Technical Report

No 47

EC 7th Amendment: "Toxic to Reproduction" Guidance on Classification

August 1992

ISSN-0773-8072-47

Technical Report

No. 47

EC 7 th AMENDMENT :

"TOXIC TO REPRODUCTION"
GUIDANCE ON CLASSIFICATION

ISSN-0773-8072-47

Brussels, August 1992

ECETOC Technical Report No 47.

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

EC 7th Amendment: "Toxic to Reproduction", Guidance on Classification

Contents

EC 7th Amendment: "Toxic to Reproduction", Guidance on Classification

SUMMARY

Classification of hazards to the reproductive process in the EC Directive on the Classification, Packaging and Labelling of Dangerous Chemical Substances is presently confined to those substances that are teratogenic. Induction of a teratogenic change is important, but it is only one of many possible hazards to the reproductive process. There is now a consensus that all hazards that may occur in the reproductive cycle should be encompassed within the classification process so that users of substances can judge all potential reproductive risks involved.

A system of classification has been proposed by the EC specialised experts for those substances which may be considered "toxic to reproduction". Fertility and toxicity to the developing foetus have been separated. Based on the general concepts of the European classification scheme for carcinogens, mutagens and teratogens it is proposed that substances harmful to reproduction are classified into three categories; Category 1 includes those substances which affect reproduction (impair fertility or cause developmental toxicity) in man, Category 2 includes those substances that should be regarded as if they will affect reproduction in man, and Category 3 includes substances which may give concern to man owing to possible effects on reproduction, but where the evidence is insufficient to support Category 2. The proposed Risk Phrases for use with each of the categories of fertility and developmental toxicity are listed. It is emphasised that the classification should be based on the occurrence of direct toxic effects on the reproductive system and not on non-specific effects which are secondary to other effects.

For making a classification data will come from human case histories and epidemiological investigations as well as animal studies, including one-, and multi- generation reproduction, developmental toxicity (including teratogenicity), and peri- and post natal studies. Supporting evidence may be

available from repeat dose standard toxicity studies eg. organ weight, histopathological changes in the reproductive and accessory genital organs, and from specialised studies on the hormonal status of animals.

Some in vivo studies are of value as screening tests for identifying substances requiring further investigation; they may also provide data to support the more definitive studies, but cannot be used alone for classification purposes.

In vitro tests are also outlined; although of no value for classification purposes they may be used for preliminary screening of substances or as aids to the investigation of mechanisms of reproductive and developmental toxicity. Problems with the design and interpretation of these tests make them of limited value at the present time.

Guidance is given on the appropriate use of human, animal and other data in classifying substances as "Toxic to Reproduction".

Section 1. INTRODUCTION

In 1979, the European Communities published Council Directive 79/831/EEC amending for the sixth time the Directive 67/548/EEC relating to the Classification, Packaging and Labelling of Dangerous Substances (the "6th Amendment"). This amendment was incorporated into legislation by the Member States in 1984 (Directives 83/467/EEC and 84/449/EEC) requiring manufacturers or importers to propose toxicity classifications for new substances.

Annex VI of the 6th Amendment gave clear guidance on the classification of substances according to their acute toxicity, but it was recognised that the classification might also have to be based on effects other than acute toxicity. The 5th Adaptation of the Directive provided further guidance on classification with respect to acute effects but also defined three categories of carcinogens, three of mutagens and two of teratogens. No detailed guidance was given on the criteria which had to be met for each classification and consequently a task force was initiated by ECETOC to prepare guidance on the classification of the substances to assist those with responsibilities in the area (ECETOC, 1986).

More recently (1989/91), a Specialised Experts Group on the Labelling of Carcinogens, Mutagens and Teratogens (CMT's) was asked by the European Commission to advise on the scientific aspects of the classification of substances under the terms of the 6th Amendment. The group concluded that the class "Teratogenic Substances" and its associated Risk Phrase, R47 (may cause birth defects) should be replaced by a class termed "Toxic to Reproduction" and that the Risk Phrase should also be replaced (EEC, 1992). ECETOC agrees with this initiative. Similar regulations have already been implemented in the United States (US EPA, 1986, 1988a, b) reflecting the general consensus within the scientific community of the need to identify substances which have a potential to interfere with any part of the human reproductive process.

Broadening of this classification introduces further complexity and consequently there is a need for further guidance on criteria for use in the classification of substances which are "Toxic to Reproduction". Classifying

will necessarily involve a critical evaluation for each individual substance of the studies undertaken and expert interpretation of information. Thus the guidance notes presented in this report presuppose that classification will involve an input from specialists in reproductive toxicology and epidemiology.

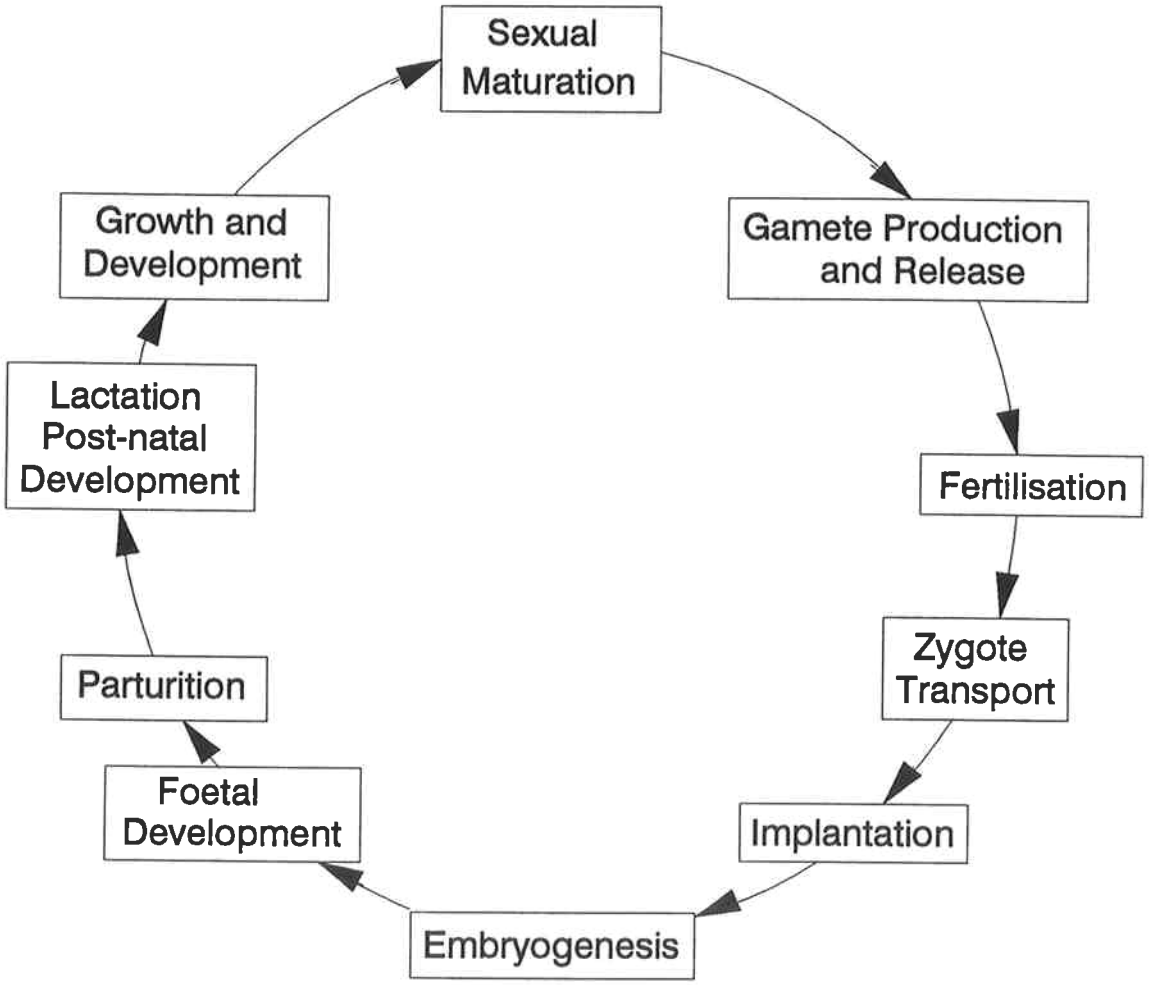
The terms of reference of the task force were:

1. to review experimental studies which provide information on the toxicity of substances which may be classified "Toxic to Reproduction";

2. to provide guidance on the use of data obtained from such studies in classifying substances as "Toxic to Reproduction" and in deciding the appropriate Risk Phrases as an aid to those who have responsibilities in this area.

Section 2. CONCEPTS OF REPRODUCTIVE TOXICITY

Reproduction is a cyclical process which is basically similar in all mammals . The cycle is represented diagrammatically below.



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

- impairment of male and/or female fertility,
- 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 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 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.

In the following sections, studies which can yield information on reproductive toxicity are reviewed. The rationale for placing a substance in one or other of the classifications and categories is then discussed and guidance is given on the types and scope of studies that can provide data to assist in the process.

Section 3. EVALUATION OF STUDIES RELEVANT TO REPRODUCTIVE TOXICITY

Information from the following sources may form the basis for classifying the potential of a chemical to affect adversely the reproductive process in man.

3.1. Epidemiological Studies

Epidemiological techniques may be used to study of the effects of chemicals on the human reproductive process. They can examine either the reproductive outcome of persons exposed to the chemical or the extent or time of chemical exposure of people suffering an adverse reproductive effect. The usefulness of these studies is dependent on such factors as the number of people studied, the background incidence of the reproductive effect, the proportion of the population exposed to the chemical and the level of exposure experienced. The most reliable data are those on fertility and fecundity, for which many countries collate data from birth records and tabulate them by area, ethnic background, age of mother, etc. More data on the incidence of congenital malformations have become available since the Thalidomide episode and, in Europe, the EC is coordinating data from a surveillance programme in 19 regional centres (De Wals et al, 1985). Data on the incidence of spontaneous abortions is generally unavailable or unreliable. Nevertheless, when such data have been based on computerised hospital admission records, as in Scandinavia, some progress has been made on its use for epidemiological purposes (Hemminiki et al, 1982).

Although there are reservations on the validity of the reproductive health data these are of less concern than the scarcity or complete absence of exposure data. It is rare for personal or even area hygiene monitoring data to have been kept for use in a study so that investigators almost always have to fall back on estimates of exposure based on occupational histories or industrial classifications of jobs. Any exposure effect can, as a result, be diluted by the inclusion of unexposed persons among those defined as exposed.

Information on reproductive dysfunction in man can be gathered from:

- historical studies of reproductive performance using a reproductive history obtained by interview or questionnaire;

- case reports which may include reproductive history, the results of investigations and the clinical outcome;

- direct measurement of parameters which reflect aspects of reproductive function;

- prospective studies of reproductive performance obtained by follow-up of exposed and control cohorts.

