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GENERATION STUDIES

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One of the important problems of toxicity evaluation is the assessment of possible adverse effects of chemical compounds on unborn and future generations of human beings. Congenital malformations, both severe and trivial, are found in about 2-4% of all human births (1). Drugs and environmental chemicals are responsible for 2-3% of developmental defects in humans, but causes of 65-70% of these defects remain unknown (2). To protect human populations, the effects of substances newly introduced to industry must be assessed on animal reproduction. Such assessment is essential for compounds which may spread into the general environment in measurable amounts, which may have occupational contact with women of childbearing potential or which are used in drugs or as food additives. These chemicals may affect fertility and general reproductive performance, embryo and foetal growth and development and postnatal growth and functional maturation.

Fertility and General Reproductive Performance

Studies in this area attempt to provide the following information concerning the influence of chemicals: gonadal function of both males and females; the estrous cycle, mating behaviour, fertility and pregnancy rate, litter size, nursing behaviour and lactation of females; and the viability, growth and development of immature animals during weaning and puberty periods.

The multi-generation test was designed mainly for food additives and pesticide residues in food. However, it may be used to evaluate the effects of other environmental chemicals on general reproduction. Keplinger et al. (3) carried out studies of the effect of pesticides on reproduction in mice for six generations. Results indicate no basis for prolonging the tests for more than three generations. The so-called three-generation test requires that animals, usually rats or mice, be exposed continuously throughout three reproductive cycles. The test is performed on both females and males. The females may be mated with control (unexposed) and exposed males.

Each generation is exposed from weaning through growth and maturation and through gestation to the end of lactation. Near the end of the period of organogenesis or at the end of gestation, half of the females from both the control and test groups are sacrificed and examined for number of embryos, implantation sites and corpora lutea as well as number of embryos or foetuses undergoing resorption. Groups of animals per dosage level consist of a minimum of 10 males and 20 females. A properly conducted three-generation test employs at least three dose levels; the largest dose represents a nearly maximum tolerated dose in chronic systemic toxicity studies. One of these three dose levels should produce no significant effect on reproduction (4). According to Wilson (5), the three-generation test is a useful procedure for an overall evaluation of low-dosage, long-lasting exposures but cannot be regarded as an adequate test for assessment of teratogenic and mutagenic properties, which should utilize specialized techniques and higher doses.

For compounds such as drugs and most chemicals used in industry, the exposure of one generation of animals seems to be a useful screening procedure for assessment of the effects of chemicals on male and female fertility and general reproductive performance. The guidelines for these studies recommended by the governments of different countries (6) require the following: exposure of males for 60 days in the United States, Sweden and Japan; treatment of females for 14 days before mating through weaning in the United States; and treatment of females for 14 days before mating and through the first week of pregnancy in Japan and Sweden. According to recommendations for such studies in the United Kingdom, either males or females should be exposed for a sufficient length of time before mating. Duration of exposure and time of exposure in relation to the time of mating may greatly influence the effects of the chemical on the general reproductive performance of animals. The inhalation exposure of female rats to mercury vapour at a concentration of 0.1 mg/m^3 only during the pregnancy period did not change the mortality rate of their progeny. However, the exposure of females to mercury vapour at the same concentration for either seven weeks before mating or three weeks before mating and through the pregnancy period increased the mortality rate of their progeny during the 60 days after parturition in comparison to that observed in control groups (7).

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Effects of Chemical Compounds on Embryo and Foetal Growth and Development

These studies investigate the potential of a chemical for embryotoxicity and/or teratogenicity. According to Staples & Wilson (8), a chemical compound is teratogenic when it measurably alters the structure or function of a statistically significant number of the offspring (versus sham controls) after administration to the male before mating, to the female before or during pregnancy or to the foetus before completion of maturation. A chemical compound which does not produce structural or functional malformations but kills developing young or causes a reduced rate of foetal growth is an embryotoxic agent; however, it is not teratogenic. A test agent may only be termed embryotoxic or teratogenic when it disturbs embryonic and/or foetal development at dose levels that do not adversely affect the mother. Embryo lethality, developmental retardation, congenital malformation and functional disorders observed only after administering doses that are overtly toxic to the mother should be considered generally toxic (6).

Although little is known about the mechanisms of action of any teratogen, teratogenic agents are generally believed to act by affecting the maternal homeostasis, the placenta or the embryo.

Many substances, both exogenous and endogenous, cross the placenta by a variety of mechanisms: simple diffusion, facilitated diffusion, active transport, pinocytosis and through physical breaks in the lipoidal membrane. Most exogenous substances penetrate the placenta by simple passive diffusion. The rate of entry into the foetal circulation, therefore, is dependent upon such physical characteristics as lipid solubility, molecular weight, degree of ionization at physiological pH (pK) and the degree of protein binding. An increase in lipid solubility and a decrease in the degree of ionization and/or protein binding facilitates the passage of a drug across the placenta.

