29 Developmental and reproductive toxicity evaluation under the European Union REACH regulations

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INTRODUCTION

On June 1, 2007, a new kind of a chemical regulation came into effect in the European Union. This law created a scheme called Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH). The provisions of REACH are administered by the European Chemicals Agency (EChA), which operates an extensive web site on REACH and its attendant requirements and guidances (http://echa.europa.eu). Hand-in-hand with REACH, the European Union instituted a new classification scheme effective January 20, 2009. REACH and the classification scheme have important commercial implications, because they apply to all substances, mixtures, and articles manufactured in or imported into the European Union.

Under REACH, a "substance" is defined as a chemical element and its compounds. A preparation is a product containing two or more substances, and an article is a product the physical form of which is more important to the function than is its chemical composition. A toy would be an example of an article. Given the global nature of commerce, most nonpharmaceutical products will be subjected to REACH unless they are manufactured or imported at volumes under 10 metric tons/year. Some nations outside the European Union have developed or are developing REACH-like legislations, increasing the impact of this kind of a regulatory scheme. China, for example, has enacted Provisions on the Environmental Administration of New Chemical Substances (PEANCS), which has REACH-like features.

WHAT HAPPENS UNDER REACH?

REACH requires that all substances, preparations, and articles be registered through the submission of a dossier. The submission is made to a publicly accessible web site (http://apps.echa.europa.eu/registered/registered-sub.aspx), using a standardized format (Table 29.1). Evaluation of the substance, preparation, or article for health and environmental risk is the responsibility of the registrant, not EChA, which will review only a sample of the dossiers.

Authorization or restriction of the substance, mixture, or article occurs according to criteria set forward in the law and its guidances. Restriction, for example, may be triggered by toxicity to reproduction, as discussed in more detail below. The computer-based submission document can alert EChA to incomplete entries or entries requiring special attention.

At this writing, the registration process is focused on substances that were previously registered in the European Union on the European Inventory of Existing Commercial Chemical Substances (EINECS). The timetable for registration depends on the amount of the substance manufactured in or imported into the European Union annually. The tonnage "bands" and deadlines for registration are ≥1000 metric tons (November 30, 2010), 100–1000 metric tons/year (May 31, 2013), and 1–100 metric tons/year (May 31, 2018).

Special Features of REACH and the Classification Scheme

The data requirements under REACH are specific for each tonnage band. The larger the volume of a substance manufactured in or imported into the European Union, the more detailed and complete must be the data set submitted for registration. The anticipated exposure level of human beings is considered in the evaluation of the substance, but exposure level does not determine which studies are required for registration.

In addition to the specific requirements for REACH, there are classification and labeling requirements. The new classification scheme is a variation on a hazard-based classification system that had been in use in the European Union for many years. The new features of the system made it compatible with the United Nations Globally Harmonized system. Classification was at one time a labeling device calling attention to hazards such as flammability. The extension of this system to toxicology end points, such as reproductive and developmental toxicity, results in labeling some substances as reproductive or developmental toxicants, irrespective of exposure levels.

Classification and labeling requirements are not linked to the tonnage band and are not strictly a part of REACH, but classification can have an impact on the evaluation and possibly the restriction of substances under REACH. Substances identified as carcinogenic, mutagenic, or toxic to reproduction are called CMR substances and may be considered to be of "very high concern" under REACH, a category discussed in more detail below. CMR substances must be registered according to the schedule for the highest tonnage band as long as the substance is manufactured or imported in quantities of at least 1 metric ton/yr.

As part of the characterization of a substance identified as a hazard, the registration dossier often must include information on exposure of downstream users of products containing the substance. In other words, a company that sells a chemical in the European Union may need to obtain manufacturing and use information from its customers and from their customers to estimate exposure to the chemical from the time of its creation or importation until its ultimate disposal. The responsibility for evaluating exposures all the way down the

