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The challenge of reproductive and developmental toxicology under REACH

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ABSTRACT

The European Union's REACH regulation has explicit requirements for reproductive and developmental toxicity data on all substances manufactured in or imported into the European Union at ⩾10 metric tons/year. Meeting the data requirements with whole-animal testing could result in the use of almost 22 million vertebrate animals for the registration of existing chemicals and cost up to several hundred thousand dollars per registered substance. The requirement for financial and animal resources can be reduced by the use of in vitro testing, quantitative structure–activity relationship models, and grouping of related substances. Although REACH strongly encourages these methods of avoiding vertebrate animal testing, it does not appear that in vitro testing or quantitative structure–activity relationship analysis will be able to replace whole-animal reproductive and developmental toxicity testing. Grouping of related compounds offers the possibility, perhaps in conjunction with in vitro testing and structure–activity analysis, of reducing vertebrate animal testing provided there is sufficient information on the related compounds and sufficient reason to believe that the related compounds will have similar toxicological properties. The designation of a substance as a reproductive or developmental toxicant follows criteria that do not consider the dose level of the substance at which reproductive or developmental effects occur, as long as excessive generalized toxicity does not occur. This method of labeling substances without consideration of effective dose level does not provide information on the actual risk of the chemical. Designation of a substance as a reproductive or developmental toxicant may have important implications under REACH and can be expected to result in the need to obtain authorization for marketing of the substance in the European Union.

1. Introduction

The European Union has enacted a new chemical regulation called REACH (Registration, Evaluation, and Authorization of Chemicals), which promises to be the most complex and comprehensive regulatory effort ever instituted. Within this law, the requirements for reproductive and developmental toxicology are particularly important, because they may result in the highest requirement for funding and for experimental animals. In addition, reproductive and developmental considerations may result in the restriction of many substances that are now in widespread use. Although REACH has what appear to be stringent requirements for experimental animal studies, the law discourages the use of vertebrate animals in testing, requiring registrants to consider alternative methods of filling data gaps. As will be discussed here, the use of alternatives to experimental animal studies for reproductive and developmental toxicity endpoints may be problematic.

2. What does REACH require?

2.1. Experimental animal test data

Under the new law, all substances manufactured in or imported into the European Union at ⩾1 metric ton/year must be registered, excluding some substances such as pharmaceuticals and pesticides that are regulated under other laws. The term, "substance," is defined as, "A chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition" (EChA, 2007). Registrations for substances already on the European Inventory of Existing Chemical Substances (EINECS) will be phased in between 2010 and 2018, depending on the volume of the substance being manufactured in or imported into the European Union.

One of the goals of REACH is to remove differences in the data bases available for the older "existing" EINECS substances, which were marketed in Europe before 1981, and newer substances, which were subject to more stringent data requirements. There

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are approximately 100,000 existing EINECS substances of which about 30,000 are expected to be registered under REACH. At least 10,000 will be registered in the ⩾10 metric ton/year volume band (Risk and Policy Analysts Limited, 2002), the band at which reproductive and developmental toxicity testing requirements begin. A similar or larger number of intermediates may need to be registered by manufacturers in the European Union.

The reproductive and developmental toxicology data requirements of REACH are summarized in Table 1. At the ⩾10 metric ton/year band, the required whole-animal (species unspecified) test data include results from either OECD Test Guideline 421 (Reproductive/developmental toxicity screening test) or OECD Test Guideline 422 (Combined repeated dose toxicity study with reproductive/developmental component) (OECD, 1995a,b). These protocols involve mating at least 10 animals of each sex per dose group in order to obtain at least 8 pregnant females per group. At least 3 dose levels and a control are recommended. Dosing begins at least 2 weeks before mating and is continued in females until postpartum day 3. Dams and pups are killed on postpartum day 4. Sires are dosed for at least 28 days before mating. Endpoints include fertility, gestation length, parental and pup weights, number of corpora lutea, litter size, external evaluation of pups, and macroscopic appearance of the male genital tract, which is preserved for histologic evaluation. The main difference between OECD Test Guideline 421 and 422 is the evaluation of neurologic, biochemical, and immunological endpoints in OECD Test Guideline 422, making it a combined screening test for reproductive and non-reproductive toxicity.

