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Workshop Report

Toward a comparative retrospective analysis of rat and rabbit developmental toxicity studies for pharmaceutical compounds

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ABSTRACT

Based on a proposal made at the ICH Workshop in Tallinn, Estonia (2010), the value of the rabbit embryo-fetal development (EFD) *versus* the rodent EFD was examined by the HESI DART group. A cross-industry data survey provided anonymised EFD and toxicokinetic data from EFD studies on over 400 marketed and unmarketed drugs (over 800 studies) that were entered by experts at RIVM into US EPA's ToxRefDB style database. The nature and severity of findings at the lowest observed adverse effect level (LOAEL) are being reviewed to quantitate the frequency with which lesser signs of embryo-fetal effects (*e.g.*, delays in ossification, minor changes in frequency of variants) are driving the LOAELs. Interpretation was based on exposure rather than administered dose. This paper provides an update of this ongoing project as discussed during a workshop of the European Teratology Society in Ispra, Italy (2013). This was the first presentation of the initial data set, allowing debate on future directions, to provide a better understanding of the implications of either delaying a rabbit EFD or waiving the need in particular circumstances.

1. Introduction – Aldert Piersma

For more than three decades, rat and rabbit embryo-fetal development studies have been the gold standard for regulatory human hazard assessment of possible developmental effects of industrial chemicals, pesticides, and pharmaceuticals. For small molecule pharmaceuticals, studies in both a rodent and non-rodent species are the default [1]. The paradigm of testing developmental toxicity in two species can be historically traced back to the thalidomide tragedy. The limb reduction defects that were observed in newborn children after thalidomide use in pregnancy could be reproduced to some extent in rabbits but not in rats [2,3]. It was concluded that the rabbit might in some cases be a better predictor of human developmental toxicity than the rat, although the rat was generally considered the more popular and more practical species for developmental toxicity testing. Current knowledge indicates that the specificity of developmental toxicity manifestations may vary widely between species and even between strains of the same species, as many rat developmental toxicity studies have clearly shown [4–6]. In

Abbreviations: AUC, area under the curve; CBG-MEB, Medicines Evaluation Board in the Netherlands; DART, Developmental and Reproductive Toxicology; EFD, embryo-fetal development; EMA, European Medicines Agency; ECVAM, European Center for Validation of Alternative Methods; HESI, Health and Environmental Sciences Institute; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ILSI, International Life Sciences Institute; ITS, integrated testing strategy; JACVAM, Japanese Center for the Validation of Alternative Methods; LOAEL, lowest observed adverse effect level; mEST, mouse embryonic stem cell test; MM, micromass test; NOAEL, no observed adverse effect level; ToxRefDB, Toxicology Reference Database; VICH, International Conference on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products; WOCBP, women of child-bearing potential.

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addition, thalidomide caused fetal death in the rat at doses similar to those causing limb malformations in the rabbit [4]. According to current standards of hazard identification, and dependent on expert judgment, these diverse effects in rat and rabbit developmental toxicity studies with thalidomide might be interpreted to indicate the same level of human hazard. This notion leads to the more general question of whether both rat and rabbit studies should be required by default for the hazard assessment of all pharmaceutical compounds. Different alternative scenarios can be envisaged. For instance, a preferred first species might be designated (perhaps selected considering prior knowledge on all characteristics of the compound under study), with the second species only tested dependent on the outcome in the first species tested, similar to the V-ICH-approved approach to testing of veterinary pharmaceuticals for food producing animals [7]. The wealth of existing knowledge on the developmental toxicity of chemicals and pharmaceuticals enables a retrospective analysis that could teach us how to optimize the testing strategy. Not only could this potentially save significant time, resources and experimental animals, it may also lead to an improved understanding of the relative responses of rat and rabbit and their relevance for human hazard identification.

Earlier comparative studies of rat and rabbit developmental toxicity studies have been carried out in the realm of chemicals and pesticides [4–7]. The general picture emerging from those data is that in the majority of cases the second species does not add significantly to the overall judgment on developmental hazard. The lowest observed adverse effect level (LOAEL) for developmental toxicity usually differs less than a factor of 10 between rat and rabbit, which can be assumed to represent general biological variation. Thus, in retrospect, the second species developmental toxicity study might often have been omitted. However, a low but significant number of cases show that either species may be insensitive in the presence of the other species showing significant developmental toxicity. In addition, the interpretation of rat *versus* rabbit developmental toxicity is complicated by maternal toxicity. The interpretation of developmental effects in the presence of maternal toxicity is particularly subject to expert judgment [8]. In view of this complexity, any proposal on changing regulatory requirements as to developmental toxicity testing in experimental animals should be preceded by a thorough retrospective analysis of the existing data of the many hundreds of compounds for which developmental toxicity has been evaluated in both rat and rabbit. Currently, an International Life Sciences Institute-Health and Environmental Sciences Institute (ILSI-HESI) Developmental and Reproductive Toxicology (DART) Technical Committee-sponsored project is ongoing, aiming at generating and analyzing a database of developmental toxicity studies of pharmaceuticals in rat and rabbit. Regulatory authorities, research institutes, and industry work together globally toward a consensus analysis that may provide a solid basis for contemplating optimization of testing strategies, improving interpretation of specific findings, and refining extrapolation to human hazard identification of developmental toxicity.

