Methods for Assessing the Effects of Chemicals on Reproductive Functions
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Effects of Chemicals on Reproductive Function

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1 INTRODUCTION

In view of a steadily increasing number of abnormal pregnancies, childless marriages and congenital developmental defects observed during the recent years, toxicologists have to improve their capability of predicting possible effects of environmental chemicals on reproductive function. The effects on reproductive function should be considered together with other long-term effects—carcinogenic, genetic and immunological—which many chemicals are capable of inducing when absorbed into the body even in very small quantities. Sometimes there is a long latent period before such effects become apparent.

Clinical observations have demonstrated a growing number of disturbances in the menstrual cycle among female workers in some industries, notably in the chemical industry. Such pathological changes frequently occur without the loss of working ability and may not be included in the routine medical records.

Sanitary standards for the majority of industrial chemicals previously tested were established without considering possible long-term effects. A convincing documentation has accumulated in the meantime which provides evidence for selective toxicity of many chemicals, and a number of already adopted hygiene regulations have therefore been revised in the Soviet Union and in the member countries of the Council of Mutual Economic Assistance (CMEA). When establishing sanitary standards for chemicals in the environment, it is now mandatory to take into account the possible long-term effects.

Disturbances of reproductive function may result from injuries in the testes and ovaries, from pathological changes in their control systems and from some diseases of the urogenital tract which impair sexual processes. These impairments may become manifest as disturbances in the individual components of the integrated reproductive cycle, such as libido, erection, ejaculation and orgasm. For example, the impairment of ejaculation—even its complete disappearance—following an experimental exposure to 3% solution of hexachlorophene, proved to be related to fibrosis of the prostate (Gellert *et al.*, 1978).

Evaluation of the effects of psychogenic factors is now considered obligatory

in clinical investigations. It is well known that neuroses with marked emotional disturbances may have a depressive effect on sexual behaviour, on spermatogenesis (Krištal and Sergienko, 1977; Vasilčenko *et al.*, 1977; Sanockij *et al.*, 1980a,b), and on reproductive capacity in general. For instance, occupational exposure to vinyl chloride may result in symptoms of asthenia, and may initiate sexual disturbances; a man with a weak sexual constitution may have a lower libido if he is asthenic. Thus some industrial chemicals may have an indirect effect on reproductive function by way of their action on the nervous, endocrine and other systems.

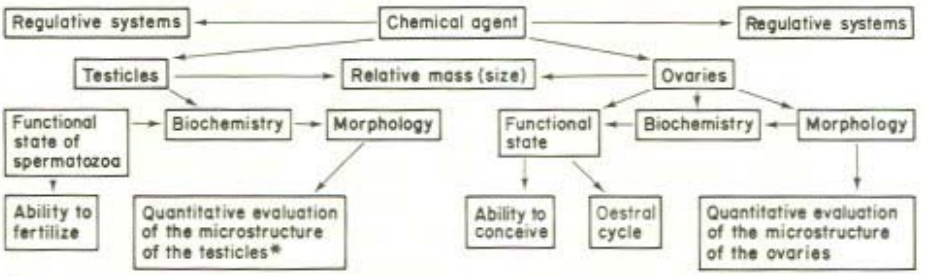
In view of this, an important task of toxicology is to determine whether the effect of a chemical agent on reproductive function is specific (selective) or not. Such toxicological studies can be successfully performed in experiments on laboratory animals (Sanockij, 1976; 1979).

2 AN OUTLINE OF METHODOLOGY FOR EVALUATING THE EFFECTS OF CHEMICALS ON REPRODUCTIVE FUNCTION

Systematic evaluation of the effects of chemicals on reproductive function should consider the following.

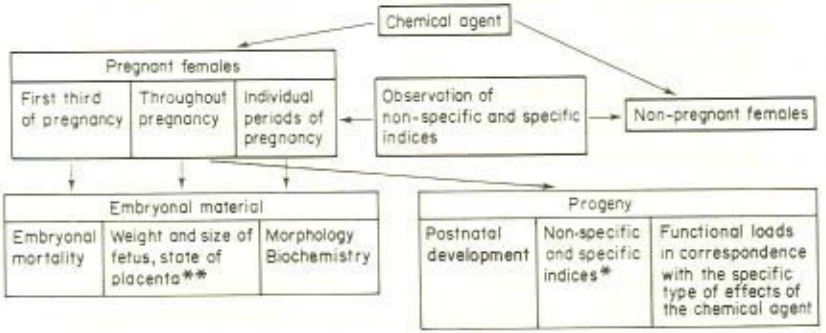
(1) Gonadotoxicity (see Figure 1):
   (a) effects on the testes and on the control of their function, and
   (b) effects on the ovaries and on the control of their function.
(2) Effects on the embryo, fetus and 'mother–embryo/fetus' system (see Figure 2):
   (a) embryotoxicity,
   (b) teratogenicity,
   (c) effects on the placenta and uterus, and on the control of their function.

Genetic effects, although conceptually a part of reproductive toxicology, are considered separately (see Figure 3).



