the CD-1 mouse, Kavlock *et al* (1985) compared a variety of maternal and developmental endpoints to test the hypothesis that acute maternal toxicity *per se* was intrinsically related to adverse developmental outcome. Pregnant females were dosed on gestation day 8 with doses of each chemical that induced maternal lethality (predicted LD_{10} and LD_{40}), and sacrificed on gestation day 18 for examination. Maternal effects that were evaluated included mortality, incidence of litters totally resorbed, number of viable litters, and weight gain.

Developmental effects included prenatal mortality, foetal weight, ossification, incidence of enlarged cerebral ventricles and renal pelvis, hydronephrosis, encephalocele, exencephaly, hydrocephaly, cranial defects, microphthalmia, cleft palate, agnathia, fused vertebrae, fused ribs, supernumerary ribs and umbilical hernia. The litter was considered to be the fundamental unit for comparison, and pooled group data was used for statistical analysis. Overall, and on the basis of the limited assessment of maternal condition, the authors found no correlation between maternal health status and developmental outcome. However, there was an increase in the incidence of supernumerary ribs, which occurred with seven of the ten chemicals and was significantly correlated ($R^2 = 0.45$, $P<0.001$) with decreased maternal weight gain.

Chahoud *et al* (1999b) quantitatively analysed the relationship between maternal bodyweight gain through gestation and several developmental parameters in the Wistar rat, using data collected in several studies of "well-known [unidentified] teratogenic substances". This review did not identify a relationship between maternal bodyweight

gain and foetal effects. There were some limitations to this analysis due to the data used and the manner in which it was treated. Firstly, the combination of control data from several studies might mask significant temporal changes in the historical control database. Secondly, the use of uncorrected bodyweight gain as the index of maternal toxicity not only includes the litter as a component, but also may mask subtle effects on bodyweight during gestation.

Where a causal relationship between maternal and developmental toxicity has been established, the level of concern for the developmental effects is reduced. Where there are insufficient data to support a causal relationship, this may lead to the conclusion that there is a specific developmental toxicity resulting from the exposure. However, where minor developmental changes are observed only in the presence of maternal toxicity, it may be appropriate for the level of concern to be reduced.

Current practice for conducting a programme of studies designed to investigate developmental toxicity encourages evaluation of maternal toxicity in greater depth than in the past. Usually one or more range-finding studies in pregnant animals are carried out, the primary aim of which is to determine maternal toxicity and to put this in the context of the toxicological profile of the substance that is often already available from other studies. In order to attain this goal, investigations including clinical chemistry, haematology and histopathology may be undertaken. Mechanism-specific endpoints such as hormone levels, oxygen levels, ion balance and renal clearance can also be assessed on a case-by-case basis in order to understand better the state of health of the dam.

4.4.3 Conclusions

A number of conclusions can be drawn from the review of the available information concerning the relationship between maternal and developmental toxicity:

- It is biologically plausible that maternal toxicity can affect developmental outcome, and this should be taken into account in evaluating developmental toxicity studies. Indeed current regulatory guidelines reflect the need to ensure that the maximum dose selected for a study limits the extent of maternal toxicity that will be evoked.
- Conclusive evidence for a causal relationship between maternal and developmental toxicities has been reported for a number of specific cases in which there was extensive study of a range of biological indices in the dams as well as in the offspring.
- Evaluations of historical datasets from developmental toxicity studies, where only relatively superficial (gross) observations on the dams are reported as compared with the more extensive study of the foetuses, do not provide conclusive correlations between maternal toxicity and developmental outcomes.
- The observation that minor developmental effects are seen only in the presence of maternal toxicity should prompt circumspection in evaluating the outcomes of developmental toxicity studies. Where major effects are observed in the dam associated with only minor effects in the offspring, this may mitigate the level of concern assigned.
- Developmental toxicity studies that evaluate maternal homeostasis in more detail (as is more frequently the case with current practice) will enable greater confidence in using maternal condition to set the level of concern over developmental findings in those studies.

5. EFFECTS ON FERTILITY AND REPRODUCTION

5.1 Purpose

The ultimate objective of single- and multi-generation reproduction studies is the determination of potential hazard to human reproduction. To achieve this goal, these studies should detect any effect that is related to the reproductive process. The endpoints of the reproductive process which are investigated mainly relate to reproductive performance and changes in the weight or morphology of reproductive organs; some study designs also evaluate sperm parameters, oestrous cycle, follicular toxicity, sexual and reproductive development and post-natal development in general. The purpose of this section is to identify and discuss these endpoints and classify them according to the concern they evoke. This should help experts identify and assess chemicals for their potential to produce adverse effects on reproduction in humans.

5.2 Studies

5.2.1 One- and two-generation studies

Findings in one- and two-generation toxicity studies, which may be indicative of a reproductive hazard include mating behaviour, the outcome of mating (fertility), and the survival of offspring both pre- and post-natally. Other findings that may indicate a reproductive hazard include sperm quality, organ weight / morphology and oestrous cycle effects. Changes in these latter endpoints may or may not be accompanied by an overt effect on reproductive outcome. Furthermore, in two-generation studies, effects on sexual maturation and the reproductive capacity of the progeny may be indicative of a reproductive hazard for fertility resulting from exposure to the chemical *in utero*, during early post-natal development and up to the time of sexual maturation.

All findings of possible effects on reproduction in such studies must be evaluated collectively. In addition, effects that may indicate a potential impairment of reproductive function should always be evaluated with reference to systemic toxicity occurring at comparable doses. Severe systemic toxicity may result in secondary effects on male and female reproductive function as well as effects on a variety of other organ systems.

5.2.2 28-Day and 90-day studies

General aspects

Test protocols for both the 28-day (OECD, 1995b) and 90-day (OECD, 1998; EPA, 1998a) toxicity studies can be used to identify a test substance as a potential reproductive hazard. This conclusion is most likely to be based on morphological parameters, as these study types do not provide information routinely on the function of the reproductive system (sexual behaviour, fertility, and pregnancy outcomes), nor on effects of the test substance on the developing animal.

Findings in 28-day or 90-day toxicity studies which may be indicative of a potential reproductive hazard include gross necropsy findings, effects on organ weights and / or histopathological findings in sexual organs of male or female animals. Additional endpoints may include sperm analyses and oestrous cyclicity. All these findings should be evaluated coherently; if available, data from one- and two-generation studies should be considered. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, level of statistical significance for intergroup differences, number of endpoints affected, relevance of route of administration to humans, and freedom from bias.

Histopathology

Histopathological findings are of greater importance in influencing hazard classification than isolated changes in weight or macroscopic findings in single organs.

In studies conducted according to the earlier (1981) version of TG 407 histopathological examination of reproductive organs and accessory sex organs was not required. Thus, in most older publications and study reports a correlation between organ weights and findings from histopathological examination is missing. In the update of this guideline (OECD, 1995b), histopathology of reproductive and accessory sex organs became a requirement. In those studies where histopathological examinations are missing, the data are of less use for assessing potential effects on the reproductive system.

In Table 5, general histopathological findings are presented; these details include different grading and distribution patterns (e.g. focal, multifocal / multiple, diffuse, unilateral or bilateral). The findings should be evaluated with respect to the animal strain used and the historical control data available for that strain. Due to the relatively short duration of the 28-day study (and, therefore possibly higher dose levels applied and tolerated than in subchronic studies) this often serves as a range-finding study to derive initial repeated dose information on the toxicity of a new (toxicologically-uncharacterised) substance. Different findings may occur compared to subchronic or chronic studies (e.g. tumours are normally not seen in 28-day or 90-day studies). Furthermore, individual laboratories use different methods e.g. for staining, grading of findings, evaluation of spermatogenesis (staging or other methods of semi-quantitative estimation of spermatogenesis). In addition, evaluation of accessory sex organs (i.e. prostate, seminal vesicle, coagulation gland) is often limited by guideline requirements and restricted to prostate only. For these reasons it is important that histopathological findings, potentially indicating an effect on the reproductive system, should be evaluated versus systemic toxicity and findings seen at comparable dose levels in other toxicity studies, especially studies of longer duration.

The significance of gross necropsy findings, organ weight changes and histopathological findings in two-generation studies is comparable to that in 28-day or 90-day toxicity studies. In contrast to 28-day / 90-day studies, two-generation studies allow a direct correlation between morphological changes and functional consequences. It also has to be recognised that the administration period for the second generation of a two-

generation study covers the whole life cycle from conception to sexual maturation. Thus effects on sexual organs or reproductive function may be induced that are not seen in 28-day / 90-day studies.

The levels of concern for those parameters of two-generation studies that are not assessed routinely in 28-day / 90-day studies, such as oestrous cycle, reproductive indices, sperm parameters and sexual maturation indices, are specified in Table 5.

5.3 Definition of terms

5.3.1 Reproductive performance

This covers most of the fertility and litter data derived from traditional reproduction studies. Standard judgements on, for example, degree of change, dose response, statistical significance and historical control range, apply in defining a change as adverse. The endpoints of interest are the various indices of male and female fertility and include gestation length, precoital interval, litter size, live-born index, pup survival and sex ratio.

5.3.2 Histopathological changes to the reproductive tract

The evaluation of histopathological changes can yield important information on effects induced by chemicals on the reproductive tract. It is acknowledged that changes in the weight and / or morphology of reproductive organs in the rat (especially the male), are important in identifying reproductive toxicants (Clegg *et al*, 1986; Morrisey *et al*, 1988; Zenick and Clegg, 1989; Linder *et al*, 1992; Moore *et al*, 1995; Takayama *et al*, 1995; Ulbrich and Palmer, 1995). Typically, such changes can be detected in standard 28-day, 90-day and reproductive toxicity studies.

Sophisticated non-standard methods of evaluation are needed if the intention is to detect low levels of change in the male reproductive system in studies of 28-day duration or less. In longer term investigations, effects on testis morphology can be accompanied by changes in epididymal sperm number, motility or morphology, which occur consequential to the initial lesion. Stage-specific analysis refers to techniques for identifying and characterising injury to the testis at specific stages of the cycle of the seminiferous epithelium. Staging (only relevant in studies of up to 28 days duration), can therefore be used to recognise abnormalities and to determine the target cell for toxicity and the stage of spermatogenesis affected. This approach is not routine and is usually undertaken as a result of observing a change in sperm quality or in the outcome of mating.

Testicular histopathology is one of the most sensitive indicators of damage, and may or may not be accompanied by a change in the functional outcome of mating. For regulatory assessment, stage specific analysis is typically conducted in order to understand the aetiology of a change identified by other endpoints.

5.3.3 Sperm parameters

Sperm number, motility and morphology

Although guidelines (OECD, 2001b; EPA,1998b) are quite clear as to the type of measurements required, there still remains a fundamental lack of standardisation of technology and statistical techniques in this field. Regulatory agencies recognise this and in particular that large scale safety assessments are likely to be undertaken using computer assisted sperm analysis (CASA). Methodologies and databases in this area are still evolving. Changes in sperm parameters in isolation should not be used to judge the reproductive toxicity of a chemical.

Sperm evaluation by manual methods

With regard to sperm number, sperm motility (% motile sperm) and sperm morphology, retrospective evaluations have indicated that these endpoints are no more sensitive than 'traditional' endpoints for assessing male reproductive function (Ulbrich and Palmer, 1995). Changes in manually assessed sperm parameters should therefore only serve to confirm those findings already detected in other endpoints (above). In the opinion of the authors, these additional data should therefore present no additional challenges in the interpretation of the results from standard studies (i.e. level of concern is high).
Analysis of sperm parameters by CASA

CASA is now increasingly used in toxicity tests. The endpoints evaluated can include percentage of motile sperm, velocity (curvilinear, straight line, average path and progressive), linearity, beat cross frequency, and amplitude of lateral head displacement. The actual value of these measurements is confounded (as mentioned above) by non-uniform methodology, lack of consensus of the appropriate statistical analyses, limited data on compound effects, lack of background control ranges, and a lack of understanding of the biological significance of altered sperm motion characteristics. However, there is evidence of, and an anticipation that, such endpoints are / may be more sensitive than other measurements.

The establishment of a LOAEL / NOAEL on the basis of small changes in sperm parameters alone is considered inadvisable. Information on such changes should only be evaluated in conjunction with other data consistent in principle with the EPA risk assessment guidelines (EPA, 1996). The EPA has signalled an intention to revisit this when significant data from studies submitted have been evaluated.

ILSI (1999) provides a valuable comment on integration of endpoints in the assessment of effect in male reproductive capability.

> "An isolated change in any one of these endpoints (testicular sperm number, epididymal sperm count, testis weight and morphology) is cause for less concern than a suite of related changes, all internally consistent. This mutual support among endpoints allows for a weight of the evidence approach to be taken when interpreting data from the male reproductive system. This weight of the evidence is clearly the

best way to view these data and is strongly recommended for all analysis of male reproduction data. The relevant US EPA requirements for all these endpoints to be routinely evaluated aid in the process and demand a weight of the evidence evaluative strategy in return".

5.3.4 Oestrous cycle

Characteristic changes in cytological composition of the vaginal smear occur in response to alterations in blood oestradiol and progesterone concentrations; these change cyclically throughout the reproductive lifespan of laboratory rodents. The purpose of quantifying cycle length is to monitor the functional status of the hypothalamic-pituitary-ovarian axis and reproductive tract. Alterations in nutritional status, stress, housing, day length and proximity of male animals are all known to alter oestrous cyclicity. Disturbances of the vaginal epithelium when taking smears can induce pseudopregnancy. There are no agreed criteria for defining 'abnormal' in the context of alterations in the regularity of changes in the cytology of vaginal samples. Changes should therefore be interpreted in conjunction with reproductive data, which includes but is not limited to, the outcome of mating studies and ovarian histology.

ILSI (1999) offers sound advice on the level of concern generated by abnormalities of ovarian cycles.

"The complete cessation of vaginal cycling in response to toxicant treatment should be considered adverse and a reflection of change in the underlying endocrine milieu. Subtle changes in the oestrous cycle pattern without associated changes in other endocrine or reproduction endpoints would not be considered adverse or sufficient to identify a compound as a reproductive hazard. Because oestrous cyclicity provides an evaluation of hormonal status and reproductive capacity, significant disruption of cyclicity should be accompanied by functional reproductive changes. Therefore, subtle changes in oestrous cycling in the absence of changes in reproductive outcome would not in and of themselves be considered an adverse reproductive effect."

5.3.5 Follicular toxicity / oocyte quantitation

This endpoint is considered a marker of female reproductive toxicity. Agents that deplete the pool of primordial follicles could lead to premature reproductive senescence. Quantitation of primordial follicles is now an endpoint in the EPA and draft OECD test guidelines. There is no agreement on the level of follicle depletion that should be considered as adverse, and the differences between the mechanisms underlying reproductive senescence in the rat and in humans make interspecies extrapolation difficult.

This endpoint has been the subject of much inconclusive debate. ILSI (1999) summarises this:

"There was no consensus regarding what degree of change in follicle number should be considered adverse. It was suggested, however, that a detectable decrease in follicle number should be considered adverse".

5.3.6 Development

Sexual and reproductive development

The level of concern is high for changes in time to preputial separation and vaginal opening that are not accounted for by bodyweight.

Measurement of the time of preputial separation and vaginal opening is required by the current reproduction toxicity guidelines of OECD and EPA (OECD, 2001b; EPA, 1998b). The requirement to measure anogenital distance is triggered by alterations in the above parameters and by a change in the sex ratio of pups in a previous generation. Chemically induced changes in all or any of these endpoints are commonly associated with other effects on the development of the genitalia and on sexual performance. Although there is no consensus on the degree of change that is considered adverse, there is concern about the endocrine dependent nature of sexual development and the implications of any changes. A distinction can be made between those changes accounted for by general alterations in the development of the offspring, and those indicating a direct and specific effect.

ILSI (1999) comment on this endpoint:

> "In general, delays in preputial separation as in vaginal opening that are accompanied by delays in the onset of other developmental markers likely suggest an overall effect on growth and development. Delay in these events in the absence of effects on body weight or other developmental marks suggest a specific effect on the development of the prepuce or vagina or the endocrine control of pregnancy. Effects on preputial separation or vaginal opening in a developmental or reproductive toxicity study should be considered as adverse for human health risk assessment, particularly if the effect is irreversible such as results from permanent malformation. There was no consensus (from ILSI workshop) regarding what degree of change in anogenital distance should be considered adverse and relevant for human health risk assessment."

Post-natal development

The level of concern is high if not accounted for by systemic toxicity or / and if observed at dose levels with no parental toxicity.

In one- and two-generation reproduction toxicity studies observations are made which allow the evaluation of post-natal developmental toxicity. These are pup bodyweight and bodyweight gain during lactation and at weaning, pup survival during lactation and at weaning and the general clinical condition. Data such as litter size, external malformations, physical and functional development and behaviour can also be used to confirm adverse findings in developmental toxicity studies. Care should be exercised in interpreting changes in offspring bodyweight, especially in studies conducted by the dietary route of exposure. A constant level of the test article (ppm) in the diet leads to peaks of exposure to the neonate from the end of the first week of lactation when the offspring is potentially exposed to the test compound by the dietary route (and consumes a high amount of diet relative to its bodyweight) and via lactation. This can be addressed experimentally by varying the dietary inclusion rate to avoid such peaks of exposure to the test substance by the offspring.

5.4 Guidance on ranking of adverse findings on reproduction for level of concern

The levels of concern indicated for the reproductive toxicity endpoints listed in Tables 5 and 6 are intended as a guide for evaluating the importance of the lesions or changes observed in the hazard assessment process. Such lesions or changes should not be considered in isolation but in the context of all other findings, following a weight-of-evidence approach.

When the individual findings are evaluated and ranked for their level of concern, several criteria should be considered which may alter the final assessment:

- The level of concern is elevated if a finding occurs more frequently together with other findings pointing to the same mechanism of action (e.g. modulation of endocrine control).
- The level of concern is elevated if the finding occurs in isolated pups from several litters rather than if all pups of only one litter are affected (e.g. post-natal developmental effects).
- The level of concern is elevated if the finding occurs at maternally non-toxic doses.
- The level of concern is elevated when the finding is corroborated by findings in other, non-reproductive toxicity studies (e.g. decrease of sperm production and histopathological changes in the testis).
- The level of concern for a given finding may be further influenced by the species in which the observation was made.
- The level of concern for a given reproductive / developmental finding is elevated when the magnitude or incidence of that finding is progressively higher in subsequent generations.