The highest two tonnage bands (100 and 1000 metric tons/year) require data from an OECD Test Guideline 414 prenatal developmental toxicity test (OECD, 2001a). This test uses at least 20 animals per dose group to achieve at least 16 pregnant females per dose group. At least 3 dose groups plus a control are used. Dosing begins around implantation, 5 days after coitus, and continues until 1 day prior to cesarean section. Fetuses are removed about 1 day before anticipated delivery and evaluated for external, visceral, and skeletal abnormalities. Other endpoints include litter size and weight, maternal weight and food consumption, number of corpora lutea and implantations, and offspring sex ratio.

The two-generation reproductive toxicity information required under REACH is not identified by OECD test guideline number, but corresponds to OECD Test Guideline 416 (OECD, 2001b). This test uses sufficient numbers of animals to ensure that at least 20 pregnant animals are available for evaluation at the end of pregnancy. At least 3 dose levels and a control are used. Parental animals are dosed for at least 10 weeks prior to mating. Dosing in females is continued through pregnancy and lactation. F1 pups are dosed from weaning and for at least 10 weeks prior to mating. F1 non-sibling animals are paired within dose groups to produce an F2 generation, which is killed after weaning. Endpoints include food consumption, parental, litter, and pup body weights, estrous cycle observations, fertility, gestational length, number of implantations and corpora lutea, litter size, gross abnormalities of pups, and attainment of postnatal developmental milestones.

There has been interest in replacing the two-generation study with a one-generation study, which is described in OECD Test Guideline 415 (OECD, 1983). An evaluation of two-generation study results showed little advantage of adding the second generation (Janer et al., 2007). There were effects on some adult F1 offspring that were not seen in the parental generation, leading to the proposal for an extended one-generation in which F1 offspring are followed to adulthood. This proposal is under consideration as a possible alternative to the two-generation reproductive data currently indicated in REACH (ECB, 2007b).

2.2. Avoidance of experimental animal testing

Although REACH is very specific about the requirements for experimental animal test data, the law discourages testing in vertebrate animals. Article 13 of REACH states, "In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure–activity relationship models or from information from structurally related substances (grouping or read-across)." Article 25 states, "In order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort".

Table 1
Reproductive and developmental toxicology testing requirements of REACH

Tonnage band, metric tons/year	Requirements	Exceptions
⩾10	Screening tests (OECD Test Guideline 421 or 422) in one species, or estimates based on structurally related substances, quantitative structure–activity relationships, or in vitro testing that the substance is developmentally toxic	Availability of prenatal developmental toxicity study or a 2-generation reproductive study Known genotoxic carcinogen or germ cell mutagen with appropriate risk management measures in place Classification as a reproductive or developmental toxicant (R60 or R62)
⩾100	Prenatal developmental toxicity study (OECD Test Guideline 414) in 1 or 2 species Two-generation reproductive toxicity study in 1 species if 28-day or 90-day study indicates adverse effects on reproductive organs	Known genotoxic carcinogen or germ cell mutagen with appropriate risk management measures in place Classification as a reproductive or developmental toxicant (R60 or R62) Studies need not be done if substance has low toxicological activity, is not systemically absorbed, and there is no significant human exposure
⩾1000	Prenatal developmental toxicity study (OECD Test Guideline 414) in 1 or 2 species Two-generation reproductive toxicity study in 1 species	Known genotoxic carcinogen or germ cell mutagen with appropriate risk management measures in place Classification as a reproductive or developmental toxicant (R60 or R62) Studies need not be done if substance has low toxicological activity, is not systemically absorbed, and there is no significant human exposure

R60 and R62 are defined in Section 5 of this paper.
From Annexes VIII, IX, and IX of REACH, available at http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_396/l_39620061230en00010849.pdf.