None of these techniques has been developed to the extent that it has the ability to detect small but important reproductive deficits, particularly in the absence of accurate exposure assessments.

Reproductive histories are critically dependent on the memory of subjects being studied. The recall of events is influenced by their perceived significance and recall may be biased if subjects associate their reproductive problems with something mentioned in an interview. Certain reproductive events, such as early spontaneous abortion, may be hard for subjects to recognise and are therefore subject to inaccurate reporting. To be useful, studies based on reproductive histories need to use large populations or lengthy study periods.

Case reports will often give accurate information on a specific adverse reproductive outcome but the evidence linking that outcome to the suggested cause will, even if quite strong, usually be circumstantial.

Direct measurements can be used to obtain a result quickly but as they are comparatively invasive there may be substantial non-participation, particularly by controls. The measurement most frequently performed is sperm count together with associated parameters, such as sperm motility and morphology (Whorton and Meyer, 1984). The precise relationship of these measurements to fertility and the prevalence of deficits in control populations are the subjects of intense

debate and doubt can be thrown on the interpretation of all but the most extreme effects, such as those induced by dibromochloropropane (Whorton et al, 1977).

A few epidemiological studies have been based on observations other than those mentioned above, for example on the incidence of foetal death (McDonald et al, 1988) but comprehensive data for such outcomes are difficult to obtain and even more difficult to evaluate.

The performance of prospective studies is relatively uncommon.

To date, there are only a few substances for which there is epidemiological data that would lead to Category 1 of the proposed EC system for the labelling of fertility effects (cf. Section 4). Whether this is a reflection of the rarity of adverse human reproductive effects due to chemical exposure, or the imprecision of epidemiological methods remains an open question.

3.2. Animal Studies Designed to Investigate Fertility and Developmental Toxicity

Experimental studies on reproductive toxicity are designed primarily to reveal direct effects on the reproductive process. The complexity of the events involved in fertility and development make it inevitable that physiological disturbances caused by the toxicity of chemicals will, in many cases, exert indirect deleterious effects. Although routine reproductive toxicity studies employ more than one dose, the highest dose level used is chosen so as to induce some overt but not excessive toxicity, in order to demonstrate that the test animals have been adequately challenged. In some studies reproductive toxicity is only observed at dose levels which induce marked systemic toxicity (eg. severe reductions in weight gain, impaired organ function or increased mortality rate). It would be inappropriate to conclude from such studies alone that there is a direct effect upon the reproductive system; such data in isolation should not be used as a basis for classification as "Toxic to Reproduction". Current standards and guidelines (OECD, 1981a,b,c, 1983a; EPA, 1984) suggest that dose levels in excess of 1g/kg body weight/day by the oral or dermal routes of exposure are not necessary when evaluating reproductive

toxicity. Although studies employing dose levels in excess of these limits may still be taken into account with other relevant data on their own they should not normally lead to classification as "Toxic to Reproduction".

The following animal studies can be used to investigate the possible adverse effects of substances on fertility and/or development. Where OECD guidelines exist for studies of the type described these are referenced as a representative example of the many national and international guidelines available. In these circumstances only a brief resume of the frequently used studies is given.

3.2.1. One-Generation Reproduction Toxicity Study (OECD, 1983a) [cf. Fig 1]

Principle: This study is designed to provide information on the effects of a substance on male and female reproductive performance over one generation.

Method: Most commonly 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 (ie. during two complete oestrus cycles) in order to elicit any adverse effects on oestrus. The animals are then mated. 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 F1 generation is weaned, typically at 21 days after birth. Males may be killed and examined at the end of the mating period or possibly after retention on test diet for the production of a second litter. Gross and histopathological examinations are made on selected organs of the parent animals.

Information Derived: The study provides information on gonadal function, oestrus cycle, mating behaviour, conception, parturition, lactation and weaning. It may also provide preliminary information about developmental toxic (including teratogenic) effects, neonatal morbidity, mortality,

growth, development, behaviour and serve as a guide for the design of subsequent tests. Reproductive performance is determined by evaluation of the litter size, pup weights and viability, and by effects on the reproductive organs (organ weights, and gross and histopathological examination of the parents). The basic study design does not cover the post-weaning phase of maturation (especially sexual) of the offspring. In the absence of follow-up investigations it does not allow determination of the cause of adverse effects or the sex of the parent adversely affected.

3.2.2. Two-Generation Reproduction Toxicity Study (OECD, 1983b) [cf Fig 2]

Principle: The study is designed to provide information on the effects of a substance on male and female reproductive performance over two reproductive generations.

Method: Most commonly the test substance is administered at three dose levels to groups of male and female rats. Males and females of the parental generation are treated as in the one-generation test except that the test substance is administered to males through the resultant pregnancies and through the weaning of the first generation (F_1) offspring. Males may be killed and examined at the end of the mating period or, may be retained on test diet for the possible production of a second litter in order to clarify the effects seen. At weaning, selected F_1 offspring continue to receive the substance during their growth into adulthood (ie. 13 - 17 weeks in rats and least 11 weeks in mice), during mating and production of an F_2 generation and up to the point when the F_2 generation is weaned, typically at 21 days after birth. Gross and histopathological examinations are made on selected organs of the parent animals.

Information Derived: This type of study provides information on gonadal function and morphology, oestrous cycle, mating behaviour, conception, parturition, lactation, weaning and on the growth and development of the offspring. Reproductive performance is determined by evaluation of the litter size, pup weights and viability, and by effects on the reproductive organs (organ weights, and gross and histopathological examination of the parents). It may also provide information about developmental toxic

effects such as neonatal morbidity, mortality, behaviour, functional deficits and preliminary data on teratogenesis. The basic design of the study does not allow determination of the cause of the adverse effects or the sex of the parent primarily affected; it can be modified in the light of findings to address these limitations.

3.2.3. Reproductive Assessment by Continuous Breeding (RACB), (Lamb et al, 1985) [cf Fig 3]

Principle: The study is designed to assess the effects of substances on male and female fertility and the reproductive performance of rats or mice when continuously bred.

Study Design and Method: The study is performed in five related tasks which are performed in a tiered sequential manner, each task depending on the outcome of the previous task. The first task is a 14-day dose finding study to determine the dose levels that can be used in task 2. In task 2, F_0 breeding pairs cohabit for 14 weeks and are continuously exposed to the test substance. Newborn litters are examined (body weights, mortality, sex, gross development) and discarded immediately after birth. If significant effects on reproductive performance are found, the affected sex is determined by crossmating (task 3). The high dose F_0 male parents are randomly paired with control females, high dose F_0 female parents with control males, and control males with control females. To avoid exposing control animals the compound is not administered during cohabitation (usually 7 days). Reproductive performance is determined by evaluation of the litter size, pup weights, and viability and by effects on the reproductive organs (organ weights, sperm analysis, gross and histopathological examination of the parents). In task 4 the fertility and reproductive performance of the F_1 generation (offspring from the final task 2 litters of control animals and the animals from highest dose group producing litters) are assessed. Where adverse effects on reproductive performance are detected in tasks 3 or 4 or when it is necessary to gain a better understanding of the toxic effects task 5 is performed. In this hormone levels (plasma follicle-stimulating hormone, luteinising hormone, testosterone, oestrogen, progesterone) may be analysed. In addition to

these observations the design provides information on the temporal development of reproductive lesions.

Information Derived: This depends on the number of tasks that it is necessary to complete. Assuming all tasks are performed the information derived is similar to that obtained from a two or more generation study (cf. Section 3.2.2.), in the case of adverse effects on fertility the affected sex(es) is (are) defined. The study is complex and difficult to conduct. It is currently not widely used and consequently a relatively small historical control data base exists.

3.2.4. Developmental Toxicity (Teratogenicity) Study (OECD, 1981d) [cf Fig 4]

Principle: The study is designed to provide information on the potential hazard to offspring arising from exposure of the dam to a substance during pregnancy.

Method: Rats or rabbits are the most commonly used species. The test substance is in general administered using at least three dose levels to groups of dams for that part of the pregnancy covering the period of major organogenesis (ie the 6th to 15th day of gestation in rats and the 6th to 18th day of gestation in rabbits). Shortly before the expected date of parturition (approximately day 20 in rats and day 28 in rabbits) the dams are killed and the uterine contents examined for external, soft tissue and skeletal abnormalities.

Information Derived: The study provides information on embryonic/foetal death, development, growth and maturation *in utero*, the sex ratio and external, soft tissue and skeletal abnormalities. The design of this study precludes the detection of functional abnormalities and morphological changes which may become manifest only after parturition.

3.2.5. Peri- and Post Natal Study (D'Aguanno, 1983) [cf Fig 5]

Principle: The study is designed to assess effects on offspring of pharmaceutical substances administered during late gestation, parturition

and lactation. For industrial chemicals these effects are generally assessed in multiple generation studies.

Method: Generally the test compound is administered at three dose levels to pregnant rats during the last third of the pregnancy and up to weaning. During this period maternal nutrition contributes maximally to the development, functional maturation and growth of the foetus. Exceptionally the dosing period may be lengthened to include organogenesis. Furthermore, the study can be extended to include measures of functional parameters such as motor activity, memory and learning and sensory functions (Japan MHW, 1984). Assessment of reproductive performance of the offspring may also be included.

Information Derived: The study provides information on foetal development, duration of gestation, parturition, litter size, lactation, pup viability, and growth and development of the newborn. The limited dosing period may not cover those periods sensitive to the induction of functional changes (eg. gonadal maturation which takes place after lactation).

3.2.6. Chernoff-Kavlock Assay (Chernoff and Kavlock, 1982) [cf Fig 6]

Principle: The study is designed as a short-term *in vivo* test to assess the potential of a substance to induce developmental toxicity. There are no regulatory guidelines for this assay but it has been suggested as a test for determining the priority of substances for further investigation. The protocol was originally described for use with mice by Chernoff and Kavlock (1982) and has been subsequently modified for use with the rat (Wickramaratne, 1987). The study is based on the assumption that most developmental toxicity becomes manifest postnatally as reduced viability and/or impaired growth of the young.

Method: Groups of pregnant nulliparous mice or rats (one or two dose groups and one control group) are dosed during the period of major organogenesis. Dams are allowed to give birth and the litters are counted, weighed at birth and on day 4 post-partum and then discarded. Dams without litters are examined for the presence of implantation sites.

Information Derived: The study provides information on foetal death, pup growth and survival, external malformations and cleft palate. The study can be used to select doses to be used in full developmental (teratogenicity) studies. The Chernoff-Kavlock assay is a screening test; it cannot replace more detailed developmental toxicity studies and should not be used as the sole basis for classification.

3.2.7. Preliminary Reproduction Toxicity Screening Test (OECD, 1990) [cf Fig 7]

Principle: This test forms part of a screening information data set (SIDS) proposed by the OECD; protocols are at present only in **draft** form. This screening test has been proposed for use with existing chemicals produced in high volume and is intended to provide a preliminary indication of reproductive toxicity in order to assign a priority for further investigation. Comments on this test approach are given in ECETOC (1992).