Table 5: Reproductive and 28-day/90-day repeated dose toxicity studies. Observations and levels of concern

Organ Weights[1]

Organ	Finding	Level of concern	Comment
Testis, epididymis, seminal vesicles, prostate, coagulation gland	Change in weight	Moderate, if not supported by other data None, in case of severe bodyweight decrease and if not supported by other data	
Uterus, ovaries	Change in weight	Moderate, if not supported by other data None, in case of severe bodyweight decrease and if not supported by other data	Depends on stage of sexual cycle
Pituitary	Change in weight	Moderate, if not supported by other data	

Macroscopic changes

Organ	Finding	Level of concern	Comment
Testis	Change in size	Moderate, if not supported by other data (testis weight excluded) None, in case of severe bodyweight decrease and if not supported by other data	
	Change in consistency	Low, if not supported by other data	
Uterus	Accumulation of fluid	High, if not dependent on sexual cycle	
Ovaries	Change in size	Moderate, if not supported by other data (ovary weight excluded) None, in case of severe bodyweight decrease and if not supported by other data	

[1] Evaluation of organ weights should be based on established statistical significance and biological relevance, taking account of historical control data. Data should be evaluated as a ratio relative to bodyweight or otherwise adjusted for bodyweight changes.

Table 5 (cont.): Reproductive and 28-day/90-day repeated dose toxicity Ssudies. Observations and levels of concern

Histopathology

Organ	Finding	Level of concern	Comment
Testis	Degeneration/necrosis of germinal epithelium	High, if multifocal or diffuse, bilateral	
	Alteration of sperm maturation	High, if multifocal or diffuse, bilateral	
	Azoospermia	High	
	Oligospermia	High, if moderate to severe, multifocal or diffuse, bilateral	
	Degeneration/necrosis/decreased number of Sertoli cells	High, if multifocal or diffuse, bilateral	Difficult to evaluate quantitatively
	Orchitis	High, if multifocal or diffuse, bilateral	
Epididymis	Inflammation (epididymis)	High, if multifocal or diffuse, bilateral	
	Fibrosis	High, if multifocal or diffuse, bilateral	
	Accumulation of debris in the lumen of the tubuli	High, if bilateral	Check testis
	Sperm granulosa	High, if bilateral	
Prostate	Atrophy	High, if diffuse	
	Inflammation	High, if multifocal or diffuse	
	Hypertrophy	Moderate, if not supported by other data	
	Amount of secretary product reduced	Moderate, if not supported by other data	
Seminal vesicle	See prostate	See prostate	Not specified in TG 407
	Hypo-/atrophy[2]	Moderate, if not supported by other data	
	Focal hyperplasia	Moderate, if not supported by other data	
Coagulation gland	See prostate	See prostate	Not specified in TG 407
	Hypo-/atrophy[2]	Moderate, if not supported by other data	
	Focal hyperplasia	Moderate, if not supported by other data	

[2] Possible findings in 90-day toxicity studies; in dogs, metaplasia of urethral epithelium may develop

Table 5 (Cont.): Reproductive and 28-day/90-day repeated dose toxicity studies. Observations and levels of concern

Histopathology (cont.)

Organ	Finding	Level of concern	Comment
Ovaries	Arrest of ovarian cycle (reduced/no secondary and/or tertiary follicles)	High	Check cycle
Ovaries	Cystic follicles, if multiple and bilateral	Moderate	Check cycle
	Cystic *corpus luteum*, if multiple and bilateral	Moderate	Check cycle
Uterus	Atrophy	High	Check cycle
	Inflammation/pyometra, if moderate or severe	Moderate	Check cycle
	Hyperplasia	Moderate, if not supported by other data	
	Hydrometra	Moderate, if not supported by other data	Check cycle
Pituitary	Cells of the anterior lobe showing a vacuolated cytoplasm,'Crooke-cells' (castration cells)	High	Sign of degenerative alterations and/or endocrine dysregulations clarify findings by electron; microscopy and/or mmunohistochemistry
	Atrophy of basophils in the anterior lobe	High	Disruption of the hypo-thalamus pituitary gonads axis

Table 6: Reproductive toxicity studies - Observations and levels of concern

Endpoint	Level of concern	Comment
Sexual / reproductive development		
1. time of vaginal opening	High for 1 and 2, if not accounted for by bodyweight changes	F_1 generation (1 and 2)
2. time of preputial separation		
3. anogenital distance	High for 3, with change in sex ratio and / or sexual development	F_2 generation (3)
Oestrous cycle		
- cycle length	Low, if not supported by other data	Depends on blood hormone concentrations
Reproduction indices		
1. period of gestation	Low / moderate for 1, unless accompanied by alteration in mating / sexual behaviour.	Prolongation of the precoital interval may occur as a result of hormonal changes (see also oestrous cycle)
2. precoital interval (time for mating)		
3. number of mated as % of those placed together (mating index)		
4. number of pregnant as % of mated (fertility index)		
5. pups stillborn as % of pups delivered (perinatal losses)	High for 2-7, if not accounted for by systemic toxicity.	
6. prenatal loss: difference of total implantations and number of pups born as % of implantations[1]		
7. litter size at birth	High if observed at a dose that does not cause parental toxicity.	

[1] Only possible if a teratology subgroup is included

Table 6 (cont.): Reproductive toxicity studies - Observations and levels of concern

Endpoint	Level of concern	Comment
Sperm parameters 1. number of homogenisation-resistant spermatids and cauda epididymal sperm reserves, respectively 2. sperm motility 3. sperm count 4. sperm morphology	Low / moderate for 1-4, if not supported by other data	Sometimes included in 28 / 90-day repeated dose toxicity study designs
Post-natal development 1. pup survival during lactation / weaning 2. pup bodyweight / gain 3. pup clinical condition	High for 1-3, if not accounted for by systemic toxicity High if observed at a dose that does not cause parental toxicity	

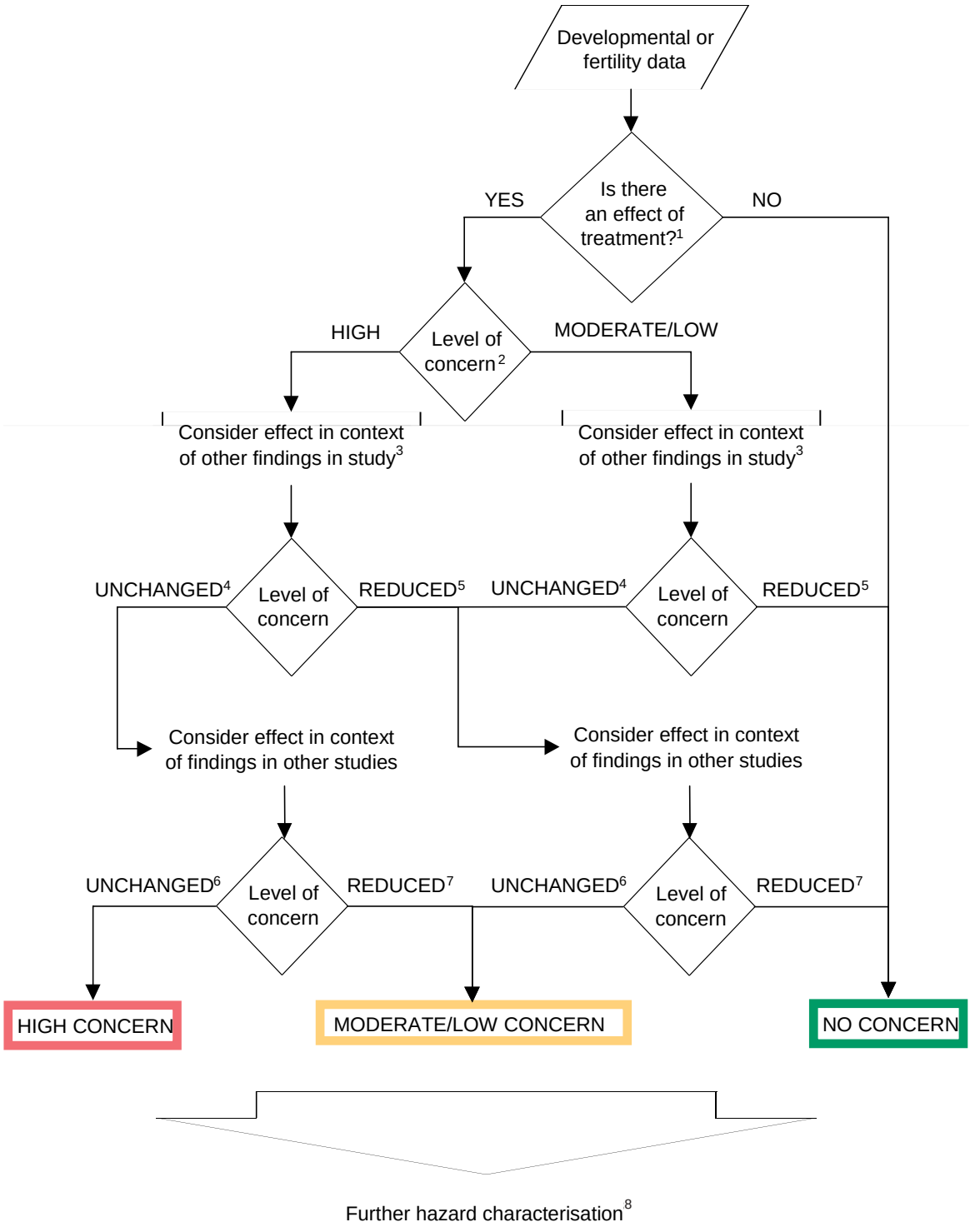
6. STRUCTURED APPROACH TO EVALUATION OF REPRODUCTIVE TOXICITY DATA

The evaluation of reproductive toxicity data is a complex process that takes account of a variety of information having different degrees of importance in defining the nature and severity of the hazard. In this Section a structured approach to the evaluation of such data has been developed (Figure 7) and applied to a number of worked examples.

- The process is initiated by the compilation of a dataset for a particular study. This dataset is analysed to determine whether there is an effect of treatment on development or fertility, and should take into account factors such as the quality of the dataset, statistical significance of changes and their biological relevance, dose-response relationship, and comparison with historical data. If there is no relevant effect of treatment, then no concern is attributed to the dataset, and the process is concluded.
- If there is an effect of treatment upon development or fertility, then a level of concern, either low, moderate or high, should be attributed in order to characterise hazard. For guidance, the reader is directed to the relevant tables in Sections 4 and 5.
- Next, the occurrence of other effects in the dataset should be considered. For developmental toxicity, findings should be considered in relation to maternal toxicity, whilst for effects on fertility, consideration should be given to systemic or parental toxicity. This will either confirm the level of concern initially set (if the developmental or fertility effects cannot be causally associated with maternal or parental / systemic toxicity) or reduce the level of concern (where maternal or parental / systemic toxicity is considered to be causal or contributory).
- The dataset should then be considered in the context of other studies, which either corroborate or counter the findings, or provide information on complimentary aspects such as kinetics, underlying mechanisms of systemic and / or reproductive toxicity, and relevance / extrapolation to man. The level of concern may be reduced if a species-specific response is demonstrated, or where mechanistic understanding indicates that reproductive toxicity is secondary to other toxic effects, or when the effect is not reproducible. The level of concern may be confirmed if similar findings at similar doses are reported in comparable studies or if, for example, complementary histological findings are reported in studies not primarily focused on reproductive toxicity.
- The process described here is directed at defining the intrinsic level of concern within a specific study. Comparison with reproductive toxicity data from other studies must include aspects such as differences in protocol (e.g. dose-response and species differences). Therefore, the evaluation of the dataset in the context of other reproductive toxicity studies should be part of an extended process, which defines the predicted hazard to man. Weight should be given to those studies that result in the greatest level of concern, unless scientific justification can be given for discounting them.

Summary data sets have been derived from study reports and publications on a broad range of chemical substances forming working examples to test the data evaluation process. These data sets are presented in Appendix B.

Figure 7: Evaluation of reproductive toxicity data



1 Statistically significant changes, dose-response relationship, biological relevance, historical data, data quality

2 Use appropriate tables in respective section

3 In context of maternal toxicity for development, in context of systemic / parental toxicity for fertility

4 Effects not causally associated with maternal or parental / systemic toxicity

5 Effects associated with maternal or parental / systemic toxicity

6 Confirmed by other data or studies

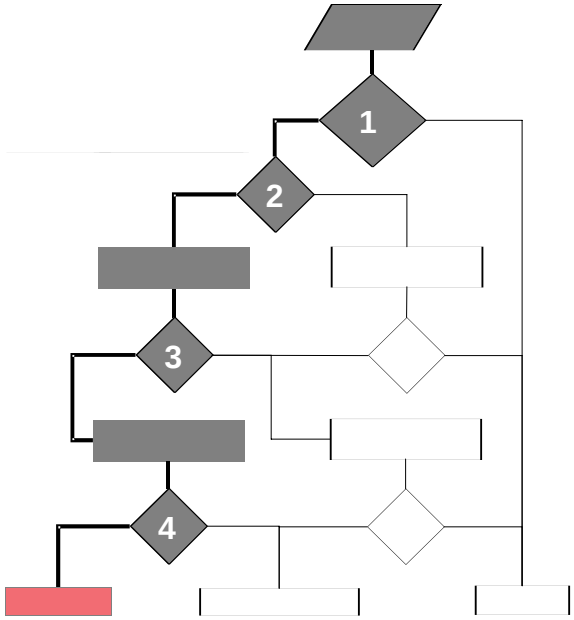
7 Species-specific response proven, mechanistic understanding gained, effect not reproducible

8 Dose levels at which development / fertility effects occur, other routes of administration, species differences

6.1 Worked examples

Several examples of substances that are toxic to development or fertility were used to illustrate the process described at the beginning of this section. These were either abstracted from the results of specific toxicology studies or are representative illustrations constructed to demonstrate a point and are derived from the combined experience of the Task Force members. The data sets used are presented as Appendix B.

Developmental toxicity - example 1



1. Is there an effect of treatment?

Increased number of resorptions (post implantation loss), decreased foetal weight, evidence of malformations.

⇒ **Yes**

2. Level of concern

External foetal effects include cleft palate, exencephaly, spina bifida. These findings are ranked in Tables 2-4.

⇒ **High**

3. Consider effect in context of other findings in study

Decreased maternal bodyweight at high dose (not adjusted) does not explain post implantation losses and the severe foetal malformations. Furthermore, increased incidences of variations at the sub-teratogenic dose indicate a dose-response relationship.

⇒ **Level of concern unchanged**

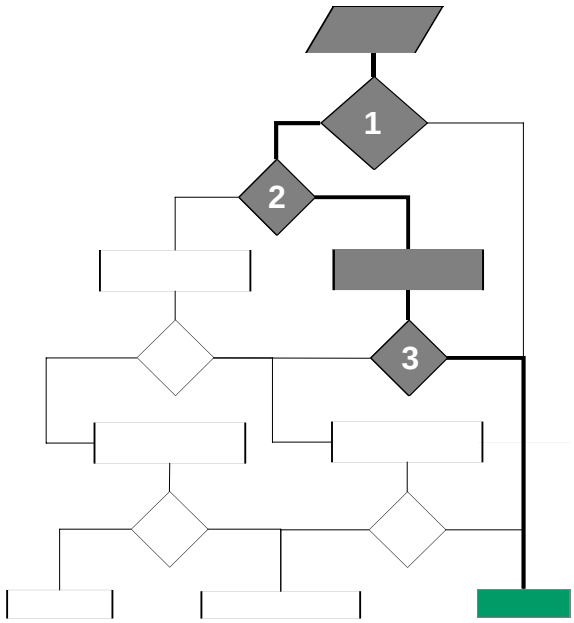
4. Consider effect in context of findings in other studies

This substance belongs to the chemical class of retinoids that is well known for teratogenic properties.

⇒ **Level of concern unchanged**

Conclusion: High level of concern

Developmental toxicity - example 2



1. Is there an effect of treatment?

Dose-related foetal growth reduction; incidence increased of incomplete ossification of sternebrae; dose-related increase in unossified sternebrae.

⇒ **Yes**

2. Level of concern

Foetal effects are limited to growth retardation and incomplete ossification. These findings are ranked in Tables 2-4.

⇒ **Low or moderate**

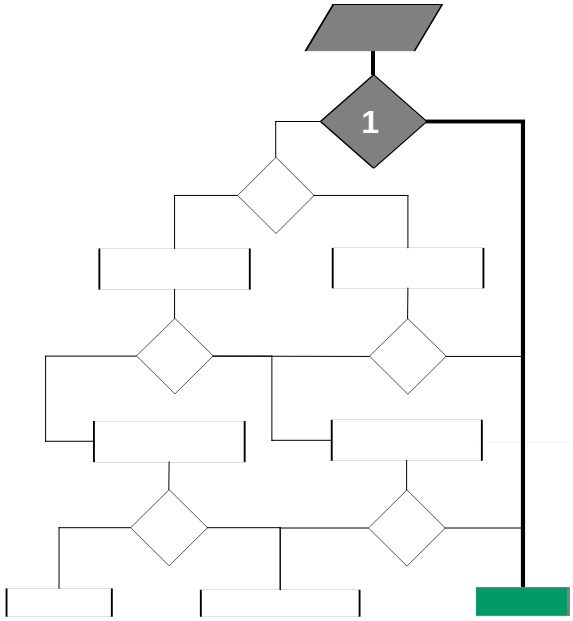
3. Consider effect in context of other findings in study

Dose-related maternal toxicity (reduced bodyweight and clinical signs of toxicity). Maternal toxicity is characterised by marked clinical effects whilst foetal findings are mild with no increase in post-implantation loss (no data shown).

⇒ **Level of concern reduced**

Conclusion: No concern

Developmental toxicity - example 3



1. Is there an effect of treatment?

No adverse affects observed in dams or foetuses

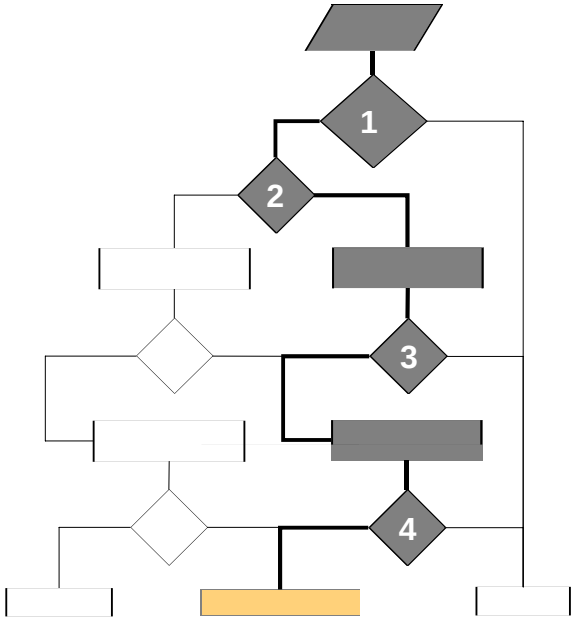
⇒ **No**

Conclusion: No concern

Still consider:

- was dose high enough?
- quality of study?
- data from other studies?

