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The extended 1-generation study (OECD 415), as a replacement of the mammalian 2-generation study (OECD 416)

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Abstract
The new EU chemicals policy REACH proposes that 30.000 existing chemicals should be evaluated within a period of 15 years. In Europe the regulatory testing requirements for chemicals depend on the production volume and include two types of reproductive toxicity test: a developmental toxicity study, and a 1-generation or 2-generation fertility study. Under the REACH legislation, these studies have to be conducted for the 10.000 chemicals with an annual production volume of more than 10 tonnes.

The 2-generation study (OECD Test Guideline (TG) 416) is a general test which allows evaluation of the effects of the test substance on the complete reproductive cycle. It has conventionally been preferred to the 1-generation study (OECD TG 415) because the latter does not test for potential effects on all phases of the reproductive cycle. The ILSI Agricultural Chemical Safety Assessment Project has proposed an extended 1-generation study (Cooper et al., 2006). This flexible study addresses the main limitation of OECD TG 415 by incorporating additional post-natal evaluations, e.g. functional observations and using an extended F1 generation dosing period (to PND day 70) to address developmental neurotoxicity. To reduce reproductive toxicity testing under REACH, we proposed in to limit fertility testing to an extended 1-generation study (Spielmann and Vogel, 2006). This approach is supported by a recent retrospective analysis of 176 several generation studies conducted by RIVM, the National Health Authority of the Netherlands, which proved that the 2-generation study had no impact on the ensuing risk assessment nor on classification and labelling (Janer et al., 2007).

Keywords: reproductive toxicity, developmental toxicity, OECD Test Guideline, REACH

Introduction

In the new European Union (EU) policy for the Registration, Evaluation and Authorisation of Chemicals (REACH), the European Commission (EC) proposes that 30,000 existing chemicals should be evaluated within a period of 15 years. Data on the hazardous properties of chemicals must be provided by industry, which must also cover the costs, if additional testing is required. There is agreement within the EC that additional toxicity testing should in the first place rely on non-animal *in silico* predictions and *in vitro* tests, for both financial and animal welfare reasons. To put the REACH policy into a realistic perspective, advisers to national governments of the EU Member States, from the EU Commission and industry have published estimates on the cost of the additional testing which is likely to be necessary. Assuming a worst case scenario, in which all the existing chemicals will have to be tested *in vivo* for all the required endpoints, more than 50 million test animals may have to be used, and reproductive toxicology will account for up to 70% of them (Pedersen et al, 2003).

The REACH system represents an attempt to increase safety from the effects of chemicals, both for humans and for the environment, by filling gaps of knowledge on the toxic properties of new chemicals and existing chemicals, i.e. those in use before 1982, when the current regulations for new chemicals came into force. For historical reasons, the spectrum of reproductive toxicity testing is markedly different for drugs and for chemicals. In drug development, during preclinical testing, "segment 1, 2 and 3" studies have to be conducted, which cover pregnancy, as well as pre-natal and post-natal toxicity, and also the lactation period. In contrast, the regulatory testing requirements for industrial chemicals are depend on the production volume. They include two types of

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reproductive toxicity test: a developmental toxicity study, and a 1-generation or 2-generation "fertility" study. Under the proposed REACH legislation, these studies will have to be conducted for an estimated 10,000 chemicals with an annual production volume of more than 10 tonnes.

From the animal welfare perspective, and also from a scientific and economical standpoint, we must ask whether the world would really be a safer place after 2-generation studies had been conducted for 30% of the existing chemicals (Spielmann and Vogel, 2006). We have evaluated the arguments carefully and propose conducting an extended 1-generation study according to the recommendations of the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI) (Cooper et al., 2006) rather than following the current REACH policy. To facilitate this approach in 2007 Germany and the US EPA - and more recently the Netherlands - have submitted to the OECD Test Guidelines Program a proposal to update the current OECD TG 415 by including well defined developmental endpoints in an "Extended F1 Generation Study". The background information for this proposal will be outlined in the present report.