Method: The test substance is administered at several dose levels to groups of male and female rats for two weeks prior to mating. Treatment is continued for both sexes throughout the mating period (maximum 14 days), pregnancy and up to day 4 of lactation when all animals are killed.

Information Derived: This study provides limited information on mating performance, conception, parturition, foetal death and perinatal mortality. This test will not provide information on all aspects of fertility or of development and should only be used to indicate the possible toxic potential of a substance. Additionally, it provides useful information to help with selecting doses to be used in subsequent experiments. It cannot replace more detailed reproductive toxicity studies. Negative results must be treated with caution and the information derived should not be used as the sole basis for classification.

3.2.8. Non-Conventional Studies

There are other types of studies that provide information on one or more aspects of reproductive function (eg. basic research studies and the dominant lethal test for germ cell mutation). The interpretation and use

of data from them, particularly for classification, must be approached with circumspection. Adherence to appropriate scientific standards for the conduct of the studies is important. They may provide both positive and negative data which could be useful for classification purposes when evaluated by experienced scientists.

3.3. Studies Providing Supporting Evidence

These studies are not designed primarily to investigate the effects on reproduction but can provide useful supporting information for the evaluation of reproductive toxicity.

3.3.1. Standard Toxicity Studies

These studies involve repeated dosage of male and female animals over periods from 14 days to 2 years. It should be noted that measurement of the indices of toxicity discussed below may not necessarily be mandatory under current Regulatory Guidelines:

1. Organ Weights: Changes in the weights of the reproductive organs may indicate an adverse reproductive effect. Increases/decreases in the weights of ovaries, uterus, testes, prostate, seminal vesicles, epididymides, pituitary gland and adrenal glands may be relevant to the assessment of reproductive toxicity.

2. Histopathology: Histopathological examination of reproductive and accessory organs and glands frequently provides information of value in the assessment of reproductive toxicity. Appropriate fixation techniques and embedding are necessary to detect subtle changes in the testes; perfusion fixation and plastic embedding is ideal, but in standard studies immersion fixation using Bouin's fluid or other appropriate fixatives followed by wax embedding is more common. Apart from gross effects, changes in ovarian histopathology are extremely difficult to detect without the serial sectioning needed to examine for follicular maturation. When interpreting histopathological information it is important to recognise that in

some of the shorter-term standard toxicity studies the animals are sexually immature. Allowance must be made also for possible differences in the sexual cycle and the occurrence of geriatric changes when interpreting histopathological data.

3.3.2. Hormonal Investigations

Investigation of hormonal status may be valuable in examining the possible mechanism of reproductive toxicity. Data on hormone levels invariably comes from specially designed studies. Concentrations of those hormones associated with reproduction can be determined readily in the plasma and target organs using specific radioimmunoassays.

3.3.3. Vaginal Cytology/Sperm Morphology

The U.S. National Toxicology Program [NTP] (Morrissey et al, 1988) has recently advocated examination of vaginal cytology (to monitor the oestrus cycle) and sperm morphology/motility/count (as indices of male reproductive function) to be included at the end of standard rat 90-day toxicity studies. The NTP has been evaluating these indices using a number of substances.

3.3.4. Structure-Activity Studies

Studies can be found in the literature in which a number of analogues of a particular chemical have been evaluated in animals using a limited number of indices of adverse effect some of which are indicative of reproductive toxicity. Such information, which relates reproductive toxicity to chemical structure can provide supporting data when attempting to predict reproductive or developmental toxicity. This is particularly so when one or more of the analogues have been studied in depth by conventional reproductive toxicity tests. Such studies tend to be useful in predicting the potential effects of other members of the same chemical series. Care must be taken in the interpretation and reliance of such approaches (ECETOC, 1989).

3.3.5. In Vitro Studies

In vitro systems, cannot on their own, provide sufficient evidence to classify an agent as a reproductive or developmental toxicant (ECETOC, 1989). Nevertheless they are useful tools for studying the mode of action of reproductive toxicants and frequently provide supporting evidence for the classification of reproductive or developmental effects. Determinations of structure-toxicity relationships and the identification of "active" metabolites can also be facilitated by such systems.

These systems also have the advantage that where human tissue can be cultured it is possible to compare the intrinsic activity and sensitivity of animal and human tissues to reproductive toxicants.

ECETOC (1989) has reviewed many of these in vitro methods, particularly those relating to developmental toxicity; in vitro methods for the study of the effects on reproduction and fertilisation were not included. They are not often used, due to their complexity and the range of expertise required. Nevertheless the systems described below represent a cross section of a large class of techniques designed to investigate the mechanisms of reproductive toxicity. They may have value in placing in perspective the results of animal studies and so may serve as an aid to classification. However, interpretation of the data obtained from these tests requires highly specialised knowledge and should be carried out by experienced scientists.

3.3.5.1. Testicular/Ovarian cell cultures: Testicular cell cultures have been employed extensively in studies designed to examine the physiology of the organ, cell-to-cell interactions and the effects of toxic agents. The in vitro systems range from the whole perfused testis to cultures of a specific cell type (eg. Gray, 1988). The most frequently used systems are those employing isolated seminiferous tubules (which can be at specified stages of the spermatogenic cycle), Sertoli cell cultures (usually only from immature animals) and Sertoli-germ cell co-cultures and Leydig cell cultures.

The effects of substances are generally assessed by examining cell morphology and viability, hormonal responsiveness, steroid output (eg. testosterone production by Leydig cells) and the production of cell specific products/ proteins (eg. inhibin by Sertoli cells) (Grey, 1988).

Ovarian cell cultures (eg. Wickings et al, 1987) most often used comprise somatic rather than germ cells (granulosa cells - equivalent to Sertoli cells in the male) and thecal cells (equivalent to Leydig-cells). Examinations carried out are similar to those employed for testicular cell cultures.

3.3.5.2. In Vitro Fertilisation in Rodents and Lagomorphs: Recently, in vitro fertilisation (IVF) in rodents has been used as a system for the assessment of the effects of chemicals (Holloway et al, 1990). This system offers the ability to study the fertilisation process in isolation, whilst maximising the sensitivity by selecting the numbers of sperms and eggs. The test protocol involves the production (by superovulation) and harvesting of ova from an immature female rodent and combining these eggs with appropriate numbers of sperm. The resulting embryos are then cultured until they reach the 8 cell stage and the success of fertilisation is scored. The system has the capability of testing the effects of an agent on the sperm, the ova or both. The technique is most easy to perform in hamsters, although it has been successfully used in the rat and rabbit.

The use of eggs freed from the zona pellucida allows the assessment of the ability of sperm from a different species to penetrate the hamster oocyte (Aitken, 1983). Hamster oocytes are incubated with the sperm sample; indications of penetration and cell cleavage are observed microscopically. The system could be equally applied to the study of sperm function from the laboratory species most often used in reproductive toxicity testing.

Section 4. CLASSIFICATION OF COMPOUNDS CAUSING REPRODUCTIVE TOXICITY

Within the European Community the classification of chemical compounds with an effect on reproduction is confined at present to those which are teratogenic (R_{47} = may cause birth defects). The EC recognised that toxicity to the developing offspring is not manifest solely as permanent structural changes but that other defects (embryo-foetal mortality, growth retardation, functional deficits and interference with sexual maturation and reproductive performance) may also result from impairment of fertility and developmental toxicity and therefore must be taken into account in classification. A recent EC proposal (EEC, 1992) takes account of this.

Owing to the complexity of the mammalian reproductive cycle, clear cut indications of reproductive toxicity are not always found in conventional toxicity studies. In addition, observed effects can be secondary to toxic action on other organ systems and not to the direct reproductive toxic activity of the chemical. Information which can be used to evaluate the potential of a substance to affect adversely the reproductive process can be obtained from a large variety of tests which have been designed specifically to investigate one or more steps of the reproductive cycle in mammalian species. Supportive evidence may be provided by *in vitro* systems or general toxicity investigations (eg. 90-day studies); it is emphasised that use of data from these studies in isolation, as a basis for classification of reproductive effects, is inadvisable.

Although the proposed amendment to the EC Directive on Classification, Packaging and Labelling requires classification on the basis of intrinsic toxicity, classification should in practice be based on an assessment of risk. According to the National Academy of Sciences, USA, (1983) such a risk assessment should include:

- hazard identification,
- knowledge of dose response relationships,
- an estimate of human exposure,
- risk characterisation.

Animal and human studies are crucial to hazard identification which involves the evaluation of data on toxic activity generated during reproduction studies. The nature, severity and extent of the biological responses should be considered in relation to any paternal or maternal toxicity and other information (including toxicokinetic parameters, metabolism, cytotoxicity, mode of action, structure activity relationships and information from other general toxicity studies) that may have a bearing on the overall assessment.

The slope and shape of the dose response curves are important factors in determining the overall risk to man at exposure levels likely to occur under conditions of intended use. It is considered that effects seen only at doses in excess of 1g/kg body weight/day when administered orally (or at the equivalent test level for inhalation and dermal routes) should not normally lead to classification as "Toxic to Reproduction". The overall weight of evidence from all sources should underpin the final judgement of the appropriate classification.

4.1. Proposed System for Classification of Substances "Toxic to Reproduction"

Substances causing adverse effects on reproduction fall into two main classes, those affecting fertility and those affecting the offspring. ECETOC generally agrees with the labelling guide (EEC, 1992) which proposes that the following two main classes be subdivided into three categories according to the weight of supporting scientific evidence.

Those substances which impair fertility are defined within the above EC guide as follows:

- Category 1, substances known to impair fertility in humans,

- Category 2, substances which should be regarded as if they impair fertility in humans,

substances falling into either of these categories would be covered by the Risk Phrase

R60, **May impair fertility,**

and

- Category 3, substances which may cause concern for human fertility,

and this category would be covered by the Risk Phrase

R62, Possible risk of impaired fertility.

The categories of substance which adversely effect the offspring are defined within the EC Guide as follows:

- Category 1, substances known to cause developmental toxicity in humans,

- Category 2, substances which should be regarded as if they cause developmental toxicity to humans,

both of these categories would attract the Risk Phrase

R61, May cause harm to the unborn child,

and

- Category 3, Substances which cause concern for humans owing to possible developmental toxic effects,

and this category would be covered by the Risk Phrase

R63, Possible risk of harm to the unborn child.

4.2. Evidence Required for Classification

4.2.1. Substances Impairing Fertility

Category 1: Substances known to impair fertility in humans

This Category will be used when there is sufficient evidence to establish a causal relationship between human exposure to a substance and subsequent impairment in fertility.