Table 29.1 Format of the REACH submission

Section	Title	Content
1	General substance information	Composition, CAS number, REACH registration number, analytical information
2	Classification and labeling	Globally Harmonized System, that is, Safety Data Sheets
3	Manufacture, use, and exposure	Manufacturing methods, production quantities, import and use, production sites, exposure scenarios, waste production
4	Physical and chemical properties	Melting point, boiling point, vapor pressure, density
5	Environmental fate and pathways	Stability, biodegradability, transport and distribution, bioaccumulation
6	Ecotoxicologic information	Endpoint data on aquatic, sedimentation and terrestrial toxicity, biological effects, degradation products
7	Toxicologic information	Endpoint data on carcinogenicity, toxicokinetics, mutagenicity, dose dependencies
8	Analytical methods	Differing matrixes or media
9	Residues in foodstuffs	Primarily for pesticides, biocides
10	Target organisms	Intended for biocides
11	Safe use guidance	First aid, handling and storage
12	Literature search	Citations
13	Assessment reports	Attachments, that is, study summaries

Abbreviation: REACH, Registration, Evaluation, Authorization, and Restriction of Chemicals.

supply chain imposes substantial burdens on manufacturers and importers, and compliance with the law may require the release of confidential business information from downstream manufacturers and distributors concerning manufacturing practices and formulation recipes.

The Substance Information Exchange Forum

REACH contains explicit language about the avoidance of testing in vertebrate animals, discussed in more detail below. In order to avoid duplication of experimental animal testing, REACH requires data sharing among registrants of the same substance if the data were obtained in vertebrate animal studies. The usual mechanism for sharing data is the Substance Information Exchange Forum (SIEF), a group consisting of all registrants of a given substance. The Guidance on Registration (1) says:

> With respect to data sharing, data must be shared for the same substance in the case of information involving tests on vertebrate animals. . . Information not involving tests on vertebrate animals must be shared if requested by a potential registrant of the same substance. The data sharing mechanisms aim to ensure that sharing of studies [that] are already available and of their related costs is agreed amongst potential registrants in a fair, transparent, and nondiscriminatory way. Importantly, in the case of lacking data, the aim of the sharing mechanism is for potential registrants of the same substance to agree who will undertake the necessary data collection to ensure that the test is carried out only once.

The SIEF system requires cooperation among registrants, many of whom are competitors. Typically, one entity becomes the lead registrant, with primary responsibility for gathering and presenting the data. Other SIEF members may contribute data or expertise. Funding of SIEF activities and payment of data owners for the use of data in registration must be worked out within the SIEF. EChA does not provide or enforce rules for the SIEFs.

Reproductive and Developmental Toxicity Testing

The testing required for reproductive and developmental effects required under REACH depends on the annual tonnage band. The requirements are presented in Table 29.2. Some of the exceptions noted in Table 29.2 rely on the classification scheme, which is presented in Table 29.3.

In the lowest tonnage band, REACH calls for data from a whole animal test (species unspecified) to include either Organisation for Economic Cooperation and Development (OECD) Test Guideline 421 (Reproductive/developmental toxicity screening test) or OECD Test Guideline 422 (combined repeated dose toxicity study with reproductive/developmental component) (4,5). These protocols include the mating of at least 10 animals of each sex per dosage group to obtain at least 8 pregnant females per group. At least 3 dose levels and a control are used. Dosing begins at least 2 weeks before mating and continues in adult females until postpartum day 3. Adult males are dosed for at least 28 days before mating. Dams and pups are evaluated on postpartum day 4 for end points including 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 saved for histologic evaluation. OECD Test Guideline 422 also includes the evaluation of neurologic, biochemical, and immunologic end points.

At ≥100 and ≥1000 metric tons/yr, REACH calls for an OECD Test Guideline 414 prenatal developmental toxicity test (6). This protocol requires at least 16 pregnant females per dosage group. There are at least three dosage groups plus a control group. Dosing begins about five days after coitus, around implantation, and is continued until just prior to cesarean section, about one day before anticipated delivery. Fetuses are evaluated for

Table 29.2 Reproductive and Developmental Toxicology Testing Under 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 toxicity 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- 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 Table 29.3.
Abbreviation: OECD, Organisation for Economic Cooperation and Development.
From Annexes VIII, IX, and X of REACH (2).