Developmental toxicity - example 4



1. Is there an effect of treatment?

No indications for foetal malformations, but dose-related reduction in foetal bodyweights and increased rib variations at the two highest doses (143 and 76 mg / kg bodyweight).

⇒ **Yes**

2. Level of concern

The observed foetal findings were reduction in foetal bodyweight and skeletal variations.

⇒ **Low / moderate**

3. Consider effect in context of other findings in study

Marginal signs of maternal toxicity (slight increases in kidney weight) at the highest dose. Slight indications for developmental toxicity without effects on the dams confirm the level of concern.

⇒ **Level of concern unchanged**

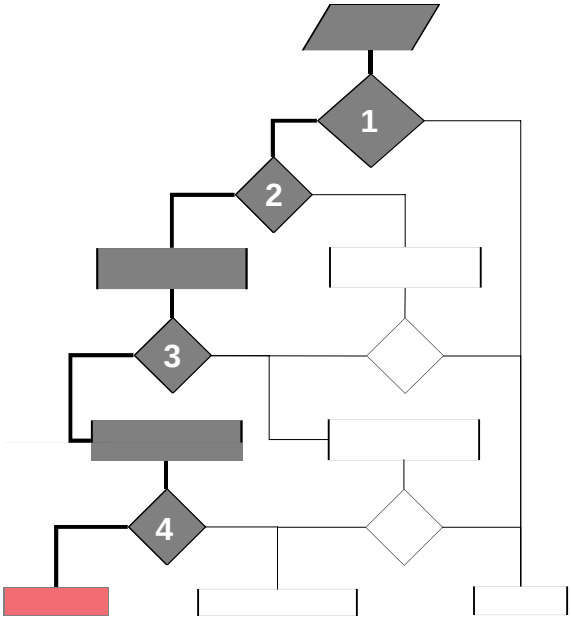
4. Consider effect in context of findings in other studies

In a second study (Example 5, below) with the same test compound with dose levels ranging from 78 - 537 mg / kg bodyweight, clear indications for foetal malformation appeared at the two highest dose levels. However, since these are outside the dose range of the present study, they do not alter the conclusion.

⇒ **Level of concern unchanged**

Conclusion: Low / moderate level of concern

Developmental toxicity - example 5



1. Is there an effect of treatment?

Increased number of resorptions at the highest dose (537 mg / kg bodyweight); dose-related decrease in foetal bodyweights at all dose levels; evidence of external and visceral malformations at the two highest dose levels; rib variations at all dose levels.

⇒ **Yes**

2. Level of concern

Gross morphological findings in the foetuses include eye, brain and tail malformations

⇒ **High**

3. Consider effect in context of other findings in study

Dose-related signs of maternal toxicity (bodyweight gain) at 163, 330 and 537 mg / kg bodyweight. The extent of maternal toxicity does not explain the severe foetal malformations at the two highest dose levels.

⇒ **Level of concern unchanged.**

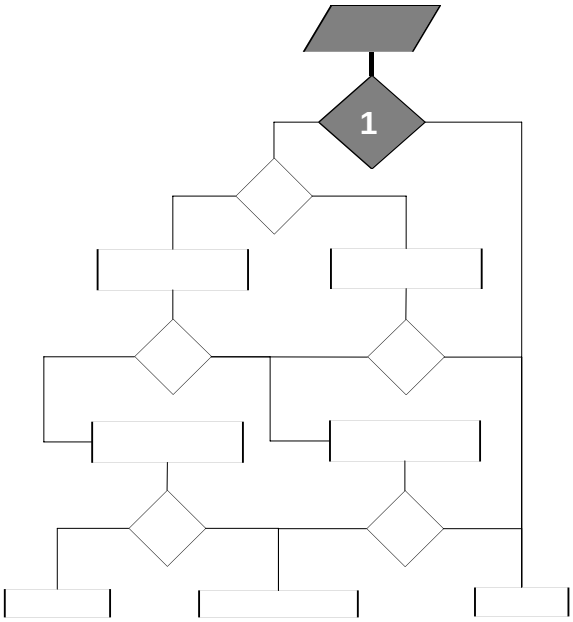
4. Consider effect in context of findings in other studies

In a previous study (Example 4, above) with the same compound at lower dose levels (19-143 mg / kg bodyweight), no foetal malformations were observed. However, there were other signs of developmental toxicity (marginal reductions in foetal bodyweights and increased rib variations) at 76 and 143 mg / kg bodyweight. The only sign of maternal toxicity was slightly increased relative kidney weights the top dose.

⇒ **Level of concern unchanged**

Conclusion: High level of concern

Developmental toxicity - example 6



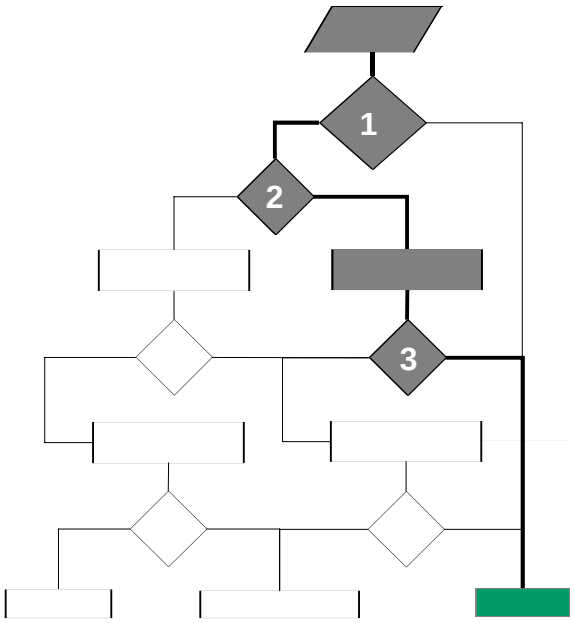
1. Is there an effect of treatment?

The data presented are insufficient for an assessment:

- type of study is not indicated
- exposure of animals only refers to concentration of chemical in drinking water and not to the dose received
- only one dose level

⇒ **No assessment possible due to insufficient data**

Developmental toxicity - example 7



1. Is there an effect of treatment?

No indications for foetal malformations, but reduced foetal bodyweights at the top dose (175 mg / kg) and skeletal findings (e.g. ribs bent) at mid (75mg / kg) and top dose.

⇒ **Yes**

2. Level of concern

The degree of the reduction in foetal bodyweights is of low / moderate concern. All observed effects on foetal morphology are either fully within or close to the historical control ranges given for the different findings. Historical controls for "sternebrae 5, 6 unossified" are given with a range of 1-37 (foetal basis). This does not seem to be reliable. However, unossified sternebrae 6 and especially 5 are common observations in controls and do not pose a concern at the 18% incidence.

The "bent limbs" which might be of some concern are observed at a low incidence (1%).

⇒ **Low / moderate**

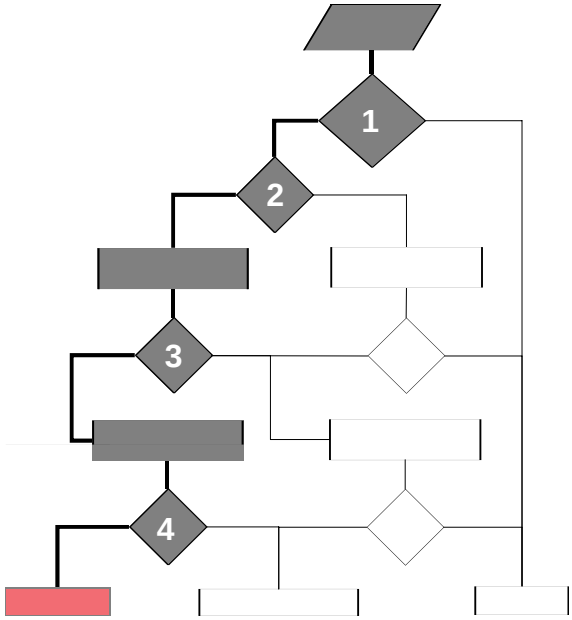
3. Consider effect in context of other findings in study

Distinct signs of maternal toxicity at mid and high dose levels (75 and 175 mg / kg bodyweight) at initiation of treatment. The distinct reductions of maternal bodyweight gains and the reduced food consumption at the mid and high dose at initiation of treatment shift the level of concern for developmental toxicity from low / moderate to no concern.

⇒ **Level of concern reduced**

Conclusion: No concern

Fertility toxicant - example 8



1. Is there an effect of treatment?

Infertility occurred in all high dose males. Distinctly reduced sperm count, sperm motility and reduced testis weights at 636 and 1,262 mg / kg bodyweight. Impaired number of live pups in all generations at 636 mg / kg bodyweight and occasionally reduced pup bodyweights and reduced pup survival.

⇒ **Yes**

2. Level of concern

Distinct effects on sperm parameters, testis weights and number of live pups.

⇒ **High**

3. Consider effect in context of other findings in study

Signs of general parental toxicity (i.e. decreased bodyweights in both sexes) at the top dose (1,262 mg / kg). Indications for impaired fertility also appeared at a dose without general toxicity.

⇒ **Level of concern unchanged**

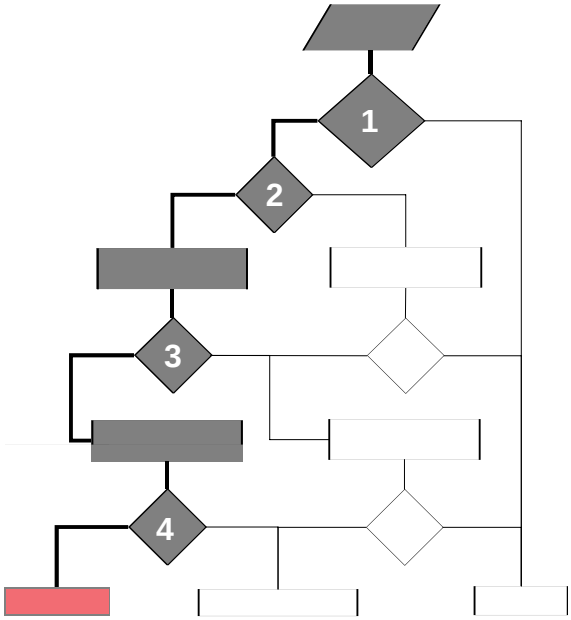
4. Consider effect in context of findings in other studies

Indications for impaired fertility (i.e. infertility) were also present in a dietary 3-generation study in rats with the same compound (Example 9, below) at the high dose level (334 mg / kg bodyweight) with only marginal effects of general toxicity.

⇒ **Level of concern unchanged**

Conclusion: High level of concern

Fertility toxicant - example 9



1. Is there an effect of treatment?

Infertility of all high-dose rats. Decreased pup survival in the low- and the mid-dose groups (10 and 33 mg / kg bodyweight) affecting F_{1a} and F_{1b} litters.

⇒ **Yes**

2 Level of concern

Infertility of all high-dose rats.

⇒ **High**

3 Consider effect in context of other findings in study

Only marginal signs of general parental toxicity (e.g. inflamed eyelids, rough fur, decreased bodyweights in both sexes on week 14) at the top dose (334 mg / kg bodyweight). Infertility occurred at a dose with only marginal signs of general toxicity.

⇒ **Level of concern unchanged**

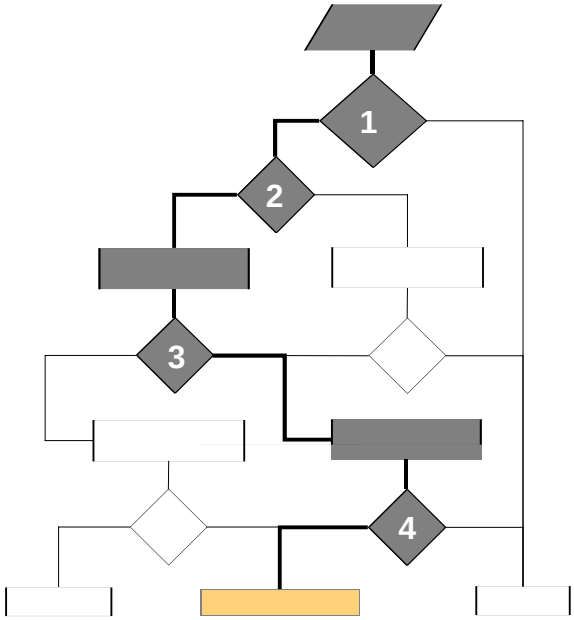
4 Consider effect in context of findings in other studies

Indications of impaired fertility (i.e. infertility) were also present in a continuous breeding study in mice with the same compound (Example 8, above).

⇒ **Level of concern unchanged**

Conclusion: High level of concern

Fertility toxicant - example 10



1. Is there an effect of treatment?

Dose-related increased post-implantation loss at all exposure levels in the first generation, but only at the top exposure level in the second generation. Reduced litter sizes, which seem to result from high post-implantation losses, and impaired pup bodyweights / weight gains at exposure levels of 33 and / or 100 ppm in both pup generations.

⇒ **Yes**

2. Level of concern

Increased post-implantation losses with reduced litter sizes are considered to be of high concern.

⇒ **High**

3. Consider effect in context of other findings in study

Signs of general parental toxicity at the mid and the top dose (33 and 100 ppm) included decreased bodyweights, impaired bodyweight gains and reduced food intake in both parental generations. No additional adverse data (e.g. foetal malformations) were observed and the effects on the reproductive parameters occurred generally at exposure levels which also induced systemic toxicity in the parental animals.

⇒ **Level of concern reduced**

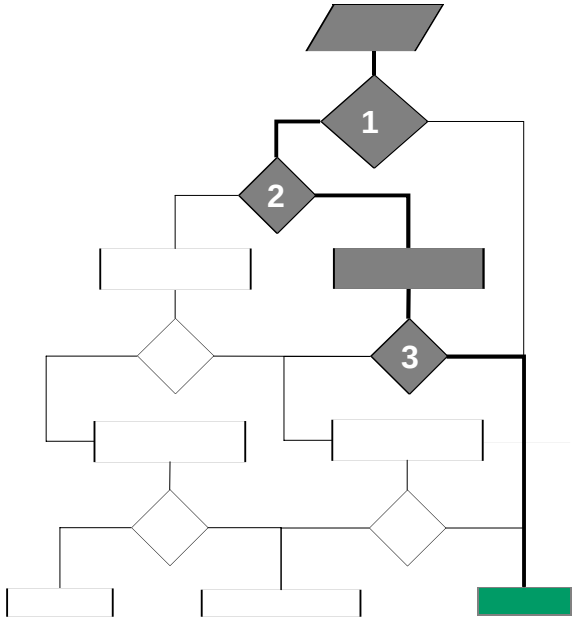
4 . Consider effect in context of findings in other studies

No other studies were available.

⇒ **Level of concern unchanged**

Conclusion: Low / moderate level of concern

Fertility toxicant - example 11



1. Is there an effect of treatment?

Reduced pregnancy rate at the second mating of the second parental generation and impaired pup bodyweights predominantly at the top dose level (600 ppm).

⇒ **Yes**

2. Level of concern

Impaired pregnancy rate occurred only after the second mating of the second parental generation; the effect on pup bodyweights was only slight.

⇒ **Low / moderate**

3. Consider effect in context of other findings in study

Parental toxicity (i.e. effects on bodyweight and food consumption as well as neurotoxic effects) in the high exposure group. Effects on reproductive parameters generally occurred at dose levels that also induced signs of marked systemic toxicity (e.g. circling movement and head bobbing in the top dose females and adverse effects on bodyweights / weight gains of the males and females in both parental generations).

⇒ **Level of concern reduced**

Conclusion: No concern

APPENDIX A: TEST GUIDELINE COMPARISON

Tables 7 and 8 compare the requirements of the test guidelines for prenatal developmental and two-generation reproduction toxicity, a general differentiation being made between 'old' (shaded pale green in the table) and 'new'.

Under the heading of 'old', the requirements of OECD (1981, 1983b), EC (1988a,b) EPA (1984a,b), EPA / TSCA (1992a,b) and Japan / MAFF (1985a,b) are listed and compared.

Under the heading 'new' the requirements of the latest OECD, the latest EPA. and, for prenatal developmental toxicity only, the respective ICH guidelines are listed.

In addition, Table 9 provides an overview of the most important requirements for OECD TG 415 [One-generation Reproduction Toxicity Study (1983a)], 421 [Reproduction / Developmental Toxicity Screening Test (1995a)] and 422 [Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (1996)].

The most important changes between 'old' and 'new' versions of the guidelines are summarised in the preamble to Tables 7 and 8 and are shaded dark green in the tables.

Table 9 summarises the key aspects of relevant guidelines for reproductive toxicity studies.

Table 7: Prenatal developmental toxicity (teratogenicity) test guidelines comparison

This table compares the latest 'new' requirements of the guidelines for prenatal developmental toxicity ie. OECD (2001a), EPA (1998d) and ICH (1994), with those of the 'old' (shaded pale green in the table) i.e. OECD (1981), EC (1988a), EPA (1984a), EPA / TSCA (1992a) and Japan / MAFF (1985b)

The most important changes between 'old' and 'new' versions are:

- Randomisation assignment: bodyweight dependent;
- non-rodent animal number: increased to "approximately 20 animals per group with implantation sites at necropsy;"
- dose volume adjustment: based upon most recent individual bodyweight;
- dosing schedule: from implantation to the day prior to scheduled caesarean section or alternatively during the entire length of gestation;
- determination of bodyweight and food consumption: at least at 3-day intervals;
- foetal skeletal evaluation: preference to include cartilage;
- foetal examinations: analyses without knowledge of treatment group;
- foetal rabbit heads: extended evaluation.