Substances should be placed in this category only when indicated by evidence from reliable epidemiological data or when a clear association between exposure and effect is evident from well documented case studies. A causal relationship between human exposure and the occurrence of a fertility deficit would be indicated epidemiologically by a markedly higher incidence of infertility than normal in men and/or women exposed to the substance. Ideally this data is strengthened by the evidence of a dose-response relationship. One of the main difficulties with epidemiological studies is the variable quality of background data on infertility. As in all such studies, confounding factors such as stress, nutrition, drug abuse, exposure to other chemicals etc. must be taken into account when interpreting findings. While strong evidence from case studies may be sufficient to support a Category 1 classification this may be strengthened by animal data adequate for Category 2 classification.

Category 2: Substances which should be regarded as if they impair fertility in humans

Substances should be placed in Category 2 when data from a well designed and conducted animal study supported by other relevant complementary information provide evidence of a strong presumption of risk to man. Classification under Category 2 should only be made where there is evidence of direct toxicity to the reproductive system.

A category 2 classification should be given when:

- the results of a study or studies in one or more species indicate a clear treatment-related effect on fertility and it should be evident that the effect is not secondary to systemic toxicity,

- and the above studies demonstrate a functional deficit in fertility (eg. fertility rate, or pregnancy rate),

- and the evidence of deficits in fertility may be supported by other data from in vivo animal studies (eg. 14-, 28- or 90-day toxicity studies) which may show gross and histopathological effects in the reproductive and associated endocrine organs of a nature and extent indicating a high likelihood of an adverse effect on fertility, eg. testicular atrophy,

- or chemical relationships to other known anti-fertility agents,

- or where there is evidence from human studies of a quality insufficient to warrant Category 1 classification.

Similar findings in more than one test species will increase the likelihood that man may be affected by exposure.

The following studies are likely to provide appropriate information:

- one generation reproduction toxicity studies,
- two generation reproduction toxicity studies,
- specific fertility studies including continuous breeding studies.

Animal studies should be evaluated to ensure they are adequate; simply adhering to regulatory guidelines may not suffice. The design (choice of species, vehicle, route of exposure etc.), conduct, interpretation and reporting must all be considered. Guidelines (US EPA, 1985; EEC, 1989) and other relevant publications (DFG, 1990; ECETOC, 1986) may be consulted for more information on the evaluation of reproductive toxicity studies.

Category 3: Substances which cause concern for human fertility

Substances should be placed in Category 3 when there is some evidence from man or appropriate animal studies but this is not of the quality needed to place the substance into category 2.

A Category 3 classification should be given when:

- some well conducted case studies or epidemiological studies suggest an effect in man but support from other human and animal studies is inadequate for a Category 2 classification,

- deficits in fertility are seen in tests on one animal species but there is no supporting evidence from human and/or other animal studies,

- histopathological examinations from toxicity studies show a consistent adverse effect on reproductive organs (including accessory genital organs or glands) of a nature and extent indicating a possible impairment of fertility but no functional deficit has been demonstrated in animal or human studies.

Reasons for Non-classification

Substances which do not meet the above criteria for Categories 1, 2 or 3 should not be classified. In addition the factors below may cast sufficient doubt on the relevance of the data to man to lead to non-classification:

- effects on fertility are seen only in association with systemic toxicity and are most likely secondary to these effects;

- evidence from animal and human studies is equivocal, eg. effects noted are unlikely to be due to exposure to the substance, or experimental studies have been inadequately performed or cannot be reproduced;

- effects are seen in experimental animals only when using routes of administration, magnitudes of exposure or physical forms which are not relevant to the human situation,

- adequate evidence exists to show that the metabolite or mode of action responsible for induction of adverse effects on fertility effect is not produced in or relevant to man;

- the basic physiological/endocrinological mechanisms impaired by the compound in the test animal species cannot be compared to those of man.

4.2.2. Substances Causing Developmental Toxicity

Development is that part of the reproductive cycle starting from fertilisation and includes implantation, embryonic and foetal development, birth, and postnatal development to the age of sexual maturity. Adverse effects include embryonic/foetal mortality, morphological changes (malformations), altered foetal and postnatal growth and development, physiological deficits and delays in maturation, behavioural and psychological deficits, abnormal sexual development or function.

The effects on offspring may result from exposure of either parent prior to conception, or of the developing organism prenatally or postnatally up to the time of sexual maturation.

Category 1: Substances known to cause developmental toxicity in humans.

Substances should be placed in Category 1 when there is sufficient evidence that they cause adverse effects in the progeny of man.

Sufficient evidence to justify classifying a substance under Category 1 may come from reliable epidemiological data or when there is a clear association between exposure and effect in well documented case studies. A causal relationship between exposure to a substance and the occurrence of adverse effects in the offspring will be indicated by:

- a markedly higher incidence of specific adverse developmental effects in the offspring of the exposed group compared with the normal population,

- a marked increase in the total of all developmental effects in offspring of exposed parents,

- a dose response relationship is evident.

Category 2: Substances which should be regarded as if they cause developmental toxicity to humans.

Substances should be placed in Category 2 when data from one or more well designed and conducted animal studies provide a strong presumption of risk of adverse effects in the offspring of man. Classification in Category 2 should only be made when the data show direct toxicity to the developing animal; it should be evident that the effect is not secondary to maternal/systemic toxicity.

To classify a substance in Category 2, one or more of the following criteria must be met:

- unequivocal evidence of a teratogenic effect in animals at a dose level not inducing maternal (parental) toxicity,

- unequivocal evidence of other severe manifestations of developmental toxicity, eg. increased lethality, severe growth depression or serious functional deficits increased in animals at a dose level producing no maternal (parental) toxicity,

- on a case-by-case basis, effects as described above in association with some, but not marked maternal toxicity, but where there is clear evidence that the effects are not secondary to maternal (parental) toxicity.

Developmental toxicity seen only in conjunction with maternal toxicity should be interpreted with caution and on a case-by-case basis, taking into account the nature and severity of both the developmental effects and the maternal toxicity.

Where the possibility cannot be excluded that the effects might be secondary to non-specific toxicity to the parent, classification in category 2 is not warranted.

The following studies are likely to provide appropriate information:

- one generation reproduction toxicity studies,
- two generation reproduction toxicity studies,
- continuous breeding studies,
- developmental toxicity (teratogenicity) studies,
- peri- postnatal toxicity studies.

Animal studies should be evaluated to ensure they are adequate; simply adhering to regulatory guidelines may not suffice. The design (choice of species, vehicle, route of exposure etc.), conduct, interpretation and reporting must all be considered. Guidelines (US EPA, 1985; EEC, 1989) and other relevant publications (DFG, 1990; ECETOC, 1986) may be consulted for more information on the evaluation of reproductive toxicity studies.

Category 3: Substances which cause concern for humans owing to possible developmental toxic effects.

Substances should be placed in Category 3 when one or more of the following criteria are met:

- unequivocal evidence of teratogenic effects in animals but only at a dose level at which there is some, but not marked, maternal (parental) toxicity,*

- unequivocal evidence of a significant increase in embryo-foetal

and/or neonatal lethality in association with some, but not marked, maternal toxicity,*

- small (but significant) changes in developmental toxicity end points in the absence of maternal (parental) toxicity (eg. an increase in common structural variants, reduced ossification and slight growth retardation), in all cases the effects should show a clear dose-response relationship.

* As noted for Category 2 developmental toxicity seen only in conjunction with maternal toxicity should be interpreted with caution and on a case-by-case basis, taking into account the nature and severity of both the developmental effects and the maternal toxicity.

Reasons for Non-classification

Substances which do not meet the above criteria for Categories 1, 2 or 3 should not be classified. A number of factors would indicate that, although developmental abnormalities have been observed, the data are inadequate for a substance to be classified. These situations include, but are not limited to:

- the developmental effects occur only in association with marked maternal toxicity,

- the significant increase in embryo-foetal or neonatal toxicity, not showing a clear dose-response relationship,

- the effects are seen in experimental animals only when using routes of administration, magnitudes of dosage or physical forms which are not relevant to the human situation,

- evidence has been obtained from animal studies which are inadequately designed, conducted or reported,

- adequate evidence exists which shows that the metabolite or mode of

action responsible for induction of developmental toxicity is not produced in or relevant to man.

4.2.3. General Comment.

A wide variety of effects are covered by the definition of developmental toxicity and studies investigating this phenomenon often reveal increases in more than one abnormality. It is most essential to evaluate the findings individually and in combination in order to establish the degree of certainty of the relevance of the findings to man and hence the correct categorisation. Supportive information such as similar findings in a second species or a high margin between the level causing the developmental effects and that causing parental toxicity will strengthen the case for categorisation.

4.3. Decision Tree leading to classification [cf Fig 8 and 9]

The proposed classifications are shown in decision trees (figures 8 and 9). These demonstrate in a simplified manner how data from epidemiological or animal studies should lead to the respective R-phrase for use in labelling. It is again emphasised that data on reproductive toxicity are often difficult to interpret and that advice from specialists in reproductive toxicology and epidemiology will be required for appropriate categorisation.

4.4. Comment on the EC Proposal for Risk Phrase R64

It is noted that the EC Labelling guide also includes a new risk phrase

R64 May cause harm to breastfed babies

for which the following descriptors apply as defined by the EC.

"For substances and preparations which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in

breast milk in amounts sufficient to cause concern for the health of a breasted child."

"Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64."

"For the purpose of classification, toxic effects on offspring resulting only from exposure via the breast milk, or toxic effects resulting from direct exposure of children will not be regarded as "Toxic to Reproduction", unless such effects result in impaired development of the offspring."

"Susbtances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64. This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk."

It is the opinion of the Task Force that data normally generated in animal tests do not discriminate between reproductive toxicity and toxicity induced through lactation, since the tests most commonly used provide for exposure throughout the reproductive cycle. The Task Force recommends that further consideration needs to be given to the use of this new Risk Phrase.