One important aspect of this type of project is transparency, allowing all relevant stakeholders to participate and give input, which may range from providing basic data to discussion of interpretation and extrapolation. The 2013 European Teratology Society Conference, held in Stresa, Italy, kindly hosted a workshop on this subject. Multiple stakeholders provided active contributions, generating an overview of objectives and approaches as briefly reproduced in the following sections. The project was introduced and placed into various contexts, and ongoing practical work was reviewed with some preliminary data for discussion. The workshop and presentations received enthusiastic and constructive feedback, indicating the general interest in the subject among a wide spectrum of stakeholders in the area.

2. ICH origins of the project – Jane Stewart

In June 2010, at the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in Tallinn, Estonia, there was a workshop entitled “Brainstorm Reproductive Toxicity: implementation of *in vitro* assays” [9]. University based scientists involved in EU funded “ReProTect” programmes were invited to describe the progress made with various *in vitro* assays to detect different reproductive toxicity endpoints. Representatives from ECVAM, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the Japanese Center for the Validation of Alternative Methods (JACVAM) were present to contribute from the perspective of validation of alternative testing approaches. In addition, invited speakers from the pharmaceutical industry (Pfizer and AstraZeneca) provided an update on the ongoing efforts to use non-mammalian systems to detect developmental toxicants and general efforts to reduce the number of animals required for reproductive toxicity testing.

At the same 2010 ICH meeting, the ICH S6 expert working group on Biotechnology-derived Proteins agreed that single species embryo-fetal development (EFD) testing would be acceptable for biotechnology-derived pharmaceuticals where only non-human primates were of relevance and it was agreed that Women of child bearing potential (WOCBP) could be entered on Phase III trials for those biopharmaceuticals without having finalized the embryo-fetal development toxicity testing. It was noteworthy that the 2010 ICH “Brainstorm” workshop followed the revision of the ICH M3 guideline in 2009, which provided a harmonised position on two points. (1) WOCBP could be entered on clinical trials of limited scope & duration based on small scale “preliminary” EFD studies in mammals provided those women were using highly effective contraception. (2) WOCBP could be entered on Phase III trials with monoclonal antibodies (mAb), for which embryo-fetal exposure during organogenesis is understood to be low in humans, without having finalized EFD testing of that mAb [10].

The above “freedoms” described in ICH M3(R2) were granted because of the increased confidence in the ability to minimize the number of pregnancies on clinical trials by effective use of modern contraceptive measures. This provided the impetus for the AstraZeneca speaker at the “Brainstorm” workshop to present an “alternative to alternatives” challenge. The second species (typically the rabbit) was often being used in EFD testing with little understanding of its pharmacological or drug metabolism relevance for humans. Therefore, the use of animals for developmental toxicity testing could substantively be reduced simply by postponing EFD testing until such times as there was reasonable proof that the candidate drug was likely to succeed.

In particular the following challenge was laid down at that ICH workshop: where rat main EFD data exist prior to Phase III AND (i) rat has been shown to be a pharmacologically relevant species, AND (ii) exposures in the rat are considered adequate, AND (iii) Phase III clinical trial can maintain effective contraception, what impact would delaying main EFD study of the (rabbit) 2nd species have on risk assessment and informed consent for the Phase III trials? This was incorporated in a subsequent proposal to base the end-of-phase II decision only upon a single species in combination with the *in vitro* data [9,10].

It is recognized that in the general population, many pregnancies are unplanned and that factors such as age, ethnicity, education and income affect those rates [11]. Those risk factors are independent of whether the female patient on a Phase III trial is receiving a novel (generally short half-life) small molecule or a novel (often long half-life) mAb. A pregnancy while on a clinical trial will invariably result in the women being excluded from the trial and, therefore, she may not receive the intended medical benefit from that trial. Pertinent