In the body of Table 7:

- An asterisk highlights identical or similar requirements on specific indices in the guidelines for prenatal developmental or 2-generation reproduction toxicity, and indicates where the wording was equivalent to the requirements of OECD TG 414 (1981);
- a dagger (†) indicates that the requirements are the same as for OECD (2001a).
- 'not mentioned' indicates where a specific requirement has been described in detail in one guideline but not in another;
- 'not specified' indicates where a certain parameter has been described in detail in one guideline, but only generally mentioned in the other.

Table 7: Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
Animals						
1. Species / strain	- commonly used laboratory strains; should not have low fecundity and should be characterised for response to teratogens	* - at least 2 mammalian species TSCA: -... for its sensitivity to developmental toxins	* - at least 2 mammalian species	- most relevant species - commonly used laboratory species and strains	†	- usually two species
- rodents	- rat preferred, mouse or hamster	TSCA: - rat, mouse or hamster FIFRA: - rat preferred	- rat preferred	- rat preferred	†	†
- non-rodents	- rabbit preferred	- rabbit	*	*	*	*
2. a) Age at start of study	- healthy young adult virgin F of comparable age and size	TSCA: - young adult animals (nulliparous F) FIFRA: - young adult pregnant animals	- young adult of first pregnancy	* - and not used previously in experimental procedures	- young adult animals (nulliparous F)	- young, mature virgin F
	- acclimatised to laboratory conditions for at least 5 d prior to the test	- not mentioned	- not mentioned	*	- not mentioned	- not mentioned

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
2. b) randomisation	- animals should be randomised and assigned to the treatment groups before mating	- not mentioned	- not mentioned	- mated F should be randomly assigned to the groups - should be as nearly as practical of uniform weight and age	- healthy animals should be randomly assigned to the control and treatment groups, in a manner which results in comparable mean bw values	- not mentioned
2. c) mating	- naturally, with M of established fertility	TSCA: - not specified FIFRA: - naturally	- naturally	- should be mated with males of the same species and strain, avoiding the mating of siblings	† if parentage is known	- not mentioned
	- by artificial insemination	*	*	- not specified for rodents - for rabbits: day 0 = day of coitus or artificial insemination	†	- not mentioned

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
3. Size of groups	- adequate to ensure that sufficient pups are produced to permit an evaluation of teratogenic potential of the substance EC: -... sufficient litters and pups ...	TSCA: -... of the potential developmental toxicity	- not mentioned	- not mentioned	†	- sufficient litters and pups
- rodents	- at least 20 pregnant rats, mice or hamsters	*	*	- sufficient number of females to result in approx 20 females with implantation sites at necropsy	- approx. 20 females with implantation sites at necropsy -	- 16 to 20 litters
- non-rodents	- at least 12 pregnant rabbits	*	*	- sufficient number of females to result in approx 20 females with implantation sites at necropsy	- approx. 20 females with implantation sites at necropsy	- 16 to 20 litters
4. Caging	- individual	- not mentioned	- not mentioned	*	- not specified	- not mentioned
- rodents and non-rodents	- pregnant F may be provided with nesting materials EC: - not mentioned	- not mentioned	- not mentioned	- not mentioned	†	†

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
Treatment						
5. Dose levels	- at least 3 plus control	* - plus concurrent control	* - plus control	*	* (untreated or sham-treated)	*
	- vehicle control if the test substance is administered in a vehicle	TSCA: - and / or where appropriate a vehicle control if the substance is administered in a vehicle FIFRA: - or vehicle group if the test substance is administered in a vehicle of unknown toxicity	- or vehicle group if the substance is - administered in a vehicle	concurrent control group should be used (sham-treated or a vehicle-control group) if a vehicle is used in administering the test substance	*	* - when the vehicle may cause effects a second (sham- or untreated) control should be considered
- low dose	- no observable effects	TSCA: - no grossly observable evidence of either maternal or developmental toxicity FIFRA: - no evidence of toxicity		- should not produce any evidence of maternal or developmental toxicity	†	- no observed adverse effect level

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
- intermediate dose(s)	- geometrically between high and low dose	TSCA: - Ideally, minimal observable toxic effects - if more than 1intermediate concentration is used, the concentration levels should be spaced to produce a gradation FIFRA: - not mentioned	*	- should produce minimal observable toxic effects	† - or should be at or near the limit of detection for the most sensitive endpoint	- should be selected in a descending sequence, depending on kinetics
- high dose	- ideally, some overt maternal toxicity (e.g. slight weight loss but not more than 10% maternal deaths) unless limited by the physical / chemical nature of biological properties of the substance	*	*	- should induce some developmental and / or maternal toxicity, but not death or severe suffering (in case of maternal mortality not more than about 10%)		- some minimal toxicity is expected to be induced in dams

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (Teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA/TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN/MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
6. Limit test	- If a low dose of at least 1,000 mg/kg produces no evidence of embryotoxicity or teratogenicity, studies at other dose levels may be considered unnecessary EC: - not mentioned	* FIFRA: *	*	- if a test at one dose level of at least 1,000 mg/kg by oral administration produces no observable toxicity and if an effect would not be expected based upon data from structurally related compounds, a full study with 3 dose levels may not be considered necessary	- highest dose level need not exceed 1,000 mg/kg/day by oral or dermal administration, or 2 mg/l (or the maximum attainable conc.) by inhalation If the limit dose level produces no observable toxicity a full study may not be considered necessary	- under most circumstances 1 g/kg/day should be an adequate limit dose †
7. a) Requirements for the vehicle	- toxicological properties should be understood	TSCA: - not mentioned FIFRA: *	*	* - vehicle control group should receive vehicle in the highest volume used	†	- dose with vehicle at the same rate as test group animals
	- should not be teratogenic or have effects on reproduction	*	*	- should neither be developmentally toxic nor have effects on reproduction	- not mentioned	- when the vehicle causes effects, a second untreated control group should be considered
7. b) Requirements for control	- animals should be handled in an identical manner to the exposed animals	*	* *	*	- not mentioned	*

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
8. Exposure conditions	- test substance should be administered at approximately the same time each day	TSCA: * FIFRA: - not mentioned	*	*	*	- not mentioned
	- when given by gavage, dose may be based on the bodyweight of the F at start of substance administration EC: - dose may be based on the bodyweight of females at start of substance administration	*	*	- dose based on most recent individual bodyweight	† when administered by gavage or dermal application	†
	- alternatively, the animals may be weighed periodically and the dosage based on the recent weight determination	*	*			

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
9. Route of administration	- orally, by gavage	*	*	*	*	- similar to those intended for human usage
-	- alternatively other routes may be used where these are more representative of likely routes for human exposure EC: - or depending in the physical properties of the test substance	- unless the chemical or physical characteristics of the test substance, or pattern of human exposure suggests a more appropriate route of administration	- not mentioned	* plus.... tester shall provide justification, and reasoning for selection, and appropriate modifications may be necessary	† based on principal route of potential human exposure	
10. Duration of treatment	- period of major organogenesis	*	*	- normally daily from implantation to the day prior to scheduled caesarian section	† at minimum	- from implantation to closure of the hard palate
	- alternatively, the period of dosing may be extended to approximately 1 d before delivery date	TSCA: - not mentioned FIFRA: *	*	- alternatively, if preliminary studies do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill	- alternatively, if preliminary studies do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from fertilization to approx. 1 day prior to the expected day of termination	

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
10. Duration of treatment (cont.)	- rat: d of gest. 6 - 15	*	* - or d 7 - 17	- not specified	†	*
- rodents	- mouse: d of gest. 6 - 15	*	*	- not specified	†	†
	- hamster: d of gest. 6 - 14	*	*	- not specified	†	†
- non-rodents	- rabbit: d of gest 6 - 18	*	*	- not specified	†	rabbit: d of gest -6 / 7-18
- definition of day 0	- day on which vaginal plug and / or sperm are observed	*	*	- for rodents - for rabbits: usually day of coitus or of artificial insemination	* in the rodent or that of insemination in the non-rodent	*
	- if based on observation of mating or artificial insemination the times stated should be adjusted by adding 1 d	TSCA: - not mentioned FIFRA: *	*	- not mentioned	†	†
11. Frequency of dosing	- daily	*	- not specified	*	*	- usually once daily (kinetic)

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
Study observations	**- I Clinical data**					
12. a) Bodyweight	- weekly	TSCA: - at least weekly FIFRA: - weekly and at the day of sacrifice	- period prior to, during and after treatment	- on day 0, or not later than day 3 if time-mated animals are supplied, on the first day of dosing, at least every 3-days during the dosing period and on the day of scheduled kill	† on day 0, at termination, and at least at 3-day intervals	- at least twice weekly
12. b) Food consumption	- weekly	* FIFRA; - in a dosed-feeding study	- period prior to, during and after treatment	- at 3-day intervals (on the same days as bodyweight is determined)	† - (preferably on the same days as body-weight is determined)	- at least once weekly

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
13. Clinical examination a) Clinical signs / mortality	- at least once daily throughout the study	TSCA: * FIFRA: - at least once each week at the same time as weighing	* - In addition thorough physical examinations at the same time maternal bodyweights are recorded	(considering the peak period of anticipated effects dosing)	*	*
	- daily additional observations to minimise loss of animals to the study	*	*	- not mentioned	†	†
	- dead, weak or moribund animals should be removed and necropsied EC: - not mentioned	TSCA: * FIFRA: - to ensure that not more than 10% of animals in any test group are lost due to cannibalism...	- not specified	- not mentioned	†	†
b) Abortion or premature delivery	- should be sacrificed and subjected to thorough macroscopic examination	*	*	*	*	*

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
14. Date of sacrifice	- shortly before the expected date of delivery	*	- not specified	- shortly before caesarean section	- immediately prior to expected day of delivery	*
	- one day prior to term	- not mentioned	- not mentioned	- one day prior to expected day of delivery	- approx 1 day prior to expected day of termination	*

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
Study observations	**- II Post-mortem examinations**					
15. Examination after caesarean section or death	- uteri should be removed immediately	TSCA: -... and weighed FIFRA: *	*	- as soon as possible after death -... and the pregnancy status of the animals ascertained. - uteri that appear non-gravid should be further examined to confirm the non-pregnant status. Gravid uteri including cervix should be weighed (not from animals found dead during the study)	* †	- for apparently non-pregnant rat, ammonium sulphide staining of uterus to identify peri-implantation death of embryos
	- number of embryonic or fetal deaths and live fetuses	*	*	*	*	*
	- estimation of time of death *in utero*	*	*	*	*	- not specified
	- number of *corpora lutea* in rats and rabbits may be determined	TSCA: - number of *corpora lutea* should be determined for all species except mice FIFRA: - number of *corpora lutea* where possible	- number of *corpora lutea* where necessary	- number of *corpora lutea* for pregnant animals	†	- number of *corpora lutea* in rats and rabbits - gross evaluation of placenta

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA/TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN/MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
16. Examination of foetuses	- externally	*	*	*	*	* - individual identification
	- sex	*	*	*	*	*
	- individual weight	FIFRA: - litter weight	- litter weight	*	*	*
	- mean weight derived	*	*	- not mentioned	†	
17. Preparation and morphological examination of foetuses				- foetal analyses should preferably be conducted without knowledge of treatment group in order to minimise bias	†	- The examination of the mid- and low-dose foetuses for visceral and for skeletal abnormalities may not be necessary where the evaluation of the high dose and controls did not reveal any relevant findings
- rodents	- 1/3 to 1/2 of each litter, for skeletal anomalies	*	*	- approx 1/2 of each litter for skeletal anomalies	† -... skeletal (preferably bone and cartilage) alterations	- 1/2 of each litter for skeletal examination
	- remaining part of each litter for soft tissue anomalies	*	*	*	(it is acceptable to examine all foetuses for soft tissue anomalies followed by an examination for skeletal anomalies)	*

* same as OECD (1981)

† same as OECD (2001a)

Table 7 (cont.): Prenatal developmental toxicity (teratogenicity) test guidelines comparison

	OLD			NEW		
	OECD (1981); EC (1988a)	**EPA / TSCA (1992a); EPA (1984a) (FIFRA)**	**JAPAN / MAFF (1985b)**	**OECD (2001a)**	**EPA (1998d)**	**ICH (1994)**
- non-rodents	- each foetus for visceral anomalies by dissection	*	*	- all foetuses should be examined for both soft tissue and skeletal alterations - the heads of one-half of the foetuses examined in this manner should be removed and processed for evaluation of soft tissue alterations	* † - for at least half of the foetuses adequate evaluation of the internal structures of the head incl. eyes, brain, nasal passages, and tongue	*
	- then for skeletal anomalies	*	*	- bodies of these foetuses (i.e. those with detailed head examination) and remaining intact foetuses should be processed and examined for skeletal anomalies	- all foetuses should be examined for skeletal alterations	- all foetuses should be examined for skeletal abnormalities
18. Gross necropsy (dams)	- examination for any structural abnormalities or pathological changes which may have influenced pregnancy	*	*	(preferably without knowledge of treatment group)	*	- macroscopic examination - preserve organs with macroscopic findings for possible histopathology

* same as OECD (1981)

† same as OECD (2001a)

Table 8: Two-generation reproduction toxicity test guidelines comparison

This table provides a comparison of the 'new' requirements of the guidelines for two-generation reproduction toxicity i.e. OECD (2001b) and EPA (1998b), with those of the 'old' (shaded pale green in the table) OECD (1983b), EC (1988b), EPA (1984b), EPA / TSCA (1992b) and Japan / MAFF (1985a)

The most important changes between 'old' and 'new' versions are:

- Limit test: possible;
- duration of mating: maximum of two weeks or three oestrous cycles;
- mating procedure: if mating has not occurred, no further opportunity for mating (EPA only);
- litter standardisation: still optional, but according to EPA 4 females / 4 males or 5 females / 5 males;
- addition of oestrous cyclicity data (EPA and OECD) and additional data for stage of the oestrous cycle at the time of necropsy (EPA only);
- addition of sperm evaluation;
- addition of sexual maturation data (F1 progeny: vaginal opening / preputial separation; F2 progeny: measurement of anogenital distance if clarified triggers);
- addition of functional tests in F1 offspring recommended (OECD only);
- addition of gross pathology for at least 1 pup / sex / litter (OECD) or 3 pups / sex / litter (in the F1 and F2 pup generation) (EPA);
- addition of selected organ weights for 1 pup / sex / litter (in both F1 and F2);
- addition of histopathological examination of treatment-related abnormalities in the pups, if appropriate;
- addition of selected organ weight determinations in all parental animals;
- extended histopathological examinations in P and F1 parental animals [in all (OECD) or 10 male / 10 females each (EPA) from at least control and high dose groups;
- histopathological characterisation of grossly abnormal tissue and target organs in weanlings not selected for mating with emphasis on the organs of the reproductive system (OECD only).

In the body of Table 8:

- An asterisk highlights identical or similar requirements on specific indices in the guidelines for prenatal developmental or 2-generation reproduction toxicity, and indicates where the wording was equivalent to the requirements of OECD TG (1983b);
- a dagger (†) indicates that the requirements are the same as for OECD (2001b).
- 'not mentioned' indicates where a specific requirement has been described in detail in one guideline but not in another;
- 'not specified' indicates where a certain parameter has been described in detail in one guideline, but only generally mentioned in the other.

Table 8: Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
Animals					
1. Species/strain	- rat or mouse	TSCA: - rat preferred FIFRA: *	- at least one mammalian species, rat preferred	- rat preferred	†
	- strains with low fecundity should not be used	*	*	*	*
	- if other species are used, appropriate modifications will be necessary EC: - not mentioned	- if another mammalian species is used, justification reasoning for its selection to be given	- not mentioned	* ... and justification should be given	†
2. Age and start of study P-generation - males	- 5-9 wk	TSCA: - at 8 wk FIFRA: - about 8 wk	- not mentioned	- 5-9 wk and of uniform weight, age and parity	†
	- after weaning, and acclimatisation for at least 5 d	- not mentioned	- immediately after weaning and acclimatisation for at least 1 wk	- acclimatisation for at least 5 d - not subjected to previous experimental procedures	†
- females	- age: not specified EC: - after weaning	TSCA: - at 8 wk FIFRA: - about 8 wk	- immediately after weaning, and acclimatisation for at least 1 wk	- 5 to 9 wk old and of uniform weight and parity - acclimatisation for at least 5 d - not subjected to previous experimental procedures	†
	- at least 5 d of acclimatisation	- not mentioned - nulliparous and nonpregnant	- nulliparous and nonpregnant		

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
3. Size of groups	- sufficient number to yield about 20 pregnant F at or near term	- at least 20 M and sufficient number of F to yield at least 20 pregnant F at or near term	- at least 20 M and sufficient number of F to yield at least 20 pregnant F at parturition	* (preferably not less than 20 pregnant F)	* (approx 20 pregnant F)
	- for substances that cause sterility this may not be possible EC: - not mentioned	- not mentioned	- not mentioned	*	- not mentioned
4. Caging	- pregnant F individually EC: - ... near parturition, separately	- pregnant F near parturition, separately in delivery or maternity cages	- pregnant F near parturition, separately in delivery or maternity cages	- individually or in small groups of the same sex - mated F shall be singly caged in delivery or maternity cages	- F presumed to be pregnant, should be caged separately in delivery or maternity cages
	- provided with nesting materials	*	*	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
Treatment					
5. Dose levels	- at least 3 plus control	*	*	*	*
	- or vehicle control receiving the vehicle in the highest volume used	*	*	*	*
	- plus pair-fed control if the test substance causes reduced dietary intake or utilisation	- not mentioned - in the low and intermediate dose groups and in the control group the incidence of fatalities should be low to permit a meaningful evaluation of the results	- not mentioned	* - a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response - 2 to 4 fold intervals frequently optimal for setting the descending dose levels - for dietary studies the dose interval should be not more than 3-fold	* - dose levels should be spaced to produce a gradation of toxic effects - not specified
- low dose	- ideally, no observable adverse effects on the parents or off-spring	- no evidence of toxicity - where there is an "usable" estimation of human exposure the lowest dose should exceed this	- no evidence of toxicity	- no observed adverse effects (NOAEL) or doses near the limit of detection that would allow the determination of a benchmark dose	- no evidence of either systemic or reproductive toxicity (i.e. NOAEL) or near the limit of detection for the most sensitive endpoint
- intermediate dose(s)	- ideally, minimal toxic effects	* - if more than one intermediate dose is used, the levels should be spaced to produce a gradation of toxic effects	*	- not specified	- minimal observable toxic effects