BIBLIOGRAPHY

- Aitken, C.R. (1983). Attributes and applications of the zona-free hamster egg penetration test. In "In Vitro fertilisation and embryo transfer." Eds Crosignani, P.G. and Rubin, B.G., pp 167-193.
- Chernoff, N. and Kavlock, R.J. (1982). An In Vivo teratology screen utilising pregnant mice. Journal of Toxicology and Environmental health, 10, 541-550.
- D'Aguanno, W. (1983). Guidelines for reproductive studies for safety evaluation of drugs for human use. FDA Introduction of Total Drug Quality, U.S. Food and Drug Administration.
- De Wals, P, Weatherall, J.A.C.& Lechat, M. (1985). Registration of Congenital Anomalies in Eurocat Centres, 1979 - 1983. An EEC Concerted Action Project, Pub Caba.
- DFG, (1990). Deutsche Forschungsgemeinschaft, Kriterien zur Beurteilung von Studien zur Reproduktionstoxizitat mit Pflanzenschutzmitteln. VCH Verlagsgesellschaft mbH, D-W6940, Weinheim.
- ECETOC, (1986), A Guide to the Classification of Carcinogens, Mutagens and Teratogens under the Sixth Amendment, Technical Report No 21, Brussels.
- ECETOC, (1989), Alternative Approaches for the Assessment of Reproductive Toxicity, Monograph No 12, Brussels.
- ECETOC, (1992), Existing Chemicals; OECD Screening Information Data Set, Proposed Testing Methods, Technical Report in preparation, Brussels.
- EEC, (1989), Guidelines for the Evaluation of Reproductive Toxicity. CEC/V/E/3/LUX/17/86, May 1989, Luxembourg.
- EEC, (1992), Draft to Annex VI labelling guide Chapter 4, section 4.2.3. DGXI/125/90-Rev 6, dated January 1992.
- Gray, T J B. (1988). Application of in vitro systems in male reproductive toxicology. in "Physiology and Toxicology of male reproduction. Eds J C Lamb and P M B Foster. Academic Press, New York.
- Hemminiki, K., Mutanen, P., Saloniemi, I., Niemi, M-L. & Vainio, H. (1982). Spontaneous Abortions in hospital staff engaged in sterilising instruments with chemical agents, Brit. Med. J., 285, 1461 - 1463.
- Holloway, A J, Moore, H D M, and Foster, P M D. (1990). The use of in vitro fertilisation to detect reductions in fertility of male rats exposed to 1,3-dinitrobenzene. Fund. Appl. Toxicol., 14, 113 - 122.
- Japan MHW (1984). Japan toxicity test guideline : Studies of the effects of drugs on reproduction. III. Study by administration during the perinatal and lactation periods. Notification No.118 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, February 1984.
- Lamb, J.C., Jameson, C.W., Choudhury, H. and Gulati, D.K. (1985). Fertility assessment by continuous breeding : evaluation of diethylstilbestrol and a comparison of results from two laboratories. Journal of the American College of Toxicology, 4(2), 173-184.
- Levine, R.J. (1983). Methods for Detecting Occupational Causes of male infertility, Scand. J. Work Environ. Health, 9, 371 - 376.

- McDonald, A.D., McDonald, J.C., Armstrong, B., Cherry, N.M., Cote, R., Lavoie, J., Nolin, A.D. & Robert, D. (1988). Foetal Death and work in pregnancy, Brit. J. Indust. Med., 45, 148 - 157.
- Morrissey, R E, Schwetz, B A, Lamb, J C, Ross, M D, Teague, J L, and Morris, R W. (1988). Evaluation of rodent sperm, vaginal cytology and reproductive organ weight from National Toxicology Program 13-week studies. Fund. Appl. Toxicol., 11, 343 - 358.
- National Academy of Sciences (USA), (1983). National Academy of Sciences, Risk Assessment in Federal Government: managing the process. Commission on Life Sciences. National Academy Press, Washington, DC.
- OECD, (1981a). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 407, Repeated Oral Dose Toxicity, Rodent, 28-day or 14-day study.
- OECD, (1981b). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 408, Sub-chronic oral toxicity, Rodent, 90-day study.
- OECD, (1981c). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 409, Sub-chronic Toxicity, Non-rodent, 90-day study.
- OECD, (1981d). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 414, Teratogenicity.
- OECD, (1983a). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 415, One-generation Reproduction Toxicity Study.
- OECD, (1983b). Organisation for Economic Cooperation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, No 416, Two-generation Reproduction Toxicity Study.
- OECD, (1990). Preliminary Reproduction Toxicity Screening Test (for use in the OECD SIDS programme), guideline circulated for discussion purposes.
- Whorton M.D. & Meyer, C.R. (1984). Sperm count results from 861 American chemical/agricultural workers from 14 separate studies, Fertility & Sterility, 42, 83 - 87.
- Whorton, D., Krauss, R.M., Marshall, S., and Milby, T.H. (1977). Infertility in male pesticide workers. Lancet, 2, 1259-1261.
- Wickings, E J, Middleton, M C, and Miller, S G. (1987). Non-steroidal inhibition of granulosa cell aromatase activity in vitro. J Steroid Biochem., 26, 641 - 646.
- Wickramaratne, G.A. (1987). The Chernoff-Kavlock Assay; its validation and application to rats. Teratog. Carcinogen. Mutagen, 7, 73.
- US EPA/FIFRA, (1984). Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation, Human and Domestic Animals, Office of Pesticide Programs, EPA 540/9-84-014; paragraph 82-1 Subchronic oral toxicity/rodent and non-rodent, 90 day study: paragraph 83-3 Teratogenicity Study: paragraph 83-4 Reproductive and Fertility Effects.
- US EPA, (1985). US Environmental Protection Agency, Health Hazard Evaluation Division, Standard Evaluation Procedure: Teratology Studies, PB86-129392.

- US EPA, (1986). US Environmental Protection Agency, Guidelines for the health assessment of suspected developmental toxicants. Fed. Register, 51 (185), 34028-34040.
- US EPA, (1988a). US Environmental Protection Agency, Proposed Guidelines for assessing female reproductive risk. Fed Register, 53 (126), 24834-24847.
- US EPA, (1988b). US Environmental Protection Agency, Proposed Guidelines for assessing male reproductive risk. Fed. Register, 53 (126), 24850-24869.