Scientific weight of evidence evaluation of the 1- and 2-generation fertility studies

In order to evaluate the contribution provided by 2-generation studies to the assessment of the safety of chemicals, the following evidence has to be taken into account:

1. The predictive value of reproductive tests in animals tests for human pregnancy is poor, e.g. according to a recent analysis by Bailey et al. (2005).
2. Moreover, only around 1% of the known animal teratogens are also active in humans and there is no substantial evidence to prove a correlation between the adverse effects observed in two-generation studies in rodents and human reproduction and fertility (Shepard and Lemire, 2004).
3. Epidemiological data on the outcome of human pregnancy for women, who have been exposed to specific drugs, shows that only 2% of malformations can be attributed to drugs and other chemicals. Thus, with a few exceptions, the human embryo seems to be more resistant to toxic chemicals than the embryos of laboratory animals (Schaefer et al., 2006).
4. It must be borne in mind that the chemicals used in drugs and pesticides are designed to interact with active molecules at the cellular level in the human body and in the pest species, respectively. In contrast, industrial chemicals are designed to improve the functions of chemical products for quite different purposes, often as a result of their physical properties, rather than their chemical reactivity. Thus, in contrast to drugs and pesticides, a large proportion of industrial chemicals are not toxic to mammals and do not interfere with human reproduction.
5. This assumption is substantiated by experience obtained with more than 3000 new chemicals in the EU during the past 20 years, since only a very few of these chemicals have been classified and labelled as toxic to reproduction.
6. When the two-generation study (OECD TG 416) was introduced as an OECD Test Guideline in 1982, the test was not formally validated with respect to its relevance for human reproduction and fertility.
7. In a literature survey of 117 two-generation studies on pharmaceutical agents, there were only two cases of specific effects being seen in the second generation (Ulbrich and Palmer, 1995). In the ILSI ACSA Project it was found that, in more than 350 2-generation studies on pesticide chemicals, there were only two exceptions to the conclusion that an F2 generation was not necessary (Cooper et al., 2006).
8. The National Health Authority of the Netherlands (RIVM) conducted a retrospective study on 176 multi-generation studies that had been submitted to authorities both in Europe and the USA to assess potential differences between the first and the second generation (Janer et al., 2007). In the 2-generation studies the effects reported in the second generation affected neither the overall NOAEL nor the critical effects. It was, therefore, concluded that the 2-genration study had no impact on risk assessment, nor on classification and labelling. However, several substances did show an increased sensitivity of the F1 adults in comparison to the parent generation.

Thus, while reproductive toxicology seems to have the greatest implications within the REACH policy in terms of the economic cost of testing and the numbers of animals that will have to be used, it certainly does not deserve this high priority when viewed from a scientific perspective.

Establishing an updated OECD TG 415 (extended 1-generation study)

The reproduction study proposed by the ILSI ACSA Committee is an *extended* 1-generation study (Cooper et al., 2006). This study uses a comprehensive range of endpoints to detect abnormalities of reproductive function and sexual development, and it specifies that larger subsets (three per sex per litter) of F1 offspring be maintained and dosed post-weaning. This allows an assessment of offspring maturation (including the

timing of sexual development), along with limited evaluations of delayed or latent manifestations of some toxicities, and of additive effects that may be related to the amount of time over which exposure has occurred. Moreover, there is the option of continuing dosing one of the three subsets of F1 offspring and allowing them to mate to produce second generation litters. The triggers for the extension of the study to a second-generation are (1) an adverse effect on fertility or fecundity of the parental generation, (2) indications of abnormal sexual development of the F1 pups, or (3) deaths or evidence of toxicity to the F1 pups pre-weaning. Because the evaluation of the reproductive system includes multiple sensitive structural, functional, and endocrine-mediated components, it is likely that any critical effect on reproduction will be detected, thereby triggering a second mating phase. Equivocal effects on these parameters or unusual control data compared to historical background may also trigger a second generation.

