Session 2. Report 2.

STANDARD PROTOCOLS BASED ON THE ICH GUIDELINES FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES : AN INTERNATIONAL IMPLEMENTATION

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Introduction

Pfizer is an international company which focuses on world-wide development of pharmaceuticals and animal health products. We have three laboratories in the United States (Groton, CT), France (Amboise) and Japan (Taketoyo, Aichi) which are engaged in preclinical studies for safety evaluation of new candidates. In June 1993, the Steering Committee of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) approved the ICH Harmonized Tripartite Guideline "Detection of Toxicity to Reproduction for Medicinal Products" (ICH guideline). Prior to the ICH guidelines there were many differences in standard procedures for reproductive and developmental toxicity studies among the sites. The ICH guideline provided an opportunity to improve and harmonize our testing procedures. As the first step, we reviewed and revised standard protocols for the reproductive and developmental toxicity studies. The standard protocols now used at our three sites are based on the 3-study design (the most probable option) recommended as general screens to identify potential treatment related

effects in the ICH guideline.

In this report, outlines of our standard protocols including the preliminary range finding studies, are presented: 1) Rabbit Toleration Study in non-pregnant animals, 2) Maternal Range Finding Studies in Rats and Rabbits, 3) Fertility and Early Embryonic Development Study (Reproductive Study I), 4) Teratology Studies in Rats and Rabbits (Reproductive Study III), 5) Toxicokinetic Studies in Rats and Rabbits and 6) Pre and Postnatal Development Study (Reproductive Study II).

1. Standard Protocols

The standard protocols of the 6 studies are summarized as follows. The outlines of each study are shown in Table 1 and the study designs in Figure 1.

1) Rabbit Toleration Study

This exploratory study is designed to establish appropriate dose levels for a subsequent maternal range finding study. Groups of 3 non-pregnant rabbits are treated once daily for 5 or 10 days. The length of the study is determined for each compound based on previous work in another species, usually rat. The animals are monitored for clinical signs, body weight and food consumption. On the last day of dosing, 4-5 blood samples are collected to determine plasma (serum) kinetics. The animals are submitted to a gross necropsy of the major organs.

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**Table 1. OUTLINE OF REPRODUCTIVE STANDARD PROTOCOLS
RABBIT TOLERATION STUDY**

Animals :	3 Non-pregnant rabbits / dose group
Study Design :	3 doses plus vehicle control <i>Duration, 5-10 days determined by the compound</i>
Endpoints :	Clinical sings, body weights, food consumption 4-5 blood samples on last day of dosing to determine plasma (serum) kinetics

MATERNAL RANGE FINDING STUDY IN RATS AND RABBITS

Animals :	7 copulated rats / dose group, 7 inseminated rabbits / dose group
Study Design :	3 doses plus a vehicle control Gestational Days 6-17 for rats, Gestational Days 6-18 for rabbits Cesarean section at term
Endpoints :	Clinical signs, body weights, food consumption Corpora lutea and implantation sites Fetal examination ; body weights, sex, external anomalies

REPRODUCTIVE STUDY I : FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY

Animals :	20 / sex / dose group
Study Design :	3 doses plus a vehicle control <i>Males treated for 4 weeks prior to and through mating until sacrifice (a minimum of 63 days)</i> <i>Females treated for 2 weeks prior to mating through implantation, Gestational Day 7</i> <i>Mating period-2 weeks, 1:1</i>
Endpoints :	Clinical signs, body weights, food consumption <i>Estrous cycle evaluation by vaginal smear for 14 days prior to mating</i> <i>Cesarean section, all females, Gestational Day 14</i> Implantation sites, corpora lutea, live and dead fetuses Retention of reproductive organs in females for optional histopathology <i>Histopathology of testis</i> Organ weights of testes and epididymides (optional) <i>Sperm count and sperm motility - cauda epididymis (optional)</i>

REPRODUCTIVE STUDY III : TERATOLOGY STUDY IN RATS AND RABBITS

Animals :	20 copulated rats / dose group, 20 inseminated rabbits / dose group
Study Design :	3 doses plus a vehicle control Gestational Days 6-17 for rats, Gestational Days 6-18 for rabbits Cesarean section at term
Endpoints :	Clinical signs, body weights, food consumption, corpora lutea and implantations sites Fetal examination ; Body weights, sex, external anomalies Visceral examination for rats : fixed specimens for rabbits : <i>fresh dissection</i> Skeletal examination : <i>double staining</i>

TOXICOKINETICS STUDY IN RATS AND RABBITS*Run in parallel with Reproductive Study III*

Animals : 4 copulated rats / dose group, 4 inseminated rabbits / dose group

Study Design : 3 doses plus a vehicle control

Gestational Days 6-17 for rats. Gestational Days 6-18 for rabbits

Endpoints : Maternal blood samples collected on last day of dosing

Cesarean section after last blood sample

*Maternal plasma (serum) and fetal tissue analyzed for presence of compound***REPRODUCTIVE STUDY II : PRE AND POSTNATAL DEVELOPMENT STUDY**

Animals : 20 pregnant rats / dose group

Study Design : 3 doses plus a vehicle control

Gestational Day 6 through Postnatal Day 21

All females allowed to litter

Endpoints : F0 dams ; Clinical signs, body weights, food consumption

F1 pups ; Clinical signs and body weights from postnatal day 1, food consumption from postnatal day 28

Postnatal developmental assessments :

Pre-weaning

All pups	Surface righting :	day 1-
	Incisor eruption :	day 7-
	Eye opening :	day 10-
	Air righting :	day 14-
	Visual cliff :	day 18

Post-weaning :