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
5. Dose levels (cont.) - high dose	- ideally, toxicity but no mortality in the parental (P) animals unless limited by the physical/ chemical nature or biological effects	- toxicity, but no mortality in the parental (P) animals - highest concentration should not exceed 5% in the diet FIFRA: - ... with the exception of nutrients	- toxicity, but no mortality in dams	- toxicity but not death or severe suffering unless limited by the physical-chemical nature or biological effects - in case of mortality not more than approx. 10% in the P animals - not specified	† - should not exceed 1,000 mg/kg/day (or 20,000 ppm in the diet) unless potential human exposure data indicate need for higher doses
6. Limit Test	OECD: - not mentioned EC: - in the case of substances of low toxicity, if a dose level of at least 1,000 mg/kg bw produces no evidence of interference with reproductive performance, studies at other dose levels may not be considered necessary - if a preliminary study at the high dose level, with definite evidence of maternal toxicity, shows no adverse effects on fertility, studies at other dose levels may be considered not necessary	- not mentioned	- not mentioned	- if an oral study at one dose of at least 1,000 mg/kg, or, for dietary or drinking water administration, an equivalent percentage in the diet or drinking water produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using several dose levels may not be considered necessary	- if the limit dose level produces no observable toxicity a full study may not be considered necessary and if toxicity would not be expected, based on data from structurally-related compounds

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
7. a) Requirements for vehicle	without toxic effect	- should not interfere with absorption of the test substance or produce toxic effects	- not mentioned	- toxicological properties should be understood	†
7. b) Requirements for control	- not specified	*	- treatment in a manner identical to the dosed group	- handled in an identical manner to the test group subjects	*
8. Exposure conditions	- when administered by gavage or capsule, dose is based on the individual animals' body-weight and is adjusted weekly	*	*	- if dosed by gavage, this should be done at similar times each day and the dose should be adjusted at least weekly	- if administered by gavage or dermal, application, the dosage administered to each animal prior to mating and during gestation and lactation should be based on individual animals' bodyweight and adjusted weekly at a minimum
	- during pregnancy, dose is based on daily bodyweight or on bodyweight at d 0 or 6 of pregnancy, if desired	- during pregnancy, dose may be based on bodyweight at d 0 and 6 of pregnancy	- during pregnancy, dose may be based on individual bodyweight at d 0 and 6 of pregnancy	- not specified	

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**US EPA Health Effects Guidelines OPPTS 870.3800 (1998b)**
9. Route of administration	- diet or drinking water	*	- diet in principle	- oral route (diet, drinking water, or gavage) preferred	† (usually administered by the oral route)
	- other routes acceptable	TSCA: * FIFRA: - alternatively gavage or capsules - oral route is preferred	- alternatively gavage or capsules	- if other routes of administration are used, justification shall be provided and appropriate modifications may be necessary	†
10. Duration of treatment a) P-generation (males)					
- rats and mice	- during growth and at least one complete spermatogenic cycle	- not mentioned	- not mentioned	*	- not specified
- rats	- for 10wk prior to and throughout the mating period until termination	- for at least 8 wk prior to and throughout the mating period until termination	- for at least 8 wk prior to and throughout the mating period, pregnancy, and up to the weaning of F_1 offspring	*	*
- mice	- for 8wk prior to the mating period	- not mentioned	- not mentioned	- not mentioned	†

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
b) P-generation (females)	two complete oestrous cycles - in rats and mice; for at least 2wk prior to mating - throughout the 3wk mating period, pregnancy, and up to the weaning of the F_1 offspring	- not mentioned - for at least 8wk prior to the mating period *	- not mentioned - for at least 8wk prior to the mating period *	- for 10wk prior to mating period - during the 2wk mating period, throughout pregnancy and up to weaning of the of the F_1 offspring	† - during their mating, during the resultant pregnancies, and through weaning of the F_1 offspring
c) F_1 generation selected for mating					
- males	- starts at weaning and ends with sacrifice	- starts after weaning, then throughout the mating period with F_1 females (11 wk) FIFRA: - ... (11 wk for mice, 17 wk for rats)	- from weaning, to weaning of F_2 offspring	*	- from weaning during their growth into adulthood, mating, and production of a F_2 generation, until the F_2 generation is weaned
- females	- starts at weaning and ends with sacrifice	- starts after weaning, then throughout the mating period with the F_1 males (11 wk), pregnancy, and to the weaning of the F_2 offspring FIFRA: ...(11 wk for mice, 17 wk for rats) ...	- from weaning, to weaning of F_2 offspring	*	- from weaning during their growth into adulthood, mating, and production of a F_2 generation, until the F_2 generation is weaned
F_2-generation	- not mentioned	*	- if necessary	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
11. Frequency of dosing	- 7 d/wk	- continuous exposure if given in diet or drinking water	- not mentioned	*	*
Procedure					
12. Mating -definition of day 0	- day on which vaginal plug or spermare found (each morning the F should be examined for presence of sperm or vaginal plug)	*	*	*	*
- P-generation	- 1 : 1; 1F is placed with the same M until pregnancy occurs or 3 wk have elapsed	- each F with a single M from the same dose level until pregnancy occurs or 3 wk have elapsed FIFRA: - ... randomly selected M ...	- F_1 with a single M from the same dose group until mating is confirmed or 3 wk have elapsed	- 1 : 1; each F shall be placed with a single randomly selected M from the same dose level until copulation occurs or 2 wk have elapsed	- 1 : 1; each F shall be placed with a single randomly selected M from the same dose level until evidence of copulation has been observed or either 2 wk or 3 oestrous periods have elapsed
	- alternatively 1 M : 2 F possible	- paired mating should be clearly identified - mixed matings with other M should be avoided	- not mentioned	- not mentioned	†
- offspring after attaining full sexual maturity	- in rats: begins at the age of at least 13 wk	TSCA: - in rats: begins at the age of approx. 14 wk FIFRA: - in rats: begins at the age of approx. 17 wk	- not mentioned	F_1 offspring should not be mated until attaining full sexual maturity	- not specified

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
12. Mating (cont.)					
- offspring, after attaining full sexual maturity	- in mice: begins at the age of at least 11 wk	- not mentioned FIFRA: *	- not mentioned	- not mentioned	†
	- 1 M and 1 F are randomly selected from each litter for cross-mating with a pup of another litter of the same dose group	TSCA: * FIFRA: - 1 M and 1 F are randomly selected from each litter for cross-mating with a pup ofanother litter	- 1 or 2 M and 1 or 2 F are randomly selected from each litter for cross-mating with a pup of another litter	*	- at least 1 M and 1 F are randomly selected from each litter for mating with another pup of the same dose level but different litter
	- mating of siblings should be avoided	*	*	*	- not mentioned
	- in certain instances such as poor reproductive performance in the controls, consideration should be given to the production of 2 litters/generation EC: - not mentioned	- not mentioned	- not mentioned	- in certain instances, such as treatment-related alterations in litter size, or an equivocal effect in the first mating, P or F_1 adults should be remated to produce a second litter	†

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
13. Proof of fertility	- pairs that fail to mate should be evaluated to determine the cause of infertility	*	*	*	- if mating has not occurred after 2 wk or 3 oestrous periods, animals should be separated without further opportunity for mating
	- additional opportunities to mate with other proven sires and dams	*	*	*	
	- examination of the oestrous cycle or spermatogenesis	TSCA: *			
		FIFRA: not mentioned	*	*	
	- microscopic examination of the reproductive organs	*	*	*	

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
14. Rearing F_1 and F_2-generation - litter size without standardization	- dams are allowed to litter normally and rear their progeny to the stage of weaning	- not mentioned	- not mentioned	- standardisation optional	†
- litter size with standardisation	OECD: - selection of 4 M and 4 F per litter on d 4 after birth EC: -... as nearly as possible	*	*	- not specified	- selection of 4 M and 4 F or 5 M and 5 F per litter on d 4 after birth
	- partial adjustment is accepted if the number of M + F pups prevents having 4 of each sex / litter. 5 M and 3 F are also acceptable	*	*	- when standardisation is done, the method used should be described in detail	- partial adjustment is accepted if the number of M + F pups prevents having 4 (or 5) of each sex per litter 5 M and 3 F (or 4 M and 6 F) are also acceptable
	- adjustments are not applicable for litters of less than 8 pups	*	*	- not specified	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
Study observations - I Clinical data					
15. a) Bodyweights P- and F_1-generation parents	- on day 1 of dosing and weekly thereafter	- at birth, and d 4, 7 (optional), 14, 21 after birth	*	* - parental F: at a minimum on gd 0, 7, 14, 21 and during lactation on same days as litters	†
b) Food consumption	- weekly during premating and mating periods	TSCA: - not mentioned FIFRA: - not specified	- not specified	- weekly during premating and gestation as a minimum	†
	- optionally, daily during pregnancy	- not mentioned FIFRA: - not specified	- not specified		
	- after parturition and during lactation on the same d as the litters are weighed	TSCA: - not mentioned FIFRA: - not specified	- not specified	- not mentioned	†
c) Water consumption	- not specified	*	*	- if test substance is administered in the water, weekly at minimum	†

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
16. Clinical examinations / mortality	- at least once daily throughout the study period	*	*	- taking into account the anticipated peak period of effects	†
	- record duration of gestation, signs of difficult or prolonged parturition. All signs of toxicity, including mortality and pertinent behavioural changes	*	*	- twice daily (weekend, once daily) observation for morbidity and mortality	†
17. Oestrous cycle	- not specified	*	*	- oestrous cycle length and normality should be evaluated by vaginal smears for all P and F_1 females prior to mating and optionally during mating	- oestrous cycle length and pattern should be evaluated by vaginal smears for all P and F_1 females during a minimum of 3 wk prior to mating and throughout cohabitation

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
18. Sperm parameters	- not specified	*	*	for a subset of at least 10 P and F_1 males of each group, sperm from testis and epididymes should be collected at termination for enumeration of homogenisation-resistant spermatids and cauda epididymal sperm reserves. In addition, sperm from cauda epididymis (or vas deferens) should be collected for evaluation of sperm motility and sperm morphology. If treatment-related effects are observed, sperm evaluation in all males in each dose group; otherwise enumeration may be restricted to control and high-dose P and F_1 males.	- for all (at least high dose and control) P and F_1 males, sperm from testes and epididymes should be collected at termination for enumeration of homogenisation-resistant spermatids and cauda epididymal sperm reserves. In addition sperm from cauda epididymes (or vas deferens) should be collected for evaluation of sperm motility and sperm morphology
19. Examination of litters at birth	- as soon as possible after delivery	*	*	* lactation day 0)	†
	- number of pups, stillbirths, live births	*	*	*	*
	- sex of pups	- not mentioned	- not mentioned	*	*
	- gross anomalies	*	*	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
20. Preservation of pups	- dead or moribund pups and pups sacrificed at d 4 should be preserved and studied for possible defects	- dead pups and pups sacrificed at d 4 should be preserved and studied for possible defects and cause of death	*	- pups found dead on day 0, should preferably be examined for possible defects and cause of death, and preserved if not macerated	- pups found dead on day 0 should be examined for possible defects and cause of death
21. Examinations during lactation	- counting of live pups	*	*	*	*
	- weighing of litters on the:	TSCA: - weighing of individual pups FIFRA: *	- weighing of individual pups	- weighing of individual pups	†
	- morning after birth	- at birth, or soon thereafter	- at birth, or soon thereafter	- at birth, or soon thereafter	†
	d 4	*	*	*	*
	d 7	TSCA: * FIFRA: * (optional)	* (optional)	*	*
	- weighing individually weekly thereafter until termination of the study	TSCA: - d 14 - d 21 after parturition FIFRA: - d 14 (optional) - d 21 after birth - individual weighing of pups	- d 14 - d 21 after birth	- d 14 - d 21	† - and at times of vaginal patency or balano-preputial separation and at termination

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
21. Examinations during lactation (cont.)	- physical or behavioural abnormalities in dams or offspring should be recorded	*	*	*	- not mentioned
22. Sexual maturation	- not specified	*	*	- age of vaginal opening and preputial separation for all F_1 weanlings selected for mating. If treatment related effects in F_1 sex ratio or sexual maturation, anogenital distance measurements on day 0 of all F_2 pups	†
23. Functional tests	- not specified	*	*	- recommended in F_1 offspring (when separate studies on neuro developmental toxicity are not considered), but may be omitted in groups that reveal clear signs of adverse effects. If these investigations are made, they should not be done on pups selected for mating	- not mentioned

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA / TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN / MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
24. Dates of sacrifice P-generation					
- males	- at end of mating period (males)	*	- after weaning of F_1 offspring	*	*
	- alternatively, may be retained on diet for possible production of 2nd litter, sacrificed and examined at some time before end of study	- not mentioned	- not mentioned	*	*
	- when no longer necessary for assessment of reproductive	TSCA: - not mentioned FIFRA: - after delivery of last litter sired - or in cases of infertility after proof of fertility	- not mentioned	*	*
- females	- when no longer necessary for assessment of reproductive effects	TSCA: - after weaning of F_1 offspring FIFRA: - after weaning of last litters - or in cases of infertility after proof of fertility	- after weaning of F_1 offspring	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
24. Dates of sacrifice (cont.)					
F_1-generation selected for mating					
- males	- when no longer necessary for assessment of reproductive effects	TSCA: - at end of mating period FIFRA: - after mating period - after delivery of the last F_1 litter sired - or in cases of infertility after proof of fertility	- after weaning of F_2 offspring	*	*
- females	- when no longer necessary for assessment of reproductive effects	- after weaning of their last litters	- after weaning of F_2 offspring *	*	
- males, females not selected for mating	- after weaning	*	*	*	(at comparable ages after weaning)
F_2 offspring	- after weaning	- at age 21 d	*	*	(at comparable ages)

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
Study observations - II	**Post-mortem examinations**				
25. Gross pathology	- at time of sacrifice or death during study, all parental animals (P and F_1) should be examined macroscopically for any structural abnormalities or pathological changes	- all animals including those which died during the experiment or were sacrificed in moribund conditions should be completely examined FIFRA: - ... to ensure that not more than 10% of the animals in any test group are lost due to cannibalism	- all animals at sacrifice, dead, or sacrificed in moribund state	- at time of termination or death during study, all parental animals (P_1 and F_1) and all F_1 generation, and all pups with external abnormalities or pathological changes as well as at least one randomly selected pup/sex/litter from both (F_1 and F_2) generations should be examined macroscopically for any structural abnormalities or pathological changes	- at time of termination or death during study, all parental animals (P_1 and F_1) and at least 3 pups/sex/litter from the unselected F_1 weanlings and the F_2 weanlings should be examined macroscopically for any structural abnormalities or pathological changes
	- special attention to the organs of reproductive system	*	*	* - uteri of all primiparous F should be examined (without compromising histopathology) for presence and number of implantation sites	* at necropsy, a vaginal smear should be examined to determine the stage of the oestrous cycle and the uteri of all cohabitated F should be examined (without compromising histopathology) for presence and number of implantation sites

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
26. Organ weights (P and F_1 parental animals)	- not mentioned	*	*	- uterus, ovaries, testes, epididymis (total and cauda), seminal vesicles (with coagulating glands and their fluids), prostate, brain, liver, kidneys,adrenals, spleen, thyroid, pituitary, known target organs	- uterus (with oviducts and cervix), ovaries, testes, epididymis (total and cauda), seminal vesicles (with coagulating glands and their fluids), prostate, brain, pituitary, liver, kidneys, adrenals, spleen, known target organs
27. Organ weights (pups)	- not mentioned	*	*	- for F_1 and F_2 pups, that are examined macroscopically (i.e. one randomly selected pup/sex/litter): brain, spleen, thymus	- for F_1 and F_2 pups, that are examined macro-scopically in 1 randomly selected pup/sex/litter: brain, spleen, thymus
28. Preservation of organs (general)	- from all P- and F_1 animals selected for mating	- in a suitable medium for possible future examination	- organs of the reproductive system of all animals - those which are prepared for future examinations should be embedded in paraffin	- all P and F_1 animals - in a suitable medium for histopathological examinations	- all parental P and F_1 animals; grossly abnormal tissues and target organs, when known, also from F_1 and the F_2 weanlings selected for macroscopic examination - in a suitable medium for histopathological examinations

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
29. Histopathology (P and F_1 parental animals)	- if necessary, or if organs (c.f. 24) have not been examined in other multiple dose studies	- not mentioned	- not mentioned	- not mentioned	†
	a) all animals in control and high dose groups (P- and F_1-generation) selected for mating	*	*	- full histopathology for all high dose and control animals (P and F_1) selected for mating	- full histopathology on 10 randomly chosen high dose and control (P and F_1) animals per *sex* selected for mating
	b) all animals dying during study (where practicable)	- not mentioned	- not mentioned	- not mentioned	†
	c) organs showing abnormalities should be examined in animals from the other dose groups	*	*	- organs demonstrating treatment-related changes also in low and mid dose groups	- organs demonstrating treatment-related changes in the remainder of the high-dose and control and all low- and mid-dose parental animals
	d) microscopy of all tissues showing gross-pathological changes	*	*	*	*
	e) microscopy of reproductive organs of animals suspected of infertility	- not mentioned	- not mentioned	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 8 (cont.): Two-generation reproduction toxicity test guidelines comparison

	OLD			NEW	
	OECD (1983b); EC (1988b)	**EPA/TSCA (1992b); EPA (1984b) (FIFRA)**	**JAPAN/MAFF (1985a)**	**OECD (2001b)**	**EPA (1998b)**
30. Histopathology (weanlings)	- not mentioned	*	*	- grossly abnormal tissue and target organs from all pups with external abnormalities or clinical signs, as well as from at least one randomly selected pup/sex/litter from both the F_1 and F_2 generation not selected for mating, shall befixed in a suitable medium; histopatho-logical examinations of all preserved tissue with emphasis on reproductive organs	- histopathological examination of treatment related abnormalities noted at macroscopic examination should be considered, if such evaluation were deemed appropriate and would contribute to the interpretation
31. Organs to be investigated (P and F_1 parental animals)	- ovaries	*	*	*	*
	- uterus	*	*	*	* (with oviducts)
	- cervix	*	- not mentioned	*	*
	- vagina	*	*	*	*
	- testes	*	*	* (one)	†
	- epididymides	*	*	* (one)	†
	- seminal vesicles	*	*	*	*
	- prostate	*	*	*	*
	- coagulating gland	*	- not mentioned	*	*
	- pituitary gland	*	*	- not mentioned	* plus adrenal glands
	- target organs	*	- not mentioned	*	*
	- grossly abnormal tissue	*	*	*	*

* same as OECD (1983b)

† same as OECD (2001b)

Table 9: Reproductive toxicity guidelines TG 415, 421 and 422

This table provides an overview of the key requirements for the following guideline studies:

- one generation reproduction toxicity study (OECD, 1983a);
- reproduction / developmental toxicity screening test (OECD, 1995a; EPA, 2000a);
- combined repeated dose toxicity study with the reproduction / developmental toxicity screening test (OECD, 1996; EPA, 2000b).