The animals used most frequently for teratogenicity testing (mice, rats, hamsters and rabbits) have a short gestation period and are easily kept in the laboratory in sufficient numbers. Quite frequently, a difference in susceptibility to teratogenic action of chemicals occurs between species, and this difference, at least in part, is

due to differences in the absorption, distribution and metabolism of the test chemical. As in other toxicological tests, animal species used for assessment of embryotoxic or teratogenic effects should absorb, distribute, metabolize and excrete the test compound in a way similar to that of humans. Knowledge of the pathways of metabolism of chemicals in the animal species used is essential when extrapolating the results to humans. At least two species, one rodent (rat or mouse) and one lagomorph (rabbit), should be used for testing. Animals used for teratological studies must be healthy and should be housed under the best possible environmental conditions.

In most experimental procedures for teratogenicity assessment, the compound is given during the period of organogenesis because the embryo is most susceptible during that period of development. Because certain chemicals are able to change the rate of their own metabolism when given repeatedly, Wilson (5) recommends treatments during organogenesis at sequential short-term periods (three to four days). This procedure avoids maternal adaptative metabolic changes that might cause fluctuations in dosage to the conceptus. The pregnant females are sacrificed at term or one to two days before term, and the number per dam of corpora lutea, implantations, living and dead foetuses, early and late resorptions and abortions is recorded. The sex of living foetuses is noted. The foetuses undergo external, gross visceral and skeletal examination for structural malformation.

Methods for these examinations were presented at the Teratology Workshop held in Berlin in 1977 (9). Using the procedures presented, Brittelli et al. (10) studied the teratogenic potential of hexafluoroacetone in rats, and Brown et al. (11) assessed the embryotoxic effects of trimethadione in mice.

In statistical analysis, two possible basic units can be considered in evaluating teratogenic effects: the litter and the individual foetus. The fate of an individual implant may be influenced by the site of implantation in the uterine horn or the number of implants in the uterus. Malformations are frequently clustered within litters, resulting in abnormal distribution. Thus, the litter is the more valid unit when statistical evaluations are involved.

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Epidemiological methods for detection of teratogens which are independent of experimental methods also exist. Different approaches to that problem are reviewed by Klingberg & Weatherall (1).

Postnatal Studies

Several compounds administered to female animals before mating and/or during the pregnancy in doses which do not produce embryotoxic or teratogenic effects can induce alterations of viability, postnatal growth and development of the newborn (7,12). Frohberg (6) assumes that postnatal observation may be a more sensitive parameter than classical testing for teratogenicity.

The period of observation of postnatal development should last from birth to puberty or even longer. Examination of young animals only from birth to weaning seems insufficient for at least two reasons. First, the majority of the methods used for measurements of behavioural parameters were designed to study adult animals: rarely can they be used to test very young animals. Second, the alterations seen in animals before weaning may only reflect retardation in their development. Thus, only examination at puberty and later may detect lasting developmental anomalies.

The effects seen during the postnatal period of development may be produced prenatally or postnatally, either directly by chemically crossing the placental barrier or by being secreted in the mother's milk or indirectly by altering the mother's metabolism during the pregnancy, thus reducing her ability to care for her offspring. In order to differentiate between prenatal and postnatal influences, the exchange of offspring between similarly treated mothers (fostering) or the exchange of offspring between exposed and control mothers (cross-fostering) may be employed. These procedures may help to evaluate maternal influence on postnatal development.

The exposure of human beings to certain chemicals widely spread in the environment or used as food additives and drugs may start shortly after birth through the mother's milk or food. To assess the adverse effects of such exposure, experimental treatment of lactating animals or the immature offspring may be necessary. In addition to

evaluating the toxic effects, the absorption, distribution and excretion of the tested compound in developing animals are important to study (13).

The extent of testing in postnatal studies should include different aspects of anatomical, physical and mental development as well as mortality rate during the observational period. When the animals under study reach sexual maturity, or even earlier, the same tests as those used to assess systemic toxicity in adult animals may be employed. Thus, the tests for evaluation of developmental processes and the tests for evaluation of systemic toxicity may be used in postnatal studies.

Physical development of young animals can be assessed by measurements of body weight and timing of such features as unfolding of the external ear, generalized hair growth, tooth eruption, eye opening, vaginal opening and testicular descensus (14). Tesh (15) presents methods for assessing auditory and visual functions, locomotor activity, learning performance, locomotor coordination and quadruped muscle development in young laboratory animals. According to Grauwiler & Leist (16), examination of postnatal development should be made in two steps. In the first step, tests used should indicate general evidence for any disturbance of development. The large number of animals and very tight time schedule require an easy, quick procedure for behavioural follow-up. The second step is necessary when relevant deviations from normal are seen in the performance of young animals in the tests at the first step.

Using a simple test, Overmann et al. (17) demonstrated altered performance of negative geotaxis and delayed eye opening and maturation of the air-righting reflex in neonatal rats exposed to lead from parturition to weaning through the milk of dams drinking 0.02% or 0.2% lead acetate solutions. In similarly exposed neonatal rats, Fox (18) detected lesions of the central nervous system by measurements of latencies of visually evoked response components. Winneke et al. (19) used behavioural tests, such as an open field test and a visual discrimination learning task, to assess neurobehavioural deficits in rats subsequent to prenatal and neonatal low level lead exposure.

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