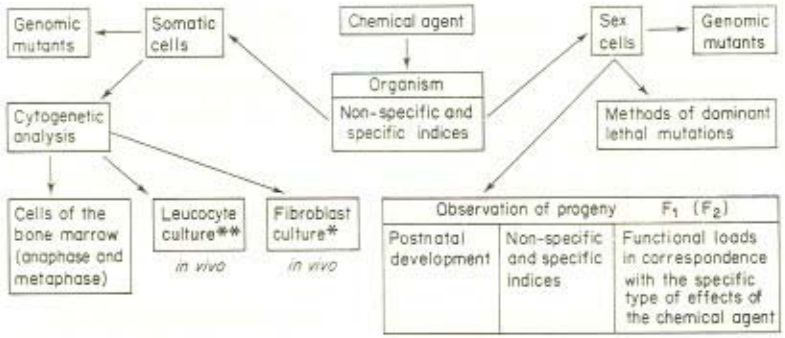
**Evaluation of the index of spermatogenesis is not obligatory.*

Figure 1 Scheme for investigating gonadotoxic effects



**Special attention should be paid to behavioural and biochemical tests.*
***Examination of the placenta is not obligatory, but data on the permeability of placenta substantially improve the quality of information.*

Figure 2 Scheme for investigating embryotropic effects



** Not obligatory*
*** When examining working persons for substances which are already in use in industry (hygienic evaluation of MAC).*

Figure 3 Scheme for investigating mutagenic effects of industrial chemicals

3 BASIC PRINCIPLES

Design of experiments in preventive toxicology involves some fundamental principles, the neglect of which may diminish the value of the work performed and make the interpretation of the results difficult. These basic principles are as follows:

(1) A system of functional, morphological and biochemical indices of effects should be used. Results obtained by using only one type of indices cannot be subject to exhaustive interpretation.

(2) Similarity in chemical structure or biological activity to other substances

known to be mutagenic, embryotoxic or gonadotoxic either directly or by way of their effects on the control mechanisms indicates the need to perform the corresponding studies.

(3) Particular attention should be paid to substances which demonstrate a pronounced ability to accumulate in the organism, and to substances with which many individuals are in contact either occupationally or in everyday life.

(4) It is indispensable to establish the threshold for the harmful effect considered (Lim_{sp}) based on the determination of the minimum effective and subthreshold doses.

(5) The risk of long-term effects can be estimated by determining the degree of selectivity (specificity) or the biological effects considered. For this purpose, the threshold for the effects on the specific function under study (Lim_{sp}) is compared with the threshold for the effect on the organism as a whole as indicated by indices of overall (integrated) toxicity (Lim_{int}). The degree of selectivity (specificity) of the effect is then determined by calculating the 'zone of specific effect' defined as $Z_{sp} = Lim_{int}/Lim_{sp}$; $Z_{sp} > 1$ indicates a selective (specific) effect on the function under study.

The threshold for a harmful effect is considered to be the smallest concentration (or dose), the exposure to which—under specific conditions of the experiment or observation—produces either changes in the organism which exceed the limits of physiological adaptation, or latent, temporarily compensated pathological conditions.

(6) If possible, the results of experimental investigation should be compared with clinical and epidemiological observations. This provides a reliable basis for setting up or correcting sanitary standards.

4 MINIMUM REQUIREMENTS FOR GONADOTOXICITY TESTING

The condition and function of the testes can be evaluated by quantitatively assessing the morphology of spermatogenic epithelium and by examining the functional state of spermatozoa (Fomenko, 1975).

The morphometric assessment can be carried out by estimating:

(1) the spermatogenesis index as determined by a method proposed by Fogg and Cowing (1951) (not obligatory);
(2) the relative number of tubules with desquamated spermatogenic epithelium;
(3) the average number of normal spermatogonia;
(4) the relative number of tubules with cells in the 12th stage of meiosis.

The functional state of spermatozoa is examined by:

(1) observing the character and duration of their movement;
(2) estimating the relative number of live spermatozoa;
(3) determining the concentration of spermatozoa in the tail of epididymis;

(4) estimating the relative number of degenerated forms of spermatozoa;
(5) determining the osmotic and acid resistance.

The control of testicular function can be evaluated by:

(1) determining the levels of gonadotropins in blood serum;
(2) determining the level of testosterone in blood plasma.

The clinical examination of male reproductive function includes the evaluation of the state of neurohormonal, psychological, erectional and ejaculatory components of the sexual cycle by using methods of structural analysis of sexual disturbances (Vasilčenko, 1977).

The examination of the ejaculate comprises the following variables:

(1) volume;
(2) colour;
(3) period of liquefaction;
(4) viscosity;
(5) sperm concentration (number per ml);
(6) total number of spermatozoa in the ejaculate;
(7) relative number of live spermatozoa;
(8) percentage of morphologically altered forms; and
(9) pH of the ejaculate.

The biochemical investigations include the determination of testosterone level in blood plasma, the amount of fructose in the ejaculate and the activity of hyaluronidase in the heads of spermatozoa.