Table 9: Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
Animals			
1. Species/strain	- rat or mouse - strains with low fecundity should not be used - if other species are used, appropriate modifications will be necessary	- rat - strains with low fecundity or well-known high incidence of developmental defects should not be used - if other species are used, appropriate modifications will be necessary	- rat - strains with low fecundity or well-known high incidence of developmental defects should not be used - if other species are used, appropriate modifications will be necessary
2. Age at start of study	- M: 5-9 wk - F: healthy young adult	- M: healthy, young adult - F: healthy, young virgin adult	- M: healthy, young adult - F: healthy, young virgin adult
3. Size of groups	- sufficient number to yield about 20 pregnant F at or near term - for substances that cause sterility this may not be possible	- at least 10 M/10 F to yield about 8 pregnant F - except in the case of marked toxic effects, at least 8 pregnant females/group are expected	- at least 10 M/10 F to yield about 8 pregnant F - except in the case of marked toxic effects, at least 8 pregnant females/group are expected
4. Caging	- pregnant F individually and provided with nesting materials	- individually or in small groups (5/cage) by sex - mating in suitable cages - pregnant F individually and provided with nesting materials	- individually or in small groups (5/cage) by sex - mating in suitable cages - pregnant F individually and provided with nesting materials

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
Treatment			
5. Dose level	- at least 3 plus control - or vehicle control receiving the vehicle in the highest volume - plus pair-fed control if the test substances causes reduced dietary intake or utilisation	- at least 3 plus control - or vehicle control receiving the vehicle in the highest volume	- at least 3 plus control - or vehicle control receiving the vehicle in the highest volume
- low dose	- ideally, no observable adverse effects on the parents or offspring	- no observed adverse effects (NOAEL)	- no adverse effects
- intermediate dose(s)	- ideally, minimal toxic effects	- demonstrating any dose-related response	- demonstrating any dose-related response
- high dose	- ideally, toxicity but no mortality in the parental (P) animals unless dose is limited by the physical/chemical nature or biological effects	- should induce toxic effects, but not death or severe suffering	- should induce toxic effects, but not death or obvious suffering
6. Limit test	- in the case of substances of low toxicity, if a dose level of at least 1,000 mg/kg bw produces no evidence of interference with reproductive performance, studies at other dose levels may not be considered necessary - if a preliminary study at the high dose level, with definite evidence of maternal toxicity, shows no adverse effects on fertility, studies at other dose levels may not be considered necessary	- if an oral study at one dose of at least 1,000 mg/kg, or, for dietary or drinking water administration, an equivalent % in the diet, or drinking water, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally-related compounds, then a full study using several dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.	- if an oral study at one dose of at least 1,000 mg/kg, or, for dietary or drinking water administration, an equivalent percentage in the diet or drinking water, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using several dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.
7. Requirements for vehicle	- without toxic effects	- aqueous solution/suspension first preference, followed by solution/emulsion in oil; toxic characteristics of vehicle must be known	- aqueous solution/suspension first preference, followed by solution/emulsion in oil; toxic characteristics of vehicle must be known

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
8. Exposure conditions	- when administered by gavage or capsule, dose is based on individual animals' bodyweight and is adjusted weekly	- when administered by gavage, dose is based on the individual animals' bodyweight and is adjusted weekly	- when administered by gavage, dose is based on the individual animals' bodyweight and is adjusted weekly
		- administration via diet or drinking water shall not interfere with normal nutrition or water balance (constant dietary concentration (ppm) or bodyweight adjusted)	- administration via diet or drinking water shall not interfere with normal nutrition or water balance (constant dietary concentration (ppm) or bodyweight adjusted)
	- during pregnancy, dose is based on daily bodyweight or on bodyweight at d 0 or 6 of pregnancy, if desired		
9. Route of administration	- diet or drinking water	- orally (gavage, diet, drinking water)	- orally (gavage, diet, drinking water)
	- other routes acceptable	- other routes acceptable	- other routes acceptable
10. Frequency of dosing	- 7 d/wk	- 7 d/wk	- 7 d/wk
11. Duration of treatment - P-generation (males)	- during growth and at least one complete spermatogenic cycle (rats and mice) - for 10 wk prior to and throughout mating period until termination (rats) - for 8 wk prior to the mating period (mice)	- M: 2 wk prior to mating, during mating and 2 wk post mating (i.e. minimum total dosing period of 28 days)	- M: 2 wk prior to mating, during mating and 2 wk post mating (i.e. minimum total dosing period of 28 days)
- P-generation (females)	- two complete oestrous cycles	- F: approx. 54 d (14 d prior to mating, up to 14 d mating, 22 d gestation, 4 d lactation)	- F: approx. 54 d (14 d prior to mating, up to 14 d mating, 22 d gestation, 4 d lactation)
	- in rats and mice; for at least 2 wk prior to mating	- for dermal route and inhalation exposure: dosing should be continued at least up to and including d 19 of gestation	- for dermal route and inhalation exposure: dosing should be continued at least up to and including d 19 of gestation
	- throughout the 3 wk mating period, pregnancy, and up to the weaning of the F_1 offspring		

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
Procedure			
12. Mating - definition of day 0	- day on which vaginal plug or sperm are found (each morning the F should be examined for presence of sperm or vaginal plug)	- day on which vaginal plug or sperm are found (each morning the F should be examined for presence of sperm or vaginal plug)	- day on which vaginal plug or sperm are found (each morning the F should be examined for presence of sperm or vaginal plug)
	- 1 : 1; 1F is placed with the same M until pregnancy occurs or 3 wk have elapsed	- 1:1 (1 F is placed with the same M until pregnancy occurs or 2 wk have elapsed)	- 1:1 (1 F is placed with the same M until pregnancy occurs or 2 wk have elapsed)
	- alternatively 1 M : 2 F possible	- not mentioned	- not mentioned
13. Proof of fertility	- pairs that fail to mate should be evaluated to determine the cause of infertility - additional opportunities to mate with other proven sires and dams - estimation of the oestrous cycle or spermatogenesis - microscopic examination of the reproductive organs	- in case of unsuccessful pairing, re-mating of females with proven males of the same group should be considered (only mentioned in TG 421)	- in case of unsuccessful pairing, re-mating of females with proven males of the same group should be considered
14. Rearing - litter size without standardisation	- dams are allowed to litter normally and rear their progeny to the stage of weaning	- dams are allowed to litter normally and rear their progeny to day 4 of lactation	- dams are allowed to litter normally and rear their progeny to day 4 of lactation
- litter size with standardisation	- selection of 4 M and 4 F per litter on d 4 after birth (as nearly as possible) - partial adjustment is accepted if the number of M + F pups prevents having 4 of each sex per litter. 5 M and 3 F are also acceptable - adjustments are not applicable for litters of less than 8 pups	- not mentioned	- not mentioned

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
Study observations - I Clinical data			
15. Bodyweights	- on day 1 of dosing and weekly thereafter	- on day 1 of dosing, weekly thereafter and at termination; pregnant F on gd 0, 7, 14 and 20 and ppd 0/1 and 4	- on day 1 of dosing, weekly thereafter and at termination; pregnant F on gd 0, 7, 14 and 20 and ppd 0/1 and 4
16. Food consumption	- weekly during premating and mating periods - optionally, daily during pregnancy - after parturition and during lactation on the same d as the litters are weighed	- weekly during premating, pregnancy and lactation - optionally during mating	- weekly during premating, pregnancy and lactation - optionally during mating
17. Water consumption	- not specified	- during the same periods as food consumption when administration via drinking water	- during the same periods as food consumption when administration via drinking water
18. Clinical examinations	- at least once daily throughout the study period	- at least once daily	- at least once daily - once before first exposure and at least once a week thereafter, detailed clinical obs. outside the home cage in a standard arena in all animals - at one time during the study functional observations and motor activity (may be omitted in certain instances) in 5M/5F per group
	- record duration of gestation, pertinent behaviour changes, signs of difficult or prolonged parturition, all signs of toxicity including mortality	- record duration of gestation, pertinent behaviour changes, signs of difficult or prolonged parturition, all signs of toxicity including mortality (twice daily)	- record duration of gestation, pertinent behaviour changes, signs of difficult or prolonged parturition, all signs of toxicity including mortality (twice daily)
19. Mortality	- at least once/day	- at least once/day	- at least twice daily

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
20. Examination of litters at birth	- as soon as possible after delivery	- as soon as possible after delivery	- as soon as possible after delivery
	- number of pups, stillbirths, live births	- number of pups, stillbirths, live births, runts	- number of pups, stillbirths, live births, runts
	- sex of pups	- sex of pups	- sex of pups
	- gross anomalies	- gross abnormalities	- gross abnormalities
21. Preservation of pups	- dead or moribund pups and pups sacrificed at d 4 should be preserved and studied for possible defects	- dead pups and pups killed at d 4 should be examined externally for gross abnormalities	- dead pups and pups killed at d 4 should be examined externally for gross abnormalities
22. Examinations during lactation	- count live pups	- count and sex live pups on day 4	- count and sex live pups on day 4
	- weigh litters on: morning after birth d 4 d 7	- weigh litters on: d 0/1 d 4	- weighing of litters on d 0/1 d 4
	- weigh individually weekly thereafter until termination of the study	- not mentioned	- not mentioned

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
Study obsrevations - II	**Haematology, clinical chemistry and urinalysis**		
23. Haematology	- not mentioned	- not mentioned	- once during study in 5M/5F/group preferably prior to or as a part of the procedure for killing[1]; F should be in a physiologically-similar state; overnight fasting is recommended
24. Clinical Biochemistry	- not mentioned	- not mentioned	- once during study in 5M/group preferably prior to or as a part of procedure for killing[1]; F should be in a physiologically-similar state
25. Urinalysis	- not mentioned	- not mentioned	- optionally in 5M/5F/group during last week of the study
Study observations - III	**Post-mortem examinations**		
26. Gross pathology	- at time of sacrifice or death during study, all parental animals should be examined macroscopically for any structural abnormalities or pathological changes - special attention to organs of reproductive system	- at time of sacrifice or death during study, all adult animals should be examined for any abnormalities or pathological changes - special attention to organs of reproductive system - F: number of implantations - F: counting of *corpora lutea* recommended	- all adult animals full detailed gross necropsy - special attention to the organs of the reproductive system - F: number of implantations - F: counting of *corpora lutea* recommended
27. Organ weights	- not mentioned	- all M: testes, epididymes	- all M: testis, epididymes - 5M/5F/group: liver, kidneys, adrenals, spleen, thymus, brain and heart
28. Preservation of organs	- ovaries, uterus, cervix, vagina, testes, epididymes, seminal vesicles, prostate, coagulating gland, pituitary gland, target organs of all P animals	- ovaries, testes[2], epididymes[2], accessory sex organs, all gross lesions	- ovaries, testes[2], epididymes[2], accessory sex organs, all gross lesions of all animals - 5M/5F/group: brain, spinal cord, stomach, small and large intestines, liver,kidneys, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, uterus, urinary bladder, lymph nodes, peripheral nerve, bone marrow, gross lesions - any organs considered likely to be target

1 if historical baseline data are inadequate, consideration should be given to determination of parameters before dosing commences

2 Bouin's fixative acceptable (formalin fixation not recommended)

Table 9 (cont.): Reproductive toxicity guidelines TG 415, 421 and 422

	OECD (1983a) TG 415	OECD (1995a) TG 421 / EPA (2000a)	OECD (1996) TG 422 / EPA (2000b)
29. Histopathology	- if necessary, or if organs (c.f. 28) have not been examined in other multiple-dose studies from: a) all animals in control and high dose groups b) all animals dying during study (where practicable) c) organs showing abnormalities in these animals should be examined in animals from the other dose groups - microscopy of all tissues showing gross-pathological changes - microscopy of reproductive organs of animals suspected of infertility	- ovaries, testes and epididymes of all animals in control and high dose group with special emphasis on stages of spermatogenesis and interstitial testicular structure; examinations should be extended to animals of other dosage groups when changes were seen in the high dose group (EPA only: additional details on histopathological examinations of testes, epididymides and ovaries) - examination of the other preserved organs (c.f. 28) when necessary	- all preserved organs and tissues of the selected animals in control and high dose group with special emphasis on stages of spermatogenesis and interstitial testicular cell-structure; examinations should be extended to animals of other dosage groups when changes were seen in the high dose group (EPA only: additional details on histopathological examinations of testes, epididymides and ovaries) - all gross lesions - target organs in the other dose groups claimed to show a NOAEL- when a satellite group is used, histopathology on tissues and organs identified as showing effects in treated groups

APPENDIX B: SUMMARY DATA SETS FOR EXAMPLE SUBSTANCES

DT Chemical 1 - Example 1 - Generic class: Retinoid

Test conditions

Type of study	teratology segment II including rearing group
GLP	yes
Animal species	rat
Route of administration	oral
Method of administration	gavage
Dose levels (mg/kg bodyweight)	0, 0.7, 2.0, 6.0, 15.0
Days of treatment during pregnancy (TDP)	7-16 (evidence of mating = day 1)
Number of animals per group	21, 19, 17, 23 (C-section) 8, 9, 8, 9, 15 (rearing)

Maternal toxicity

Dose (mg/kg)	Effect	Change towards control (%)
0-15	↘ bw, TDP11-15 (females of rearing group only)	28 (not corrected for uterus weight)

Prenatal developmental toxicity, gestational parameters

Dose (mg/kg)	Effect	Mean % by Litter
15	↗ postimplantation loss	34.5 1.8 -

Prenatal developmental toxicity, foetal growth parameters

Dose (mg/kg)	Effect	Change towards control %
15	↘ foetal wt	20

Prenatal developmental toxicity, external effects

Dose (mg/kg)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
15	cleft palate	60.9	0	<0.01	87.5	0	<0.01
	exencephaly	50.3	0	<0.01	75.0	0	<0.01
	spina bifida	3.1	0	<0.01	18.8	0	<0.01
	open eyes	16.8	0	<0.01	56.3	0	<0.01

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, skeletal effects

Dose (mg/kg)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
6	occipital bone, incised and/or bipartite	4.6	0	0-0.2	18.2	0	0-1.2
	vertebral arches cervical, bipartite	8.3	4.2	0-25.0*	36.4	22.2	
	rib, supernumerary (14th)	41.7	6.3	0-29.3	90.9	33.3	
15	not examined						

Test = Treated animals; Cont. = Control; Hist. = Historical controls

* includes split neural arches at cervical / thoracic / lumbar location

Litters and foetuses examined

Dose (mg/kg)	Litters	Foetuses		
		External	Visceral	Skeletal
0	20	195	99	96
0.7	19	196	95	101
2	17	171	87	84
6	23	231	123	108
15	16	161	-	-

Pup parameters (rearing sub group)

Dose (mg/kg)	Pups born alive (total/group)	Pups surviving weaning N (%)
0	86	83 (96.5)
0.7	96	90 (93.8)
2	92	89 (96.7)
6	84	57 (67.9)
15	0	0

DT Chemical 2 - example 2 - Generic class: Aromatic hydrocarbons

Test conditions

Type of study	developmental toxicity test OECD 414
GLP	yes
Animal species	rat
Route of administration	inhalation
Method of administration	whole body
Concentration level (ppm, inhalation)	0, 250, 750, 1500, 3000
Exposure time per day (hours)	6
Days of treatment during pregnancy (TDP)	6-15
Number of animals per group	25

Maternal toxicity

Conc. (ppm)	Effect	Change towards control (%)
750	hunched posture, eyelids closed / half closed	
	↗ rel liver wt	6
1500	hunched posture, eyelids closed / half closed, limb tremors, lacrymation, salivation, nystagmus, ↗ inspiration rate, lateral recumbency / uncontrolled movements	
	↘ bw gain, TDP20	4
	↗ rel liver wt	3
3000	hunched posture, eyelids closed / half closed, limb tremors, lacrymation, salivation, nystagmus, ↗ inspiration rate, lateral recumbency / uncontrolled movements	
	↘ adj bw, TDP20	8
	↘ bw gain, TDP20	29
	↗ water consumption, TDP19	45
	↘ food consumption, TDP6-15	18
	↗ rel liver wt	7

Prenatal developmental toxicity, foetal growth parameters

Conc. (ppm)	Effect	Change towards control (%)
250	↘ foetal wt	4
750	↘ foetal wt	1
1500	↘ foetal wt	8
3000	↘ foetal wt	13
	↘ gravid uterine wt	12

Prenatal developmental toxicity,skeletal effects

Conc. (ppm)	Effect	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
250	sternebrae, incomplete ossification	45.4	28.5	-	-	-	-
	sternebrae, unossified	44.4	37.0				
1500	sternebrae, incomplete ossification	44.3	28.5	-	-	-	-
	sternebrae, unossified	52.7	37.0				
3000	sternebrae, incomplete ossification	44.9	28.5	-	-	-	-
	sternebrae, unossified	60.1	37.0				

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Litters and foetuses examined

Conc. (ppm)		Litters	Foetuses	
		External	Visceral	Skeletal
0	24	293	147	146
250	22	272	136	131
750	20	233	117	116
1500	19	236	114	116
3000	22	277	134	138

DT Chemical 3- example 3 - Generic class: Aromatic hydrocarbons

Test conditions

Type of study	developmental toxicity test, EPA test guideline
GLP	no data
Animal species	rat
Route of administration	inhalation
Method of administration	whole body
Concentration level (ppm, inhalation)	0, 100, 400
Exposure time per day (hours)	6
Days of treatment during pregnancy (TDP)	6-15
Number of animals per group	27

Maternal toxicity

Conc. (ppm)	**Effect**	**Change towards control (%)**
100, 400	no adverse effects	

Litters and foetuses examined

Conc. (ppm)	**Litters**	**Foetuses**		
		External	**Visceral**	**Skeletal**
0	26	212	108	212
100	27	221	105	221
400	27	224	104	224

no adverse effects observed in foetuses

DT Chemical 4, study 1 - example 4

Test conditions

Type of study	developmental toxicity test OECD 414
GLP	yes
Animal species	rat
Route of administration	oral
Method of administration	diet
Vehicle	none
Dose levels (mg/kg bodyweight)	0, 19, 36, 55, 76, 143
Dose levels (ppm in feed)	0, 250, 500, 750,1000, 2000
Days of treatment during pregnancy (TDP)	0-20
Number of animals per group	29, 27, 29, 30, 27