Figure 1. One-Generation Reproduction Toxicity Study

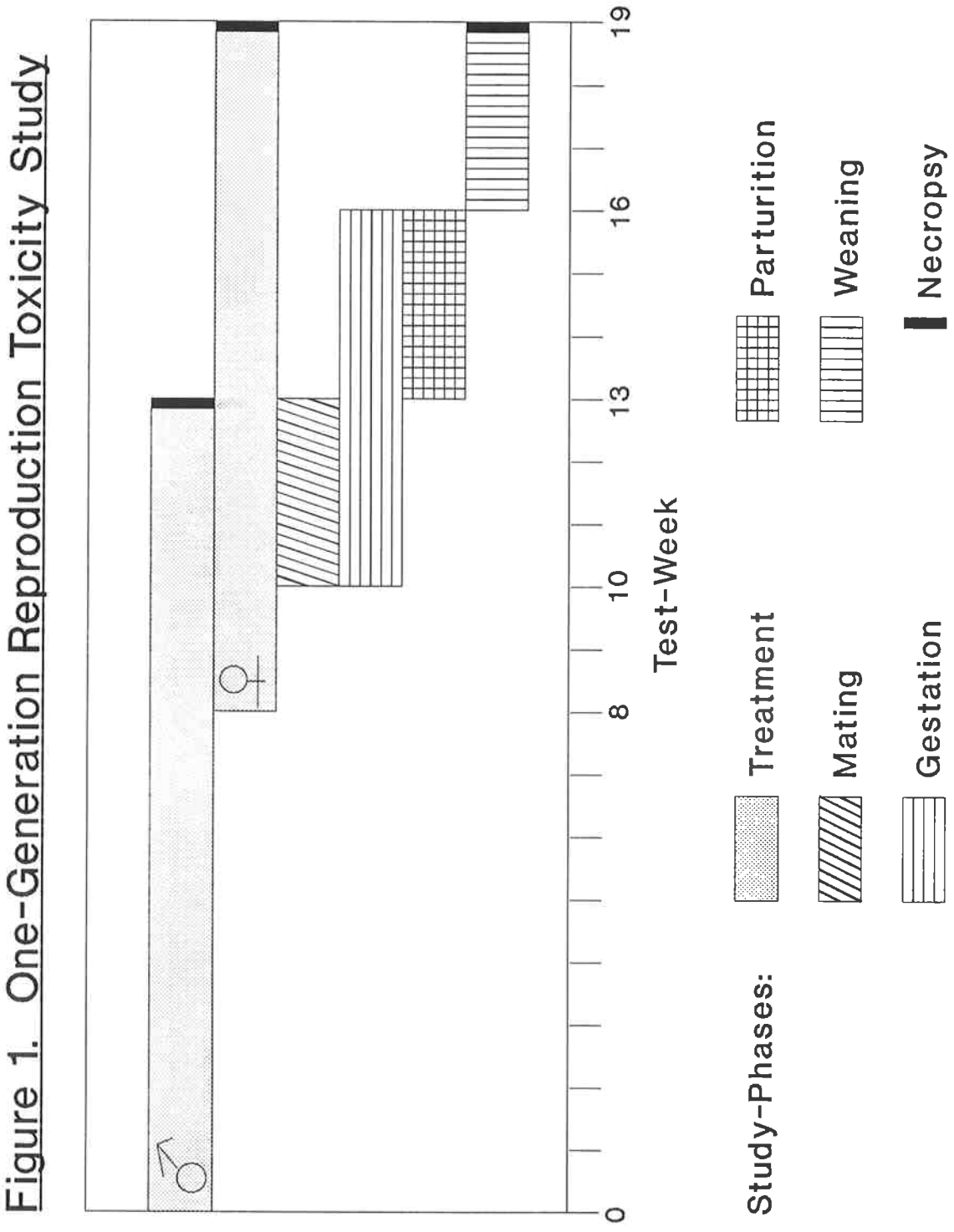


Figure 2. Two-Generation Reproduction Toxicity Study

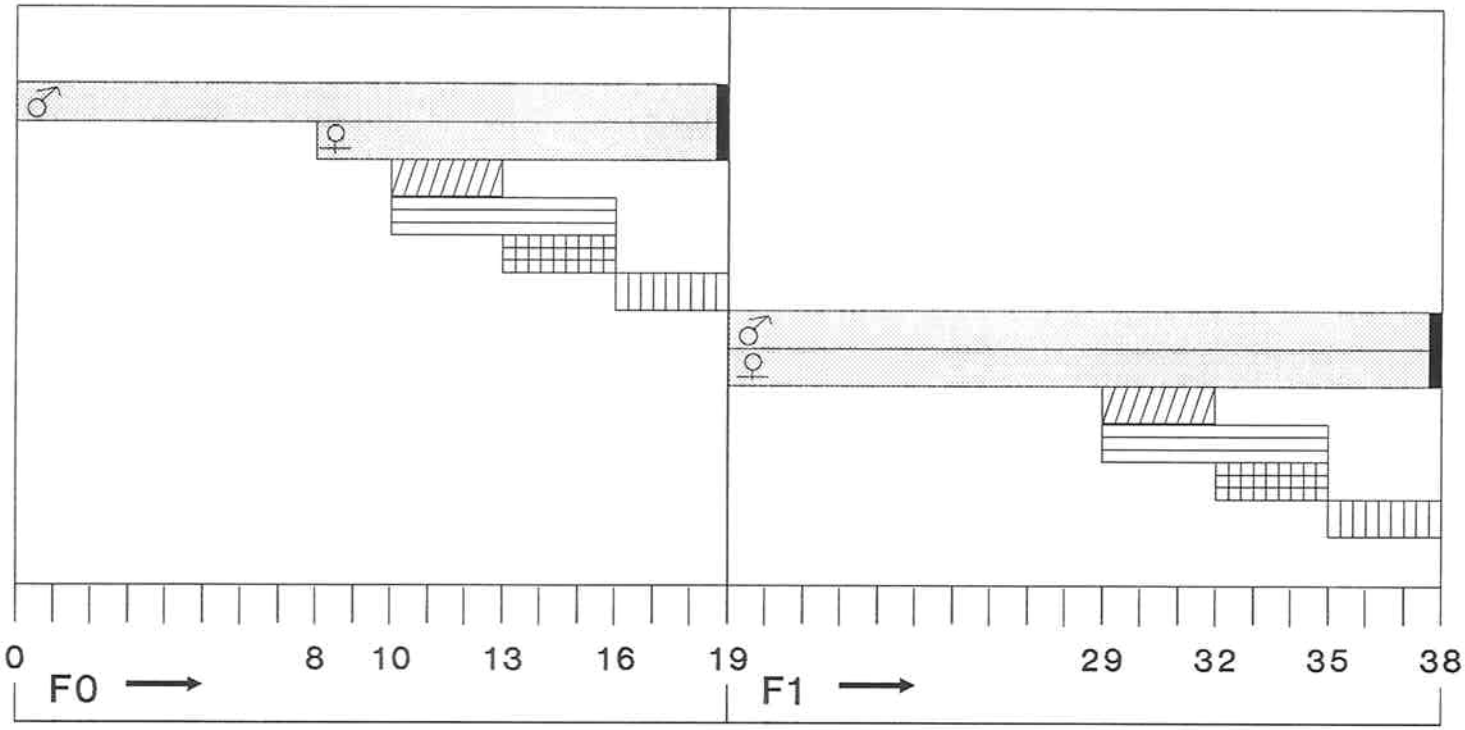


Figure 3. Reproductive Assessment by Continuous Breeding

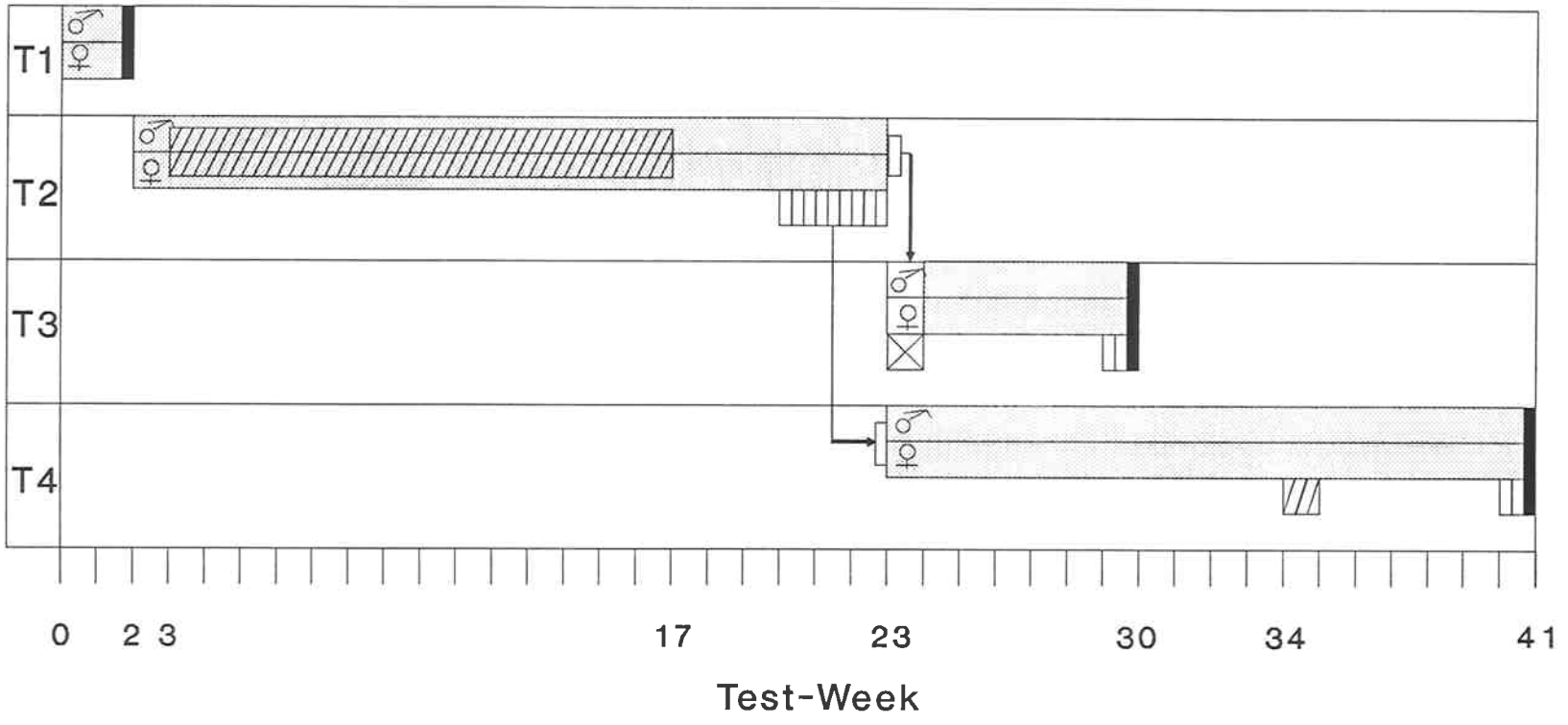


Figure 4. Developmental Toxicity (Teratogenicity) Study-Rat

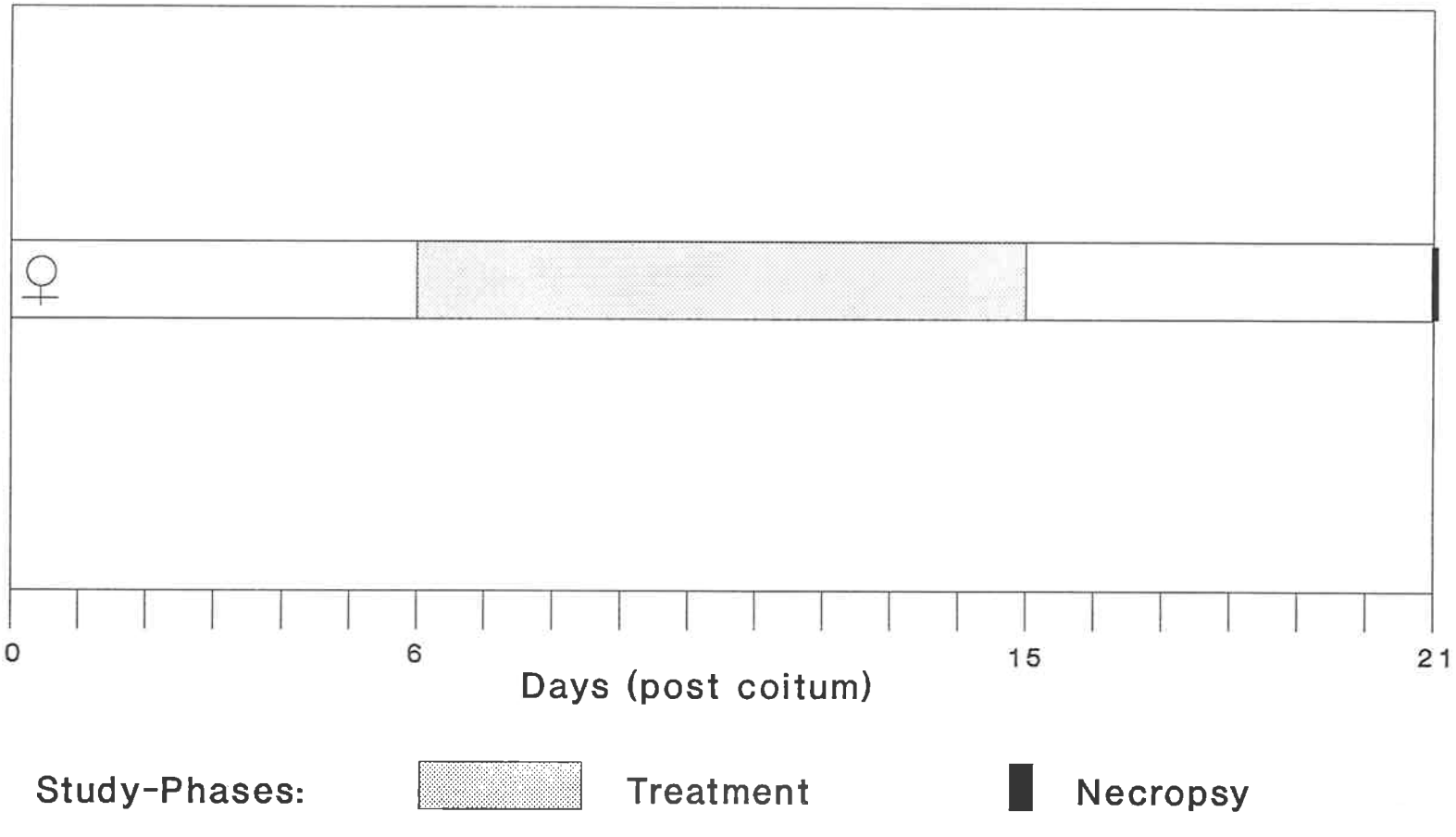


Figure 5. Peri- and Post-Natal Study

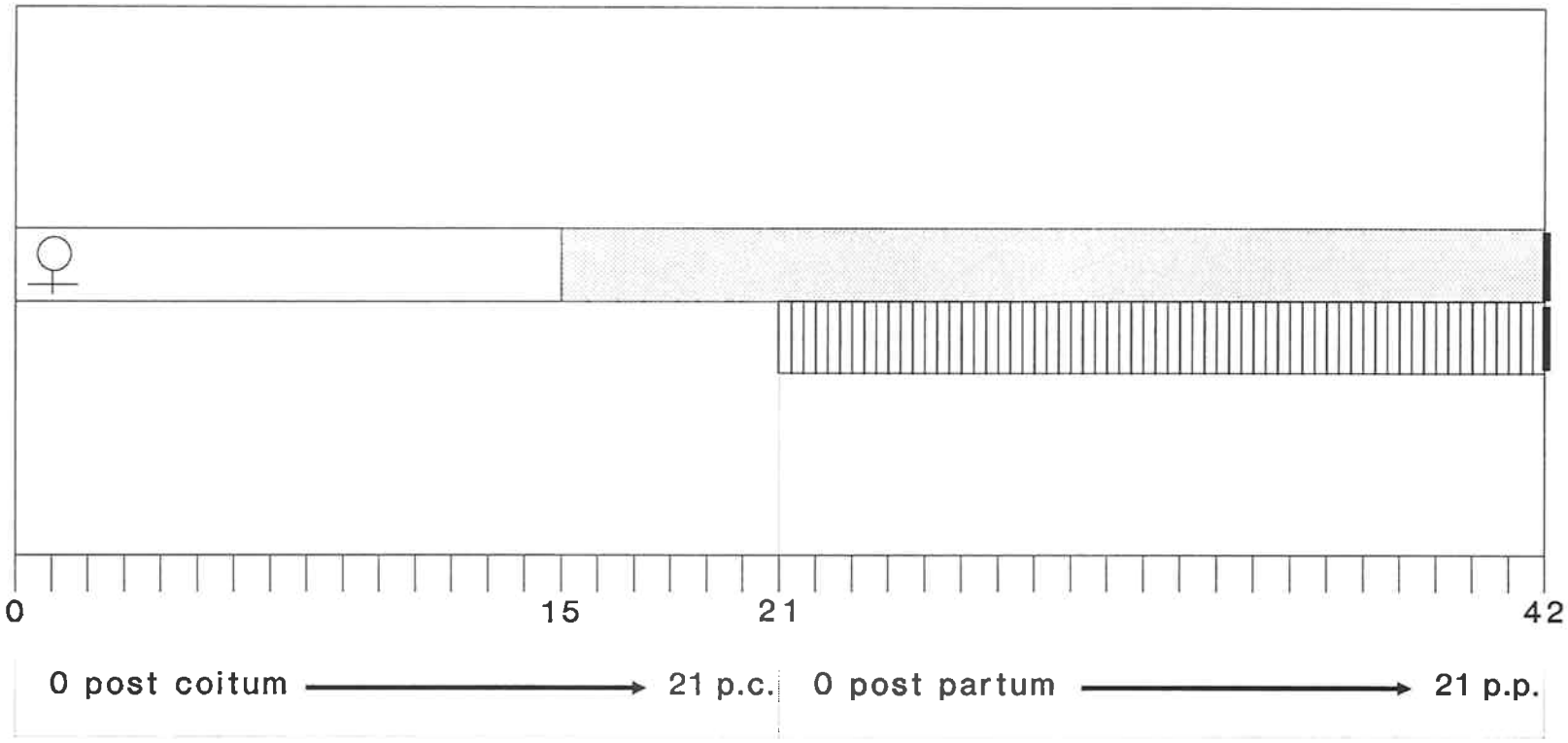


Figure 6. Chernoff-Kavlock Assay - Rat

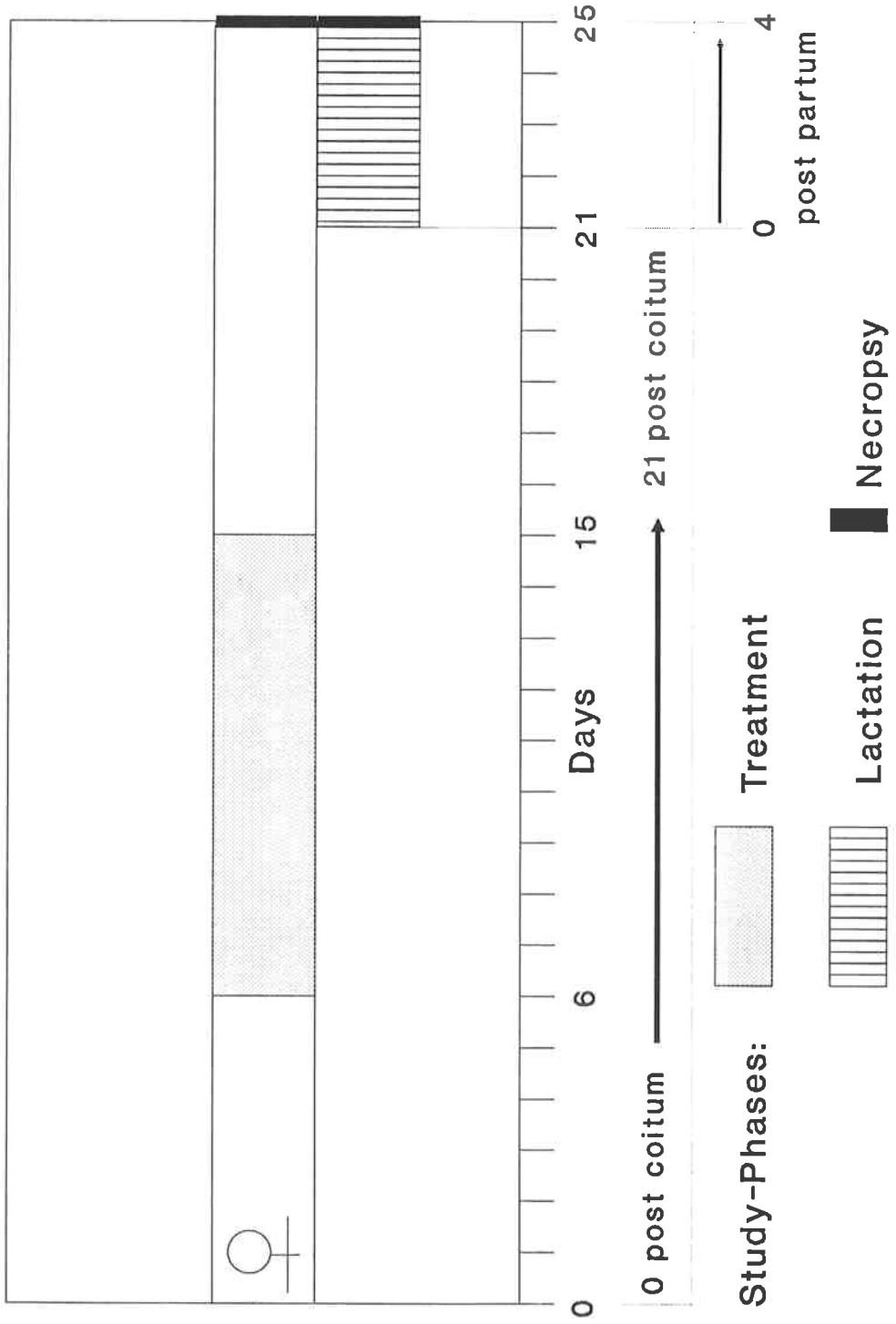


Figure 7. OECD Preliminary Reproduction Toxicity Screening Test

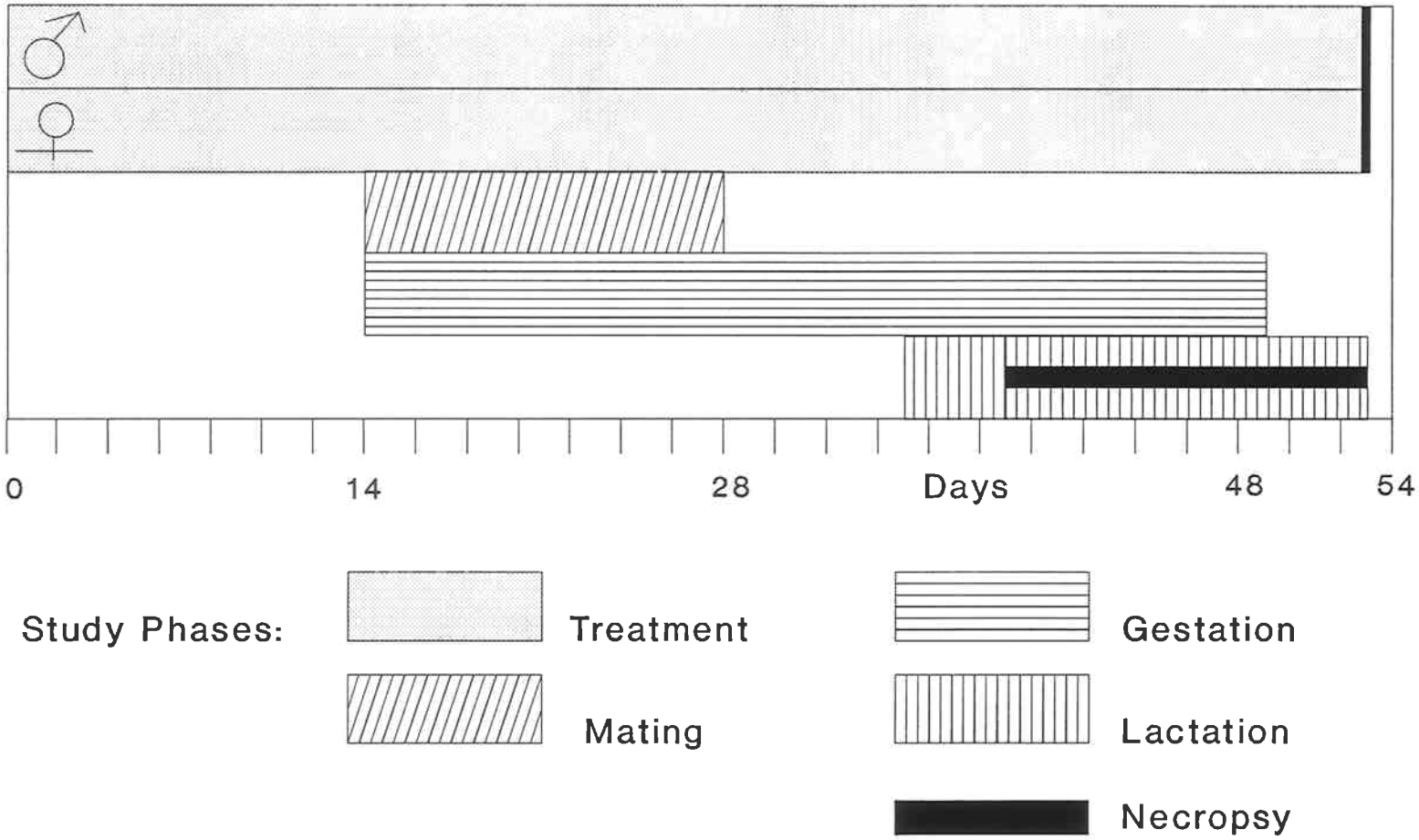


Figure 8 EFFECTS ON FERTILITY

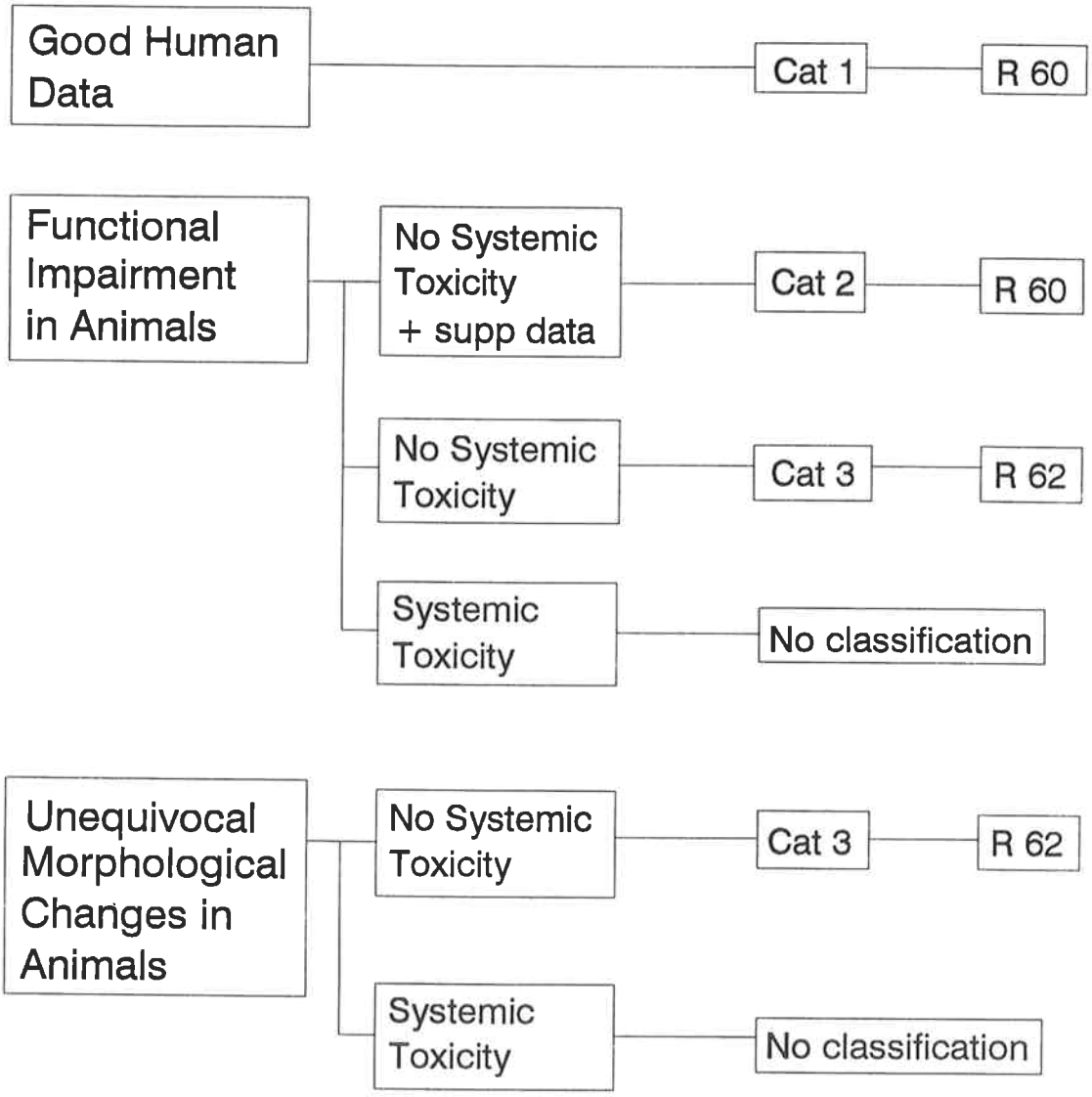
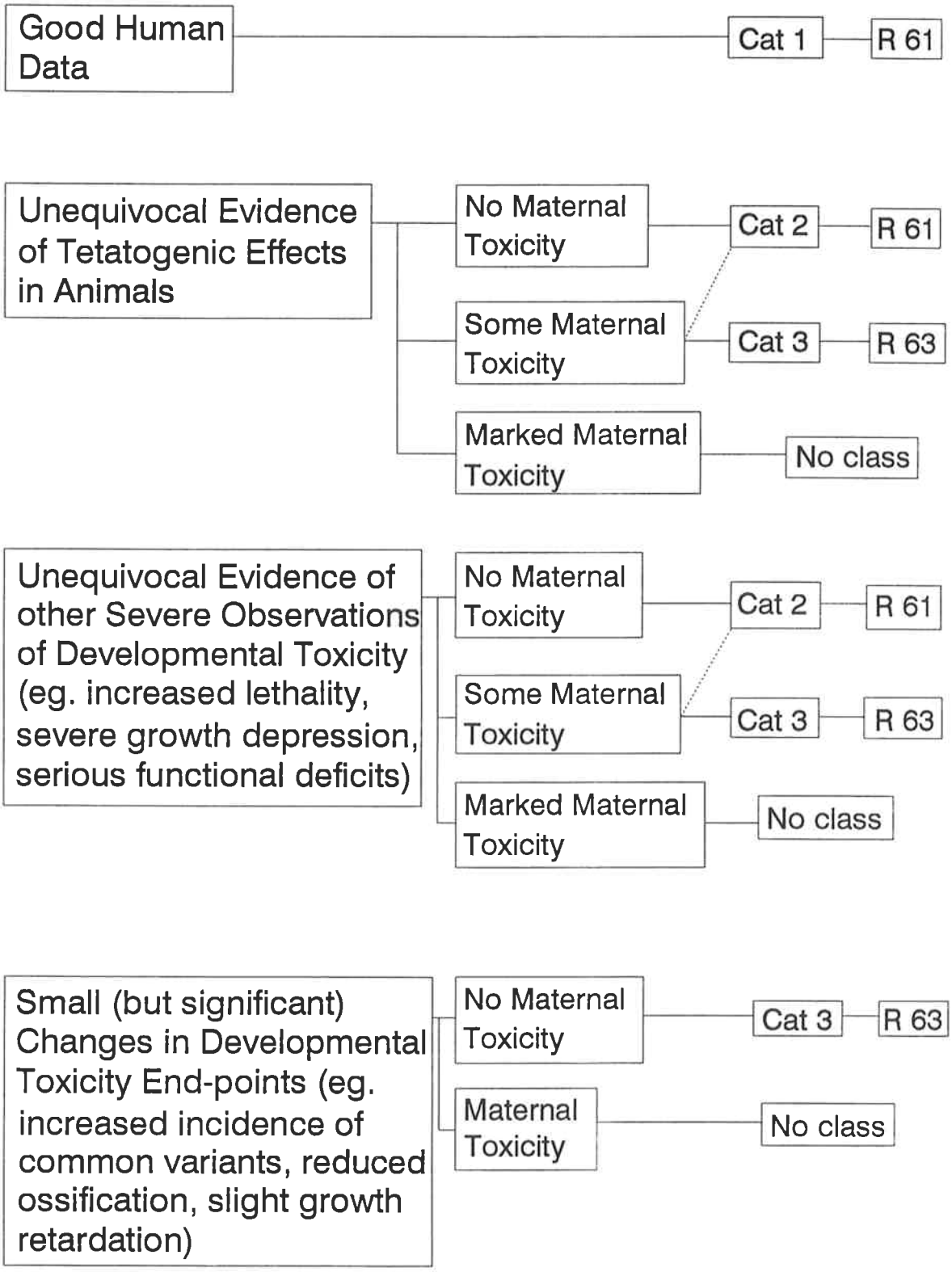


Figure 9 DEVELOPMENTAL TOXICITY



APPENDIX 1
Members of the Task Force

F.M.B. Carpanini (Chairman)	BP International Plc GB - Guildford
P.M.D. Foster	ICI Plc GB - Macclesfield
J. Hellwig	BASF AG D - Ludwigshafen
K-H. Leist	Hoechst AG D - Frankfurt
W. Mellert	BASF AG D - Ludwigshafen
H. Weber	Ciba-Geigy AG CH - Basel
D. A. Stringer	ECETOC B - Brussels

MEMBERS OF THE ECETOC SCIENTIFIC COMMITTEE
(Peer Review Committee)

W.F. TORDOIR (Chairman), Head of Occupational Health and Toxicology Division	SHELL NL - Den Haag
H. VERSCHUUREN, (Vice-Chairman) Head of Toxicology Department	DOW CHEMICAL CH - Horgen
O.C. BOECKMAN, Scientific Advisor	NORSK HYDRO N - Porsgrunn
H. DE HENAU, European Technical Centre Professional and Regulatory Services	PROCTER AND GAMBLE B - Brussels
A. DE MORSIER, Head, Ecotoxicology	CIBA-GEIGY CH - Basel
P.A. GILBERT, Head, Environmental Relations	UNILEVER UK - Port Sunlight
I.J. GRAHAM-BRYCE, Head of Environmental Affairs	SHELL NL - Den Haag
B. HILDEBRAND,* Director, Experimental Toxicology	BASF AG D - Ludwigshafen
J.R. JACKSON, Director Medicine and Health Science	MONSANTO EUROPE B - Brussels
K. KUENSTLER, Head of Toxicology Department	HENKEL D - Duesseldorf
H. LAGAST, Chief Medical Officer	SOLVAY B - Brussels
E. LOESER, Head of Institute of Industrial Toxicology	BAYER D - Wuppertal
R. MILLISCHER, Chief Toxicologist	ATOCHEM F - Paris La Défense
I.F.H PURCHASE, Director, Central Toxicology Laboratory	ICI UK - Alderley Park
M. SHARRATT,* Group Toxicology Advisor	BP UK - Guildford

* Stewards - responsible for primary peer review

Responsible editor: D.A. Stringer
Avenue E. Van Nieuwenhuyse 4
B - 1160 Brussels
D-1992- -

LIST OF ECETOC PUBLICATIONS

MONOGRAPHS

No. Title

No.1 Good Laboratory Practice
No.2 Contribution to Strategy for Identification and Control of Occupational Carcinogens
No.2 Definition of a Mutagen, for 6th Amendment
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, LD_{50}(LC_{50}) 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
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 Mutation Research, Special Issue: Early Indicators of Non-Genotoxic Carcinogenesis
No.17 Hepatic Peroxisome Proliferation

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 the 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 n°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 n°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 Pattern
No.30(4) Existing Chemicals : Literature Reviews and Evaluations
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 Tetrachloroethylene : 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 Contaminents in Soil