The examination of ovaries comprises the determination of their weight and quantitative evaluation of their microstructures (Mandl and Zuckerman, 1951a,b,c, 1952) including:

(1) primordial follicles and follicles with one layer of granulosa cells;
(2) follicles with two or more layers of granulosa cells;
(3) Graafian follicles;
(4) corpora lutea;
(5) total number of generated forms.

When evaluating the control of ovarian function in the preliminary experiment, the gonadotropic function of the pituitary gland should be examined.

5 EMBRYOTOXICITY

The exposure to chemicals during pregnancy can result in various abnormalities in the development of embryo which can be provisionally classified as being the results of:

(1) *teratogenicity* when histomorphological, biochemical, functional and other abnormalities—sometimes incompatible with life—of organ and system functions of the embryo occur; these abnormalities can become manifest in the postnatal development;
(2) *embryotoxicity* when intrauterine death or reduced size and weight of embryos occur but the tissues are normally differentiated.

As a rule, when the doses or concentrations of different chemical substances are small a, weak embryotoxicity is observed. Teratogenicity is also rare at exposures to low concentrations of chemicals. Nevertheless, some specific embryotoxic substances (for example, chloroprene) may be teratogenic even when administered at low concentrations during specific days of pregnancy (Sanockij and Sal'nikova, 1979).

The correlation between teratogenicity, the stage of embryogenesis and the duration of exposure is particularly significant. In our own studies of chloroprene, predinsolone, vinyl chloride and other substances we were able to establish an important fact, i.e. that for some chemicals the relative embryotoxicity is higher when the exposure during specific periods of embryogenesis is compared to the total exposure during pregnancy. We consider this typical for substances which have a specific embryotoxic action. Undoubtedly, this question needs further study since it is of practical importance for recommending the work regimen of pregnant women in the chemical industry.

In some cases embryotoxicity can be related to the transfer of the chemical through the placenta. Direct and indirect methods may be used for studying the degree of placental permeability to chemical substances. The direct methods consist in determining the amount of substance transferred through the placenta and its distribution in embryonic tissues. For example, the determination of *N,N'*-dimethylformamide (DMFA) in the embryonic tissues, placenta and liver of a mother showed that the inhalation exposure to DMFA even at the maximum allowable concentration and at minimum chronic effects levels (Lim_{ch}) leads to the accumulation of the chemical in embryonal tissues.

The change of placental permeability with the stage of pregnancy and with the concentration (dose) of the chemical is an important problem. As demonstrated by Dr. Sivočalova in 1975, the placental permeability can be used for determining the threshold for adverse effects of tetracycline and other substances.

Apart from using a direct measurement of the transfer of chemicals through the placenta, the permeability can be estimated by means of test substances such as ^{139}I, ^{35}S and others. The combination of the direct and indirect methods enables a better understanding of the degree of permeability of placental barrier to foreign substances and normal metabolites.

Evaluation of disturbances of compensatory mechanisms of maternal and fetal organisms plays an important role in determining the selectivity of embryotoxic effects of chemicals. For example, during inhalation exposure to DMFA, the

cardiac function of the fetus of experimental animals exposed to hypoxia at the intermediate and late stages of pregnancy was damaged; at the same time the functional state of the uterus was not impaired. This fact confirms the selective action of DMFA on the fetus. Similar data were obtained when the embryotoxic action of tertiary butyl hydroperoxide was investigated.

Taking into account the interconnection between the organs of the mother and the fetus, it is necessary to examine thoroughly those organs of the fetus and offspring which were injured to a larger degree in their mothers. In an experiment with hydrogen chloride, it was established that regardless of the stage of pregnancy the most serious alterations take place in the lungs and kidneys of experimental animals. The alterations in the fetuses and offspring followed the 'organ to organ' relationship; the degree of damage depended on the degree of injury of the same organs in their mothers.

6 MINIMUM REQUIREMENTS FOR EMBRYOTOXICITY AND TERATOGENICITY TESTING

Methods for evaluating embryotoxicity include the determination of:

(1) preimplantation losses;
(2) postimplantation losses;
(3) total embryonal mortality.

Methods for evaluating teratogenicity comprise:

(1) analysis of the pathology of internal organs by Wilson's microanatomical method;
(2) analysis of the cardiovascular pathology according to Staples' method.

Evaluation of the health status of the offspring:

(1) postnatal loss (coefficients of survival and lactation);
(2) evaluation of the functional state of individual organs and systems of the offspring;
(3) application of functional loads.

The application of methodological approaches mentioned above was validated by testing several dozens of chemicals. This provided an opportunity to elaborate the main criteria for the evaluation of long-term effects of the industrial chemicals and establishing limits for their concentration in the environment.

7 CONCLUSIONS

The methods described represent only the minimum testing requirements sufficient for solving some practical tasks, such as sanitary standardization of toxic chemicals in the air, soil, food and some other environmental components;

determination of the degree of toxicity of chemicals; and the prediction of related long-term effects.

This list of methods should not be considered dogmatically. New methods are being developed, and as the amount of information they provide, the difficulties in their use and their reliability are evaluated, they gradually supplement or replace currently used methods.

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