Maternal toxicity

Dose (mg/kg)	Effect	Change toward control (%)
143	↗ rel kidney wt	8

Prenatal developmental toxicity, foetal growth parameters

Dose (mg/kg)	Effect	Change towards control %
76	↘ foetal wt	6
143	↘ foetal wt	13

Prenatal developmental toxicity, skeletal effects

Dose (mg/kg)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
76	supernumerary rib, lumbar	1.5	3.1	0.8-14.4	20.7	30.8	-
	rib 13, short	1.5	0	0.3	13.8	0	
	rib, wavy	2.1	0	0.3	20.7	0	
143	supernumerary rib, lumbar	0.2	3.1	0.8-14.4	3.7	30.8	-
	rib 13, short	3.4	0	0.3	22.2	0	
	rib, wavy	10	0	0.3	48.2	0	

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Litters and foetuses examined

Dose (mg/kg)	Litters	Foetuses		
		External	Visceral	Skeletal
0	26	417	211	417
19	29	461	226	461
36	27	437	220	437
55	29	437	221	437
76	29	471	236	471
143	27	411	209	411

DT Chemical 4, study 2 - example 5

Test conditions

Type of study	developmental toxicity test OECD 414
GLP	no
Animal species	rat
Route of administration	oral
Method of administration	diet
Vehicle	none
Dose levels (mg/kg bodyweight)	0, 78, 163, 330, 537
Dose levels (ppm in feed)	1000, 2000, 4000, 8000
Days of treatment during pregnancy (TDP)	0-20, 6-15 (8000 ppm)
Number of animals per group	29, 14 (8000ppm)

Maternal toxicity

Dose (mg/kg)	Effect	Change toward control (%)
163	↗ food, TDP0-20	5
	↗ rel liver wt	5
	↗ rel kidney wt	11
330	↘ bw gain TDP 0-20	11
	↗ bw gain TDP 0-20 (corrected)	14
	↘ gravid uterine wt	30
	↗ food consumption, TDP0-20	7
	↗ water consumption, TDP18-20	23
	↗ rel liver wt	6
	↗ rel kidney wt	12
537	↘ bw gain TDP 0-20	35
	↘ gravid uterine wt	59
	↘ gestation wt gain	58
	↘ food, TDP6-15	13
	↗ food, TDP15-18	22
	↘ water consumption, TDP6-9	17
	↗ rel liver wt	13
	↗ rel kidney wt	30

Prenatal developmental toxicity, gestational parameters

Dose (mg/kg)	Effect	Mean % by litter		
		Test	Cont.	Hist.
537	resorptions (post implantation loss)	36.2	4.43	5.4±0.78
	litters with resorptions	100	36	
	late foetal deaths	2.38	0	

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, foetal growth parameters

Dose (mg/kg)	Effect	Change towards control %
78	↘ foetal wt	6
163	↘ foetal wt	13
330	↘ foetal wt	37
537	↘ foetal wt	54
	↘ viable foetuses	37

Prenatal developmental toxicity, external effects

Dose (mg/kg)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
537	anophthalmia	4.41	0.47	0	35.71	7.14	-
	microphthalmia	5.15	0	0	35.71	0	
	tail, shortened and curly	11.0	.	.	42.9	0	

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, vicseral effects

Dose (mg/kg)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
330	brain, enlarged lateral ventricles	5.44	0	0.07	24	0	-
537	brain, enlarged lateral ventricles	17.6	0	0.07	64.3	0	-

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, skeletal effects

Dose (mg/kg bw)	Effects	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
78	supernumerary rib, lumbar	2.1	14.8	6.3	17.9	67.9	
	rib 13 ns, short	2.5	0.2	1.4	17.9	3.6	
163	supernumerary rib, lumbar	0	14.8	6.3	0	67.0	
	rib, wavy	4.4	0.2	2.1	30.8	3.6	
	rib 13, short	6.9	0.2	1.4	38.5	3.6	
330	supernumerary rib, lumbar	0.5	14.8	6.3	8.0	67.9	
	rib, wavy	13.1	0.2	2.1	64	3.6	
	rib 13, short	39.4	0.2	1.4	100	3.6	
	rib 13, absent	6.2	0.2		36	3.6	
537	supernumerary rib, lumbar	0	17.2	6.3	0	50	
	rib 13, short	36.8	0.5	1.4	64.2	7.1	
	rib 13, absent	12.5	0		42.8	0	

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Litters and foetuses examined

Dose (mg/kg)	Litters	Foetuses		
		External	Visceral	Skeletal
0	28	431	431	431
78	28	432	432	432
163	26	408	408	408
330	255	386	386	386
0	14	215	215	215
537	14	136	136	136

DT Chemical 5 - example 6

Test conditions

Type of study	unknown
GLP	unknown
Animal species	rat
Route of administration	oral
Method of administration	drinking water
Vehicle	water
Dose levels (mg/kg bodyweight)	not specified
Dose levels (mg/l water, ppm)	30
Days of treatment during pregnancy (TDP)	6-15
Number of animals per group	20

Maternal toxicity

Dose (ppm)	Effect	Change towards control (%)
30	↘ bw, TDP20	1.3

Prenatal developmental toxicity, gestational parameters

Dose (ppm)	Effect	Mean % by litter		
		Test	Cont.	Hist.
30	↗ post-implantation loss	10.4	5.3	-

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, foetal growth parameters

Dose (ppm)	Effect	Change towards control (%)
30	↗ runt foetuses	437

Prenatal developmental toxicity, external effects

Dose (ppm)	Effect	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
30	ecchymoses	31.7	19.8	-	-	-	-

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Prenatal developmental toxicity, skeletal effects

Dose (ppm)	Effect	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
30	sternum, misshapen	9.6	5.2				
	parietals, misshapen	8.0	3.3				

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Litters and foetuses examined

Dose (ppm)	Litters	Foetuses		
		External	Visceral	Skeletal
0	20	no data	no data	no data
30	20	no data	no data	no data

DT Chemical 6 - example 7

Test conditions

Type of study	developmental toxicity test OECD 414
GLP	yes
Animal species	rat
Route of administration	oral
Method of administration	gavage
Vehicle	corn oil
Vehicle dose rate (ml/kg bodyweight)	5
Dose levels (mg/kg bodyweight)	0, 25, 75, 175
Days of treatment during pregnancy (TDP)	6-15
Number of animals per group	25

Maternal toxicity

Dose (mg/kg)	Effect	Change towards control (%)
75	↘ bw gain, TDP6-9	67
	↘ food consumption, TDP6-9	7
175	↘ bw gain, TDP6-9	56
	↘ food consumption, TDP6-9	11

Prenatal developmental toxicity, foetal growth parameters

Dose (mg/kg)	Effect	Change towards control (%)
175	↘ gravid uterine wt	15
	↘ foetal wt	14

Prenatal developmental toxicity, skeletal effects

Dose (mg/kg)	Effect	Incidence of effects (%)					
		Foetal basis			Litter basis		
		Test	Cont.	Hist.	Test	Cont.	Hist.
75	ribs, bent	6	1	0-4	17	8	0-21
175	ribs, bent	14	1	0-4	63	8	0-21
	limb bones, bent	1	0	0-1	12	0	0-5
	sternebrae 5,6, unossified	18	6	1-37	50	33	5-
100	vertebral arches, incomplete ossification	5	0	0-2	25	4	0-10

Test = Treated animals; Cont. = Control; Hist. = Historical controls

Litters and foetuses examined

Dose (mg/kg)	Litters	Foetuses		
		External	Visceral	Skeletal
0	24	340	340	340
25	25	352	352	352
75	23	316	316	316
175	24	321	321	321

Fert Chemical 7, study 1 - example 8

Test conditions

Type of study	continuous breeding, NTP RACB protocol
GLP	no
Animal species	mouse
Route of administration	oral
Method of administration	diet
Vehicle	feed
Dose levels (mg/kg bodyweight)	0, 152, 636, 1262
Dose levels (mg/kg feed, ppm)	0, 1000, 4500, 9000
Generations	P, F_{1a}, F_{1b}, F_{1c}, F_{1d}, F_{1e}, F_{2a}
Number of animals per group	40 M, 40 F for controls 20 M, 20 F for dose groups
Other relevant details	14 week treatment with 1 week pretreatment period, final litter of the lowest dose level was taken to produce the F_2 generation

P- Generation, toxicity

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
636	M,F	↗ food consumption, wk 13	15
		↗ water consumption, wk 13	43
	F	↘ relative kidney wt	6
		↘ relative liver wt	6
1262	M,F	↗ food consumption, wk13	32
		↗ water consumption, wk 13	90
	M	↘ bw, wk 2-18	11
	F	↘ bw, wk 2-18	31

P- Generation, reproductive parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
636	M	↘ testis wt	40
		↘ sperm count	72
		↘ sperm motility	31
1262	M	↘ testis wt	70
		↘ sperm count	95
		↘ fertility	100
		↘ sperm motility	45

F_{1a} Pup parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
636	M,F	↘ live pups/litter	23
		↘ live pup wt	12

Fert Chemical 7, study 2 - example 9

Test conditions

Type of study	three generation reproduction study
GLP	no
Animal species	rat
Route of administration	oral
Method of administration	diet
Vehicle	feed
Dose levels (mg/kg bodyweight)	0, 33, 100, 334
Dose levels (mg/kg feed, ppm)	0, 670, 2000, 6700
Generations	P, F_{1a}, F_{1b}, F_{2a}, F_{2b}, F_{3a}, F_{3b}
Number of animals per group	8M, 16F
Other relevant details	animals were maintained on diet for 14 weeks until maturity, then mated for 21 days for F_{1a} and remated 21 days later for F_{1b}, F_{1b} was maintained on treated diet to produce F_{2a} and F_{2b}, F_3 was obtained from F_{2b} animals

P- Generation, toxicity

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
334	M,F	inflamed eyelids, staining of fur, scaliness of tails, rough fur.	
	M	↘ bw (wk 14)	18
	F	↘ bw (wk 14)	10

P- Generation, reproductive parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
334	M,F	↘ fertlity	100

F_{1a} Pup parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
33	M,F	↗ lactation index	71
100	M,F	↗ lactation index	25

F_{1b} Pup parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
33	M,F	↗ lactation index	46
100	M,F	↗ lactation index	36

F_{2a} Pup parameters

Dose level (mg/kg)	Sex (M/F)	Effect	Change towards control (%)
33	M,F	↗ lactation index	31
100	M,F	↗ lactation index	34

Fert Chemical 8 - example 10

Test conditions

Type of study	two-generation reproduction, EPA, FIFRA 83-4,
GLP	yes
Animal species	rat
Route of administration	inhalation
Method of administration	whole body
Vehicle	none
Concentration level (ppm, inhalation)	0, 10, 33, 100
Exposure time per day (hours)	6
Generations	P, F_{1a}, F_{2a}
Number of animals per group	28 M, 28 F
Other relevant details	exposure for 7 days/week, litters were weaned on TDL 28

P- Generation, toxicity

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
33	M	↘ bw, wk 6-7	3
		↘ bw gain, wk 2-3	11
	F	↘ bw, TDP14	4
		↘ food consumption, TDL 0-14	7
100	M	↘ bw, wk 2-14	5
		↘ bw gain, wk 0-3	15
		↘ food consumption, wk 1-3	6
		↗ rel liver wt	6
	F	↘ bw, TDP14	5
		↘ bw, TDP20	8
		↘ bw gain, TDP0-20	18
		↘ food consumption, TDL0-14	14

P- Generation, reproductive parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
0	F	post implantation loss	7
33	F	post implantation loss	14
100	F	post implantation loss	41

F_{1a} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
33	M,F	↘ litter size (total), TDL0 (NS)	9
		↘ litter size (alive), TDL0 (NS)	9
		↘ bw, TDL7-21	7
		↘ bw, TDL28	5
		↘ bw, TDL4-14	13
100	M,F	↘ litter size (total), TDL0	35
		↘ litter size (alive), TDL0	36
		↗ bw, TDL1-4	10
		↘ bw, TDL14-21	13
		↘ bw, TDL28	9
		↗ bw gain, TDL1-4	14
		↘ bw gain, TDL4-21	24

F_1- Generation, toxicity

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
33	M	↘ bw, wk 0-15	9
		↘ abs liver wt	11
		↘ abs lung wt	6
		↘ rel lung wt	6
100	M	↘ bw, wk 0-15	12
		↘ abs liver wt	10
		↘ abs lung wt	8
		↘ rel lung wt	7
	F	↘ bw, TDP20	9
		↘ bw change, TDP0-20	19
		↘ bw, TDL14	5
		↘ bw gain, TDL0-14	51
		↘ bw loss, TDL14-21	69
		↘ bw loss, TDL21-28	31
		↘ bw loss, TDL0-28 (NS)	10
		↘ food consumption, TDL0-14	20

F_1- Generation reproductive parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
100	F	↗ gestational length	1
		post implantation loss	42

F_{2a} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
33	M,F	↘ bw, TDL7-14	6
		↘ bw gain, TDL4-7	13
		↘ bw gain, TDL7-14	9
100	M,F	↘ litter size (total), TDL0	44
		↘ litter size (alive), TDL0	45
		↗ bw, TDL1-4	10
		↘ bw, TDL14-21	12
		↘ bw, TDL28 (NS)	7
		↗ bw gain, TDL1-4	15
		↘ bw gain, TDL4-14	26

Fert Chemical 9 - example 11

Test conditions

Type of study	two-generation reproduction test OECD 416
GLP	yes
Animal species	rat
Route of administration	oral
Method of administration	diet
Vehicle	feed
Dose levels (mg/kg bodyweight)	not specified
Dose levels (mg/kg feed, ppm)	0, 50, 150, 600
Generations	P, F_{1a}, F_{1b}, F_{2a}, F_{2b}
Number of animals per group	25 M, 25F

P - Generation, toxicity

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
600	M	↘ bw (study day 196)	8
	F	circling movement, head bobbing (wk 20-)	
		↘ bw (study day 71)	6
		↘ bw, TDL21, 1st mating	5
		↘ bw, TDP21, 2nd mating	7
		↘ bw, TDL21, 2nd mating	7
		↘ food consumption, TDP0-21, 2nd mating	9

F_{1a} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
150	M, F	↘ bw, TDL21	10
	F	↘ bw, TDL0	10
600	M, F	↘ bw, TDL21	9

F_{1b} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
600	M,F	↘ bw, TDL21	7
	F	↘ abs kidney wt, TDL21	12

F_1- Generation, toxicity

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
150	M	↘ bw (study day 310)	7
600	M	↘ bw (study day 310)	12
		↗ abs kidney wt	9
		↗ rel kidney wt	22
		↗ rel brain wt	10
		↗ rel liver wtkidney, chronic nephritis	15
	F	circling movement, head bobbing (week 12-)	
		↘ bw (study day 191)	6
		↘ bw, TDP21, 1st mating	8
		↘ bw, TDL21, 1st mating	8
		↘ bw, TDP21, 2nd mating	13
		↘ bw, TDL21, 2nd mating	9
		↘ bw gain, TDP0-21, 1st mating	11
		↘ bw gain, TDP0-21, 2nd mating	17
		↘ food consumption, TDP0-21, 1st mating	11
		↘ food consumption, TDP0-21, 2nd mating	13
		↗ rel brain wt	10
		↗ rel kidney wt	16
		↗ rel ovary wt	18

F_1- Generation, reproductive parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
600	F	↘ pregnancy rate, 2nd mating	26
		↘ gestational length, 2nd mating	2

F_{2a} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
600	M,F	↘ bw, TDL21	9
	M	↗ rel testes wt, TDL21	16

F_{2b} Pup parameters

Dose level (ppm)	Sex (M/F)	Effect	Change towards control (%)
600	M,F	↘ bw, TDL21	10
	M	↘ abs kidney wt, TDL21	15

BIBLIOGRAPHY

Baker JH, Lindsey JR and Weisbroth SH (eds). 1980. *The Laboratory Rat, Vol II. Research Applications.* Academic Press.

Beyer PE, Chernoff N. 1986. The induction of supernumerary ribs in rodents: Role of the maternal stress. *Teratogen Carcinogen Mutagen* 6: 419-429.

BGVV. 2000. Third Berlin workshop on terminology in developmental toxicology. Manuscript in preparation.

Black DL, Marks TA. 1986. Inconsistent use of terminology in animals developmental toxicology studies: a discussion. *Teratology* 33:333-338.

Brent RL, 1990. Editorial comment: Relationship between uterine vascular clamping, vascular disruption syndrome, and cocaine teratogenicity. *Teratology* 41: 757-760.

Carney EW, 1997. Maternal physiological disruption. In: Kavlock RJ and Daston GP, eds, *Drug toxicity in embryonic development. Part 1: Advances in understanding mechanisms of birth defects: morphogenesis and processes at risk.* Vol 1. Springer, 573-594.

Chahoud I, Buschmann J, Clark R, Druga A, Falke H, Faqi A, Hansen E, Heinrich-Hirsch B, Hellwig J, Lingk W, Parkinson M, Paumgartten FJR, Pfeil R, Platzek T, Scialli AR, Seed J, Stahlmann R, Ulbrich B, Wu X, Yasuda M, Younes M, Solecki R. 1999a. Classification terms in developmental toxicology: need for harmonisation. *Reprod Toxicol* 13: 77-82.

Chahoud I, Ligensa A, Dietzel L, Faqi AS. 1999b. Correlation between maternal toxicity and embryo / fetal effects. *Reprod Toxicol* 13: 375-381.

Chernoff GF, Golden JA. 1988. Hyperthermia-induced exencephaly in mice: effect of multiple exposures. *Teratology* 37: 37-42.

Chernoff N, Miller DB, Rosen MB, Mattscheck CL. 1988. Developmental effects of maternal stress in the CD-1 mouse induced by restraint on single days during the period of major organogenesis. *Toxicology* 51: 57-65.

Clark RL, Robertson RT, Minsker DH, Cohen SM, Tocco DJ, Allen HL, James ML, Bokelman DL. 1984. Diflunisal-induced maternal anemia as a cause of teratogenicity in rabbits. *Teratology* 30, 319-332.