No.41 Human Exposure to N-Nitrosmaines, 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 EC7th Amendment: "Toxic to Reproduction": Guidance on Classification (not yet published)
No.48 Eye Irritation: Reference Chemicals Data Bank

JACC REPORTS

No. Title

No.1 Joint Assessment of Commodity Chemicals, Melamine
No.2 Joint Assessment of Commodity Chemicals, 1,4-Dioxane
No.3 Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone
No.4 Joint Assessment of Commodity Chemicals, Methylene Chloride
No.5 Joint Assessment of Commodity Chemicals, Vinylidene Chloride
No.6 Joint Assessment of Commodity Chemicals, Xylenes
No.7 Joint Assessment of Commodity Chemicals, Ethylbenzene
No.8 Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone
No.9 Joint Assessment of Commodity Chemicals, Chlorodifluoromethane
No.10 Joint Assessment of Commodity Chemicals, Isophorone
No.11 Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane
No.12 Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane
No.13 Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane
No.14 Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane
No.15 Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1,1-Dichloroethane
No.16 Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane
No.17 Joint Assessment of Commodity Chemicals, (HFA-142b) 1-Chloro-1,1,Difluoroethane
No.18 Joint Assessment of Commodity Chemicals, Vinylacetate
No.19 Joint Assessment of Commodity Chemicals, Dicyclopentadiene
No.20 Joint Assessment of Commodity Chemicals, Tris-/Bis-/Mono-(2-ethylhexyl)phosphate
No.21 Joint Assessment of Commodity Chemicals, Tris-(2-butoxyethyl)-phosphate