2 / sex	Motor activity :	day 22 or 23
	<i>Functional observational battery :</i>	day 24 or 25
All	Vaginal opening in females :	day 28-
	Auditory function, Preyer's reflex :	day 30
	Preputial separation in males :	day 35-
1 / sex	<i>Cincinnati Water Maze</i> for learning :	day 55 to 65
	<i>Passive Avoidance</i> for memory :	day 55 to 61

Reproductive performance - mating 1 / sex for F2 generation

F2 Generation - body weight through postnatal day 21

Fig. 1. HARMONIZED REPRODUCTIVE STUDY DESIGNS

2) Maternal Range Finding Study in Rats and Rabbits

This range finding study is designed to establish appropriate dose levels for subsequent definitive reproduction studies. Groups of 7 copulated animals are treated during organogenesis, Gestational days 6-17 (rat) or 18 (rabbit). The animals are monitored for clinical signs, body weight, and food consumption. Cesarean sections are conducted just prior to term. Viable fetuses are weighed, sexed (rat only) and examined for external anomalies.

3) Fertility and Early Embryonic Development Study (Reproductive Study I)

Groups of 20 male rats are treated beginning 4 weeks prior to mating for a minimum of 63 days. Groups of 20 female rats are treated for 2 weeks prior to mating, and through Gestational Day 7. The animals are monitored for clinical signs, body weight, and food consumption. The estrous cycle of the females is monitored by vaginal smear for 14 days prior to mating. This length of time usually provides observation of 2-3 cycles for each female. All females are sacrificed on Gestational Day 14. The numbers of corpora lutea, implantation sites and resorptions

are recorded for each dam. The testes from males in all dose groups are examined microscopically. If it is determined, based on fertility outcome in this study or from data obtained in subchronic toxicity studies, that a more extensive examination of the male reproductive system is needed, organ weights of the testes and epididymides are recorded and sperm from the cauda epididymides are examined for sperm count and sperm motility.

4) Teratology Studies in Rats and Rabbits (Reproductive Study III)

Groups of 20 copulated females are treated during organogenesis, Gestational days 6-17 for the rat and Gestational days 6-18 for the rabbit. The animals are monitored for clinical signs, body weight, and food consumption. Cesarean sections and gross necropsy of the dams are performed just prior to term. The numbers of corpora lutea, implantation sites and resorptions are recorded for each dam. Viable fetuses are weighed, sexed and examined for external anomalies. For rats, half of the fetuses of each litter are fixed in Bouin's solution and examined for visceral anomalies. The remaining fetuses are fixed in alcohol, stained with alizarin red S and

alician blue and examined for degree of ossification and skeletal anomalies. For rabbits, all fetuses are examined for visceral anomalies using a fresh dissection method and are subsequently stained for skeletal evaluation.

5) *Toxicokinetic Studies in Rats and Rabbits*

This study is conducted in parallel with the Reproductive Study III. Groups of 4 copulated females are treated during the organogenesis period as above. Maternal blood samples are collected on the last day of dosing. After the last sampling, fetuses are obtained by cesarean section. Drug concentrations are determined for maternal plasma (serum) and fetal tissue homogenates.

6) *Pre and Postnatal Development Study (Reproductive Study II)*

Groups of 20 pregnant rats (F0) are treated from Gestational Day 6 through Postnatal Day 21. The animals are monitored for clinical signs, body weight, and food consumption. All females are allowed to litter. The F1 pups are regularly weighed beginning on postnatal day (PND) 1 and are examined for the appearance of postnatal developmental indices and reflex behaviors. Two pups of each sex/litter are assessed for open-field locomotor activity on PND 22 or 23. A video-tracking activity monitor (San Diego Instruments, San Diego, CA) is used to monitor up to 4 males or 4 females simultaneously for 4 intervals of 5 minutes each. A functional observational battery (FOB) is conducted on PND 24 or 25 for the two pups of each sex/litter. The FOB is a series of non-invasive observational and interactive measures which assess the neurobehavioral and functional integrity of an animal. The Cincinnati Water Maze Test is performed for one male and one female F1 offspring per litter to assess learning ability around PNDs 55-65. The Cincinnati maze is composed of nine choice T's with channel widths of approximately 15 cm. Memory is also tested in a computer controlled passive avoidance chamber (San Diego Instruments, San Diego, CA)

using a one-trial learning, step-through procedure. Testing begins around PNDs 55-61. Selected F1 offspring, one male and one female / litter, are mated to nonsiblings within dose groups to assess reproductive performance. The F₂ litters are measured for viability and body weight through PND 21.

2. **Benefits of the ICH guidelines and Harmonization of Laboratories**

The implementation of harmonized standard protocols based on ICH guidelines has improved and streamlined the collection of reproductive and developmental toxicity data among the Pfizer sites for global registration of pharmaceutical products. The elimination of redundancy between studies has resulted in a decrease of approximately 40% in the number of animals used and eased the workload for reproductive and developmental toxicity studies.

In order to implement these changes, we have begun to hold technical training sessions for the staff from all sites. Among the techniques for which sessions have been held are fresh dissection examination procedures, behavioral testing and measurement of developmental indices for rat pups, and sperm analysis using a CASA system. These methodological harmonizations have been beneficial in the implementation of the new protocols and allows the expertise from the individual laboratories to be shared across the company. The result is the ability for Pfizer to conduct reproductive and developmental toxicity studies at any one of the three international sites and produce a uniform package of studies for registration.

From the success of these initial steps in harmonization, it is clear that this will be an ongoing process to maintain and monitor current study designs. As new techniques become available and older techniques are modified and improved, we will continue to share information to provide preclinical reproductive and developmental toxicity safety assessments.