The comparative consideration on recently proposed test methods for reproduction and developmental toxicity

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The method of reproductive and developmental toxicity study is dividing into two types according to the type of human exposure. One is three segment reproductive and developmental toxicity study for drugs (Fig. 1). In this study, effects of drug on reproductive and developmental parameters such as gametogenesis, estrus cycle, mating behavior, ovulation (luteinization), fertilization, implantation, embryogenesis in early gestation period, fetal growth in late gestation period, embryonic/fetal death, developmental retardation, teratogenesis, parturition, weaning, retardation of postnatal growth and functional

Fig. 1. Time Schedule of the Three Segment Reproductive and Developmental Toxicity Study for Drugs in Japan

■ Without dosing □ Dosing period * Necropsy

development are able to detect. Another is multigeneration reproduction study for new chemical substance control act in Japan (Fig. 2). In this study, effects on gametogenesis, estrus cycle, mating behavior, ovulation (luteinization), fertilization, parturition, weaning are able to detect. Teratogenicity study is also required for evaluation such as embryonic/fetal death, developmental retardation or teratogenesis.

Fig. 2. Time Schedule of the Multigeneration Reproduction Study and Teratogenicity Study for New Chemical Substance Control Act in Japan

Without dosing
  Dosing period
 * Necropsy

In the USA, National Toxicology Program (NTP) have been proceed for protect the health of American public from exposure to hazardous chemicals. Reproductive Assessment by Continuous Breeding (RACB) study in mice have been developed and operated for evaluation of NTP reproductive and developmental toxicity (Fig. 3 and 4). The RACB protocol is consist of four tasks. Task 1 is a 14 days dose setting study. Task 2 is the continuous breeding phase. In this phase, the test substance is administered to both sexes throughout 7 days pre mating period and 98 days mating period. Fertility, parturition and teratogenicity of newborns during this period are examined on each gestation. After 98 days mating, the pairs are separated but dosing are continued for 21 days. Final litter during 98 days mating period is delivered and F₁ kept until weaning. Mother is dosed until weaning. Task 3 is crossover mating trial. When significant adverse effects on fertility are observed in 98 days mating period, a crossover mating trial in F₀ generation is performed between control and affected dose group. Task 3 is performed after the last Task 2 litter is weaned. Task 4, offspring assessment phase, is performed whether or not Task 2 shows reproductive toxicity. F₁ mice are dosed throughout growing up to sexual maturation after weaning, 7 days mating period and gestation period. Fertility of F₁ parent and teratogenicity of F₂ newborns are

Fig. 3. Time Schedule of the Reproductive Assessment by Continuous Breeding Protocol

Fig. 4. Reproductive Assessment by Continuous Breeding Flow Diagram

gametogenesis, estrus cycle, mating behavior, ovulation (luteinization), fertilization, parturition, weaning, teratogenesis are able to detect. In this test method, mating begin after only 7 days of dosing and continues

98 days, so can provide some information on roughly which stages of spermatogenesis are affected. It is said that RACB has a characteristic fact that direction of after test is selected by investigator from result of test.

The large number of existing chemicals still have little or no toxicological information in the world. Consequently, OECD has selected 147 chemicals, as priority No. 1, for safety assessment out of approximately 1500 high production volume (HPV) chemicals in 1989. Data on repeated dose, genetic and reproductive toxicity are necessary as a screening for toxicity of the HPV. OECD recently (1990) proposed a protocol named the 'Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test (Repro-Tox)' (Fig. 5). This new test is intended to obtain toxicological data on HPV chemicals in order to their priority for further toxicity tests. Repro-Tox is designed to obtain the information on the general toxic effects and reproductive/developmental toxic effect by repeated dosing on both males and females in identical test. In Repro-Tox, the test substance is administered to both sexes throughout 14 days pre-mating period, 14 days mating period, gestation period and lactation day 3, i.e., totally 42-54 days, and on day 4 of lactation parental animals and offspring are killed and examined. Effects on estrus cycle, mating behavior, ovulation

Fig. 5. Time Schematic Of The Repro-Tox Protocol

▨ Dosing period   □ Without dosing

(luteinization), fertilization, implantation, parturition, teratogenesis or physical and behavioral abnormalities of pups are able to detect. External abnormalities are examined at birth, and visceral abnormalities are examined on day 4 of lactation. In this test method, effects on early stage of spermatogenesis is supplement with microscopical examination on testis and epididymis.

The parameters of four reproductive and developmental toxicity test methods described above are compared in Table 1. In the

Table 1. Comparison of required items among four reproductive and developmental toxicity test methods

Items	3-Seg.	Multi./Terato.		RACB	Repro-Tox
Gametogenesis	+	+	-	+	+
Estrus cycle	+	+	-	+	+
Mating behavior	+	+	-	+	+
Ovulation (luteinization)	+	+	-	+	+
Fertilization	+	+	-	+	+
Implantation	+	-	-	-	+
Embryogenesis in early gestation period	+	-	-	-	-
Fetal growth in late gestation period	+	-	-	-	-
Embryo/fetal death	+	-	+	-	-
Developmental retardation	+	-	+	-	-
Teratogenesis	+	-	+	+	+
Parturition	+	+	-	+	+
Weaning	+	+	-	+	-
Retardation of postnatal growth and functional development	+	-	-	-	+

3-Seg. : Three segment reproductive and developmental toxicity study.

Multi. : Multigeneration reproduction study.

Terato. : Teratogenicity study.

Repro-Tox : Combined repeat dose and reproductive/developmental toxicity screening test.

RACB : Reproduction assessment by continuous breeding.

+: Detect - : Not detect.

multigeneration study, effects on implantation, retardation of postnatal growth and functional development are unable to detect. In the teratogenicity study, the test substance is administered to pregnant animals during organogenesis. So, embryogenesis in early gestation period or fetal growth in late gestation period are unable to detect. In the RACB, effects on implantation, fetus, retardation of postnatal growth and functional development have not been examined. In the Repro-Tox, effects on fetuses is unable to detect.