Taking into account these recommendations and also the results obtained in the retrospective study conducted at the RIVM in the Netherlands (Janer et al., 2007) the OECD WNT (Working Group of National Coordinators of the Test Guidelines Program) discussed the need to update the existing OECD TG 415. To achieve this goal the USA and Germany submitted two SPSFs (Standard Project Submission Forms) entitled "New mammalian level 5 test involving various life stages" (US EPA) and "Update of the 1-generation study OECD 415" (Federal Institute for Risk Assessment (BfR), Germany). It was agreed that a single project will be included in the workplan of the OECD Test Guidelines Program and that the USA and Germany will submit a single, common SPSF. The updated SPSF recommends that in a first step the TG for the "extended 1-generation study" should be revised according to the proposal received from Germany. An OECD Expert Group was formed to reach agreement on all major elements of the revised version of this OECD TG in October 2007. In a second step the inclusion of extensions as proposed by the USA will be elaborated.

The new guideline for 1-generation reproduction testing is designed as a basic test to provide general information on the effects of a test substance on the integrity and performance of the male and female reproductive systems, including gonadal function, the oestrus cycle, mating behaviour, conception, pregnancy, parturition, lactation, and weaning, and the growth and development of the offspring up to day 70 pp. The study may also provide information on effects of a test substance on neonatal morbidity and mortality, preliminary data on prenatal and postnatal developmental toxicity and serve as a guide for subsequent tests. For further information on developmental toxicity and functional deficiencies, additional study extensions can be incorporated into this protocol. They will be elaborated in a second module of this guideline.

Thus, the 1-generation reproduction toxicity study will provide information on the effects of repeated exposure to a substance during all phases of the reproductive cycle. In particular, the study provides information on the reproductive parameters, and on development, growth and survival of offspring up to day 70 pp. The results of the study should be interpreted in conjunction with the findings of subchronic, prenatal developmental and toxicokinetic and other available studies. The results of this study can often be used in assessing the need for further testing of a chemical. Extrapolation of the results of the study to man is valid to a limited degree and may be improved if comparative data on metabolism and mechanisms of toxicity can be incorporated. They are best used to provide information on no-effect-levels and permissible human exposure.

The way forward

The result of our critical evaluation of the current limitations of the 2-generation reproductive toxicity study supports the substitution of the current 2-generation test by an extended 1-generation study following the F1 generation until sexual maturity. The ILSI/HESI ACSA has put forward a realistic proposal for an extended 1-generation study in which parental males will be treated for 4 weeks and females for 2 weeks before mating, during mating, and for females during gestation and lactation. Selected F1 generation pups will be treated continuously until PND 70 and the same observations as in a 2-generation study will be performed with additional endpoints including developmental neurotoxicity and developmental immunotoxicity. In addition, the extension towards a second generation could be justified when doubtful effects are observed in the first generation or when this extension is designed to obtain data on mechanisms of action. Moreover, an extended 1-generation study would include a more comprehensive evaluation of first generation, especially of F1 adults, than the current 2-generation test. Therefore, when clear effects are already observed in the first generation, it is most unlikely that the extension towards a second generation would provide any additional information.

The validity of the new approach is currently undergoing experimental validation in a joint project of the European crop protection industry and the US EPA. The results will definitely have an impact on the proposed update version of OECD TG 415 "Extended 1-generation study" currently discussed by the National Coordinators of the OECD TG Program. As soon as agreement is reached at the OECD level, the

new OECD TG 415 "Extended 1-generation study" should be used when testing according to European REACH policy rather than the current OECD TG 416 "2-generation study". This will both reduce the cost of testing existing chemicals according to REACH and significantly reduce animal numbers used in regulatory toxicity testing without reducing the safety of chemicals to humans and the environment.

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