Clark RL, Robertson RT, Peter CP, Bland JA, Nolan T, Oppenheimer L, Bokelman DL. 1986. Association between adverse maternal and embryo-fetal effects in norfloxacin-treated and food-deprived rabbits. *Toxicol Appl Pharmacol* 7: 272-286.

Clegg ED, Salsai CS, Voytek. 1986. *Bio Reprod* 34: 5-16.

Danielsson BRG, Reiland S, Rundqvist E, Danielsson M. 1989. Digital defects induced by vasodilating agents: relationship to reduction in uteroplacental blood flow. *Teratology* 40: 351-358.

Danielsson BRG, Danielsson M, Reiland S, Rundqvist E, Dencker L, Regård C-G, 1990. Histological and in vitro studies supporting decreased uteroplacetal blood flow as explanation for digital defects after administration of vasodilators. *Teratology* 41: 185-193.

Daston GP. 1994. Relationships between maternal and developmental toxicity. In: Kimmel CA, Buelke-Sam J, eds, *Developmental Toxicology* 2nd ed. Raven Press, NY, pp 189-212.

EC. 1967. Council Directive 67/548/EEC, on the classification, packaging and labelling of dangerous substances. OJ Eur Comm L196.

EC. 1988a. Commission Directive 87/302/EEC of 18 November 1987. Part B. Methods for the determination of toxicity. Teratogenicity study (rodent and non-rodent). O J Eur Comm L133, pp 24-26.

EC. 1988b. Commission Directive 87/302/EEC of 18 November 1987. Part B. Methods for the determination of toxicity. Two-generation reproduction toxicity test. O J Eur Comm L133, pp.47-50.

ECETOC. 1983. Identification and assessment of the effects of chemicals on reproduction and development (reproductive toxicology). Monograph No.5.

ECETOC. 1992. EC 7th amendment:"toxic to reproduction". Guidance on classification. Technical Report No. 47.

ECETOC. 1996. 'Environmental oestrogens'. A compendium of test methods. Document No. 33.

EPA. 1984a. Pesticide assessment guidelines. Teratogenicity study. Subdivision F, §83-3. NTIS rev ed, pp 126-130.

EPA. 1984b. Pesticide assessment guidelines. Reproductive and fertility effects. Subdivision F, §83.4. NTIS rev ed, pp130-137.

EPA. 1992. Guidelines for developmental toxicity risk assessment. CHM-165, 39.3401.

EPA. 1996. Reproductive Toxicity Risk Assessment Guidelines: Notice. 61 (212) Federal Register 56274-56322.

EPA. 1998a. Health Effects Test Guidelines OPPTS 870.3100. 90 day oral toxicity in rodents.

EPA. 1998b. Health Effects Test Guidelines OPPTS 870.3800. Reproductive and fertility effects.

EPA. 1998c. Health Effects Test Guidelines. OPPTS 870.6300. Developmental neurotoxicity study.

EPA. 1998d. Health Effects Test Guidelines. OPPTS 870.3700. Prenatal developmental toxicity study.

EPA. 2000a. Health Effects Test Guidelines. OPPTS 870.3550. Reproduction/developmental toxicity screening test.

EPA. 2000b. Health Effects Test Guidelines. OPPTS 870.3650. Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test.

EPA / TSCA (Environmental Protection Agency / Toxic Substances Control Act). 1992a. Developmental toxicity study. 40 CFR §798.4900, pp 498-500.

EPA / TSCA (Environmental Protection Agency / Toxic Substances Control Act). 1992b. Reproduction and fertility effects. 40 CFR §798.4700, pp 495-498.

Harding AJ, Edwards MJ. 1993. Microencephaly In rats caused by maternal hyperthermia on days 13 and 14 of pregnancy. *Cong Anom* 33: 203-209.

Hood RD (ed). 1997. *Handbook of developmental toxicology*. CRC Press.

Hood RD, Miller DB. 1997. Maternally mediated effects on development. In: Hood RD, ed, *Handbook of developmental toxicology*. CRC Press, pp 61-90.

ICH. 1994. Guideline on detection of toxicity to reproduction for medical products. Food and Drug Administration, HHS. Fed Reg 59:48746-48752.

ILSI. 1999. Health and Environmental Sciences Institute. Daston G, Kimmel C, eds. An evaluation and interpretation of reproduction endpoints for human health risk assessment.

IPCS(International Program of Chemical Safety) / OECD, 1994. Workshop on the Harmonisation of Risk Assessment of Reproductive and Developmental Toxicity, 17-21 October, BIBRA Toxicology International, Carshalton, United Kingdom (First Draft August 1994).

Japan / MAFF. 1985a. Testing guidelines for toxicology studies. Reproduction study, pp 45-48.

Japan / MAFF. 1985b. Testing guidelines for toxicology studies. Teratogenicity study, pp 48-49

Kaufmann W. 2000. Developmental neurotoxicity. Chapter 12. In Krinke, GJ, ed, *The laboratory rat*. Academic Press.

Kavlock RJ, Chernoff N, Rogers EH. 1985. The effect of acute maternal toxicity on fetal development in the mouse. *Teratogen Mutagen Carcinogen* 5: 3-13.

Khera KS. 1981. Common fetal aberrations and their teratologic significance: a review. *Fund Appl Toxicol* 1:13-18.

Khera KS. 1984. Maternal toxicity: a possible factor in fetal malformations in mice. *Teratology* 29: 411-416.

Khera KS. 1985. Maternal toxicity: a possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. *Teratology* 31: 129-153.

Kimmel CA, Buelke-Sam, J. 1994. Developmental toxicology. In Hayes AW, Thomas JA, Gardner DE, eds, *Target organ toxicology series*, 2nd ed, Raven Press, NY.

Knobil E, Neill JD (eds). 1998. *Encyclopedia of reproduction*, Academic Press.

Linder RE, Strader LF, Stott VL, Suarez JD. 1992. *Reprod Toxicol* 6: 491-505.

Manson JM, Kang YJ. 1994. Test methods for assessing female reproductive and developmental toxicology. In Wallace Hayes A, ed, *Principles and methods of toxicology*, 3rd ed, Raven Press, NY, pp 10-37.

Millicovsky G, Johnston MC. 1981. Maternal hyperoxia greatly reduces the incidence of phenytoin-induced cleft lip and palate in A/J mice. *Science* 212: 671-672.

Moore JA, Daston GP, Faustman E, Golu MS, Hart WL, Hughes C, Kimmel CA, Lamb JC, Schwetz BA, Sciallie AR. 1995. *Reprod Toxicol* 9: 61-65.

Morrisey RE, Schwetz BA, Lamb JC, Ross MD, Teague JL, Morris RW. 1988. *Fund Appl Toxicol* 11: 343-358.

Nakatsuka T, Fujikake N, Hasebe M, Ikeda H. 1993. Effects of sodium bicarbonate and ammonium chloride on the incidence of furosemide-induced fetal skeletal anomaly, wavy rib, in rats. *Teratology* 48: 139-147.

Nelson BK, Conover DL, Brightwell WS, Shaw PB, Werren D, Edwards RM, Lary JM. 1991. Marked increase in the teratogenicity of the combined administration of the industrial solvent 2-methoxyethanol and radiofrequency radiation in rats. *Teratology* 43: 621-634.

OECD. 1981. Guideline for Testing of Chemicals. 414. Teratogenicity.

OECD. 1983a. Guideline for Testing of Chemicals. 415. One-generation reproduction toxicity study.

OECD. 1983b. Guideline for Testing of Chemicals. 416. Two-generation reproduction toxicity study.

OECD. 1995a. Guideline for Testing of Chemicals. 421. Reproduction/developmental toxicity screening test.

OECD. 1995b. Guideline for Testing of Chemicals. 407. Repeated dose 28-day oral toxicity study in rodents.

OECD. 1996. Guideline for Testing of Chemicals. 422. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test.

OECD. 1998. Guideline for Testing of Chemicals. 408. Repeated dose 90-day oral toxicity study in rodents.

OECD. 1999. Guideline for Testing of Chemicals. 426. Proposal for a new guideline. Developmental neurotoxicity study. Draft document.

OECD. 2001a. Guideline for Testing of Chemicals. 414 Updated. Prenatal developmental toxicity study.

OECD. 2001b. Guideline for Testing of Chemicals. 416 Updated. Two-generation reproduction toxicity study.

Rasco JF, Hood RD. 1994a. Differential effect of restraint procedure on incidence of restraint-stress-induced rib fusion in CD-1 mice. *Toxicol Lett 71*: 177-182.

Rasco JF, Hood RD. 1994b. Effects of maternal restraint stress and sodium arsenate in mice. *Reprod Toxicol* 8: 49-54.

Rasco JF, Hood RD. 1995a. Maternal restraint stress-enhanced teratogenicity of all-trans-retinoic acid in CD-1 Mice. *Teratology* 51: 57-62.

Rasco JF, Hood RD. 1995b. Enhancement of the teratogenicity of all-trans-retinoic acid by maternal restraint stress in mice as a function of treatment timing. *Teratology* 51: 63-70.

Robertson RT, Minsker DH, Bokelman DL, Durand G, Conquet P. 1981. Potassium loss as a causative factor for skeletal malformations in rats produced by indacrinone: a new investigational loop diuretic. *Toxicol Appl Pharmacol* 60: 142-150.

Takayama S, Akaike M, Kawashima K, Takahashi M, Kurokawa Y. 1995. *J Am Coll Toxicol* 14: 266-292.

Ulbrich B, Palmer AK. 1995. Detection of effects on male reproduction. A literature survey. *J Am Coll Toxicol* 14: 293-327.

Watkinson W P, Millicovsky G, 1983. Effect of phenytoin on maternal heart rate in A / J mice: possible role in teratogenesis. *Teratology* 28: 1-8.

Weaver TE, Scott WJ Jr. 1984a. Acetazolamide teratogenesis: association of maternal respiratory acidosis and ectrodactyly in C57BL / 6J mice. *Teratology* 30: 187-193.

Weaver TE, Scott WJ Jr. 1984b. Acetazolamide teratogenesis: interaction of maternal metabolic and respiratory acidosis in the induction of ectrodactyly in C57BL / 6J mice. *Teratology* 30: 195-202.

Wise LD, Beck SL, Beltrame D, Beyer BK, Chahoud I, Clark RL, Clark R, Druga AM, Feuston MH, Guittin P, Henwood SM, Kimmel CA, Lindstrom P, Palmer AK, Petrere JA, Solomon HM, Yasuda M, York RG. 1997. Terminology of developmental abnormalities in common laboratory mammals. (Version 1). *Teratology* 55: 249-292.

Witorsch RJ (ed). 1995. Reproductive toxicology, 2nd ed. In Hayes AW, Thomas JA, Gardner DE, *Target organ toxicology series*, Raven Press, NY.

Zenick H, Clegg ED. 1989. In: Hayes AW, ed, *Principles and methods of toxicology*. Raven Press, NY, pp 285-309.

Zenick H, Clegg ED, Perreault SD, Klinefelter GR, Gray LE. 1994. Assessment of male reproductive toxicity. In Wallace Hayes A, ed, *Principles and methods of toxicology*, 3rd ed, Raven Press, NY, pp 937-988.

DATABASES CONSULTED

Atlas of the International Federation of Teratology Societies (IFTS): http://www.ifts-atlas.org/

Provides the entire terminology for foetal morphological findings published by Wise LD *et al* in Teratology 55 (see above). External, visceral and skeletal findings are illustrated with pictures (not yet complete). Excellent links to all relevant home pages in the field.

Teratology Society homepage: http://teratology.org/

Dynamic Development, A Modular Resource to Facilitate Learning in Developmental Biology http://www.acs.ucalgary.ca/~browder/dev_biol.html

OECD List of Chemical Test Guidelines: http://www.oecd.org/ehs/test/testlist.htm

US EPA, Office of Prevention, Pesticides and Toxic Substances: http://www.epa.gov/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/

NTP (National Toxicology Program Homepage): http://ntp-server.niehs.nih.gov/
Federal Register 1995, 1996, 1997 and 1998: http://www.access.gpo.gov/su_docs/aces/aces140.html

MEMBERS OF THE TASK FORCE

D. Owen (Chairman)	Shell Chemicals UK-London
H. Bürgin	F. Hoffmann-La Roche CH-Basel
G. Daston *	Procter & Gamble USA-Cincinnati
J. Hellwig	BASF D-Ludwigshafen
S. Hubbard	Borax UK-Cheshire
S. Khalil **	Syngenta CH-Stein
R. Lewis	Syngenta UK-Cheshire
M. Martens	Monsanto B-Brussels
N. Moore	BP Chemicals UK-Middlesex
F. Carpanini	ECETOC B-Brussels

* Corresponding member

** now at RCC Ltd, CH-4452 Itingen, Switzerland

MEMBERS OF THE SCIENTIFIC COMMITTEE

B. Hildebrand (Chairman)	Consultant D-Weinheim
N. Carmichael (Co-chairman) Head, Toxicology	Aventis CropScience F - Sophia Antipolis
G. Randall (Co-chairman) Director, Environmental Laboratory	AstraZeneca UK - Brixham
C. Braun Occupational Toxicologist	Akzo Nobel NL - Arnhem
E. Bomhard Head, Industrial Toxicology	Bayer D - Wuppertal
C. d'Hondt Head, Environmental Safety Department	Syngenta CH - Basel
T. Feijtel Manager, Professional and Regulatory Services	Procter & Gamble B - Brussels
J. Jackson Senior Associate, Medical Adviser	Monsanto B - Brussels
R. Millischer Head, Industrial Toxicology Department	Atofina F - Paris
A. Sarrif Director, Toxicology Affairs, Europe	DuPont D - Bad Homburg
J. Solbé Head, SEAC Environment	Unilever UK - Bebington
L. Smith Director, Central Toxicology Laboratory	Syngenta UK - Macclesfield
B. van Ravenzwaay Director, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
H-J. Wiegand Head, Product Safety Department	Degussa D - Marl
Stewards: B. Hildebrand	Consultant D -Weinheim
E. Löser	Bayer D - Wuppertal

ECETOC PUBLISHED REPORTS

Monographs

No.	Title
No. 1	Good Laboratory Practice
No. 2	A Contribution to Strategy for Identification and Control of Occupational Carcinogens
No. 3	Risk Assessment of Occupational Chemical Carcinogens
No. 4	Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD50 (LC50) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary)
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity / teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Allergy
No. 20	Percutaneous Absorption
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation
No. 22	Evaluation of Chemicals for Oculotoxicity
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis
No. 24	Risk Assessment for Carcinogens
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances
No. 27	Aneuploidy
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment
No. 30	Genetic Susceptibility to Environmental Toxicants
No. 31	Guidance on Evaluation of Reproductive Toxicity Data

Technical Reports

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man

No. 6 Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2
No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8 Biodegradation Testing: An Assessment of the Present Status
No. 9 Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11 Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5
No. 12 The Phototransformation of Chemicals in Water: Results of a Ring-Test
No. 13 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment
No. 14 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health
No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16 A Review of Recent Literature on the Toxicology of Benzene
No. 17 The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4
No. 18 Harmonisation of Ready Biodegradability Tests
No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20 Biodegradation Tests for Poorly-Soluble Compounds
No. 21 Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment
No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23 Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24 The EEC 6th Amendment: Prolonged Fish Toxicity Tests
No. 25 Evaluation of Fish Tainting
No. 26 The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride
No. 27 Nitrate and Drinking Water
No. 28 Evaluation of Anaerobic Biodegradation
No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns
No. 30 Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available)
No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment
No. 32 Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data
No. 33 Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34 Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man
No. 35 Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36 Biomonitoring of Industrial Effluents
No. 37 Tetrachlorethylene: Assessment of Human Carcinogenic Hazard
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40 Hazard Assessment of Chemical Contaminants in Soil
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43 Emergency Exposure Indices for Industrial Chemicals
No. 44 Biodegradation Kinetics

No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models
No. 51 Environmental Hazard Assessment of Substances
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment
No. 55 Pulmonary Toxicity of Polyalkylene Glycols
No. 56 Aquatic Toxicity Data Evaluation
No. 57 Polypropylene Production and Colorectal Cancer
No. 58 Assessment of Non-Occupational Exposure to Chemicals
No. 59 Testing for Worker Protection
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard
No. 61 Environmental Exposure Assessment
No. 62 Ammonia Emissions to Air in Western Europe
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man
No. 65 Formaldehyde and Human Cancer Risks
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs
No. 68 Assessment Factors in Human Health Risk Assessment
No. 69 Toxicology of Man-Made Organic Fibres
No. 70 Chronic Neurotoxicity of Solvents
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members)
No. 72 Methyl tert-Butyl Ether (MTBE) Health Risk Characterisation
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank
No. 78 Skin Sensitisation Testing: Methodological Considerations
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data)
No. 80 Aquatic Toxicity of Mixtures
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy
No. 82 Risk Assessment in Marine Environments
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union

Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine
No. 2 1,4-Dioxane
No. 3 Methyl Ethyl Ketone

No. 4 Methylene Chloride
No. 5 Vinylidene Chloride
No. 6 Xylenes
No. 7 Ethylbenzene
No. 8 Methyl Isobutyl Ketone
No. 9 Chlorodifluoromethane
No. 10 Isophorone
No. 11 1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12 1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13 1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14 1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15 1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16 Dichlorofluoromethane (HCFC-21)
No. 17 1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18 Vinyl Acetate
No. 19 Dicyclopentadiene (CAS: 77-73-6)
No. 20 Tris- / Bis- / Mono-(2 ethylhexyl) Phosphate
No. 21 Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1)
No. 23 Polycarboxylate Polymers as Used in Detergents
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27 n-Butyl Acrylate (CAS No. 141-32-2)
No. 28 Ethyl Acrylate (CAS No. 140-88-5)
No. 29 1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30 Methyl Methacrylate (CAS No. 80-62-6)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33 1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34 Acrylic Acid (CAS No. 79-10-7)
No. 35 Methacrylic Acid (CAS No. 79-41-4)
No. 36 n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37 Methyl Acrylate (CAS No. 96-33-3)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39 Tetrachloroethylene (CAS No. 127-18-4)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions

Special Reports

No. Title

No. 8 HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9 Styrene Criteria Document
No. 10 Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11 Ecotoxicology of some Inorganic Borates
No. 12 1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13 Occupational Exposure Limits for Hydrocarbon Solvents
No. 14 n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15 Examination of a Proposed Skin Notation Strategy
No. 16 GREAT-ER User Manual

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP / INC / CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction