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

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"TOXIC TO REPRODUCTION"
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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

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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.

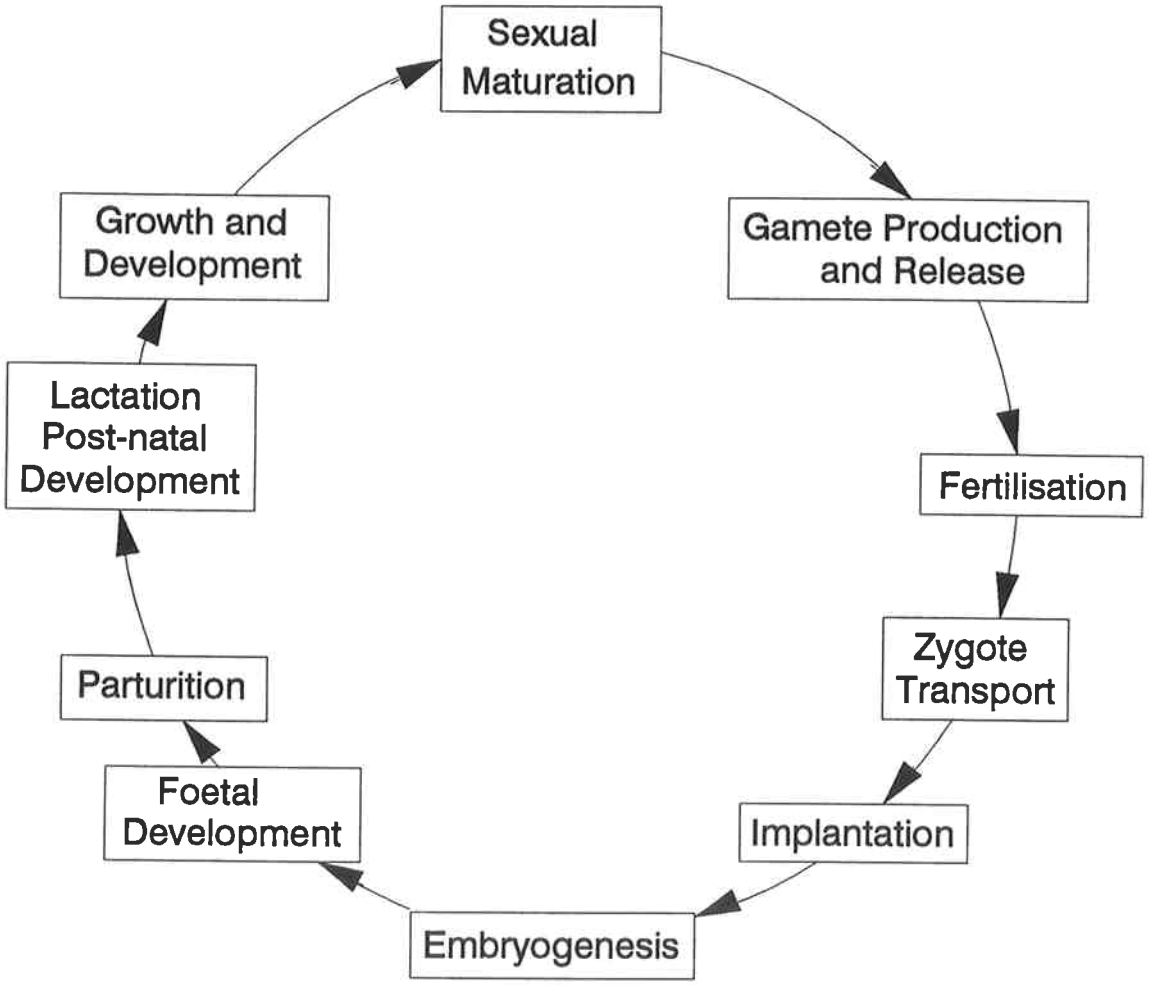
The terms of reference of the task force were:

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