Table 29.3 Reproductive and developmental toxicity classification

Code	Designation	Criteria
R60	May impair fertility	Substance impairs fertility or should be regarded as if it impairs fertility in humans (data from epidemiology studies or from well conducted experimental animal studies in which the reproductive effects do not appear to be due to generalized toxicity, poor animal husbandry, infection, or nutritional deficiencies)
R61	May cause harm to the unborn child	Substance causes developmental toxicity or should be regarded as if it causes developmental toxicity in humans (data from epidemiology studies or from well-conducted experimental animal studies in which the developmental effects do not appear to be due to generalized toxicity, poor animal husbandry, infection, or nutritional deficiencies).
R62	Possible risk of impaired fertility	Substance causes concern for human fertility (data from experimental animal studies with design deficiencies or in which the reproductive effects may be due to generalized toxicity, poor animal husbandry, infection, or nutritional deficiencies).
R63	Possible risk of harm to the unborn child	Substance causes concern for humans owing to possible developmental toxic effects (data from experimental animal studies with design deficiencies or in which the developmental effects may be due to generalized toxicity, poor animal husbandry, infection, or nutritional deficiencies).
R64	May cause harm to breastfed babies	Absorbed by women, may interfere with lactation or may be present in milk in amounts sufficient to cause concern for the health of the child.

Source: From Ref. 3.

external, visceral, and skeletal abnormalities, and information is collected on other end points (litter size and weight, maternal weight and food consumption, number of corpora lutea and implantations, and offspring sex ratio).

REACH also requires a two-generation reproductive toxicity study for the higher two tonnage bands. Although the protocol is not identified by the OECD test guideline number in the legislation, this testing requirement is satisfied by OECD Test Guideline 416 (7). This protocol requires 20 pregnant animals in each dosage group, including at least three dose levels plus a control group. Adults are dosed for at least 10 weeks prior to mating, and dosing of females is continued through pregnancy and lactation. Direct dosing of F_1 pups begins at weaning and is continued for at least 10 weeks prior to mating. Nonsibling F_1 animals are mated within dosage groups to produce an F_2 generation, which is evaluated after weaning.

Study end points include food consumption, parental, litter, and pup body weights, estrous cycle observations, fertility, gestational length, numbers of implantations and corpora lutea, litter size, gross abnormalities of pups, and attainment of postnatal developmental milestones.

Although REACH indicates the need for a two-generation study at the higher tonnage bands, it may be possible to replace this study with a one-generation study as described in OECD Test Guideline 415 (8). Little additional information appears to be gained from adding the second generation (9). The effects on some adult F_1 offspring may be identified that are not noted in the parental generation; therefore, it has been proposed that a reasonable substitute for the two-generation study is an extended one-generation study, in which F_1 offspring are followed to adulthood. Additional F_1 end points could include clinical pathology, a functional observation battery, immunotoxicity end points, estrous cyclicity, semen analysis, and neurobehavioral end points. At this writing, this proposal is under consideration as a possible alternative to the two-generation reproductive test currently required by REACH (10).

Substances of Very High Concern

REACH calls for a listing of substances of very high concern (SVHC), to include chemicals known to be carcinogenic, mutagenic, or toxic to reproduction (essentially R60 and R61; see Table 29.3). The SVHC list as of June 2011, appears in Table 29.4.

The placing of a chemical on the SVHC list does not require the conditions of human exposure to be considered in the studies that were used to list the chemical. For example, an experimental animal study that shows reproductive toxicity at an exposure level four orders of magnitude higher than the anticipated human exposure levels may be sufficient to list the chemical, as long as the reproductive effect in the experimental study is not believed to be because of generalized toxicity, poor husbandry, infection, or inadequate nutrition.

Chemicals on the SVHC list must be authorized before marketing. Authorization of these chemicals requires a showing that there is not a less toxic substance that can be used in place of the SVHC chemical and that the marketing of the SVHC chemical entails a benefit to the public that offsets the risk. Authorization, if granted, may be restricted to certain conditions, and a risk management plan will be required. The manufacturer or importer of any article that contains >0.1% of an SVHC must notify customers if the amount of the SVHC being introduced into the European Union in the article is ≥1 metric ton/yr.

Restrictions on Experimental Animal Testing

REACH appears to require a great deal of experimental animal testing. The reproductive and developmental toxicity testing protocols require more animals than all the other protocols combined. It has been estimated that for 20,000 chemicals at the 1-ton level, 4600 chemicals at the 10-ton level, 2900 chemicals at the 100-ton level, and 2600 chemicals at the 1000-ton level, the requirements outlined in Table 29.2 would

Table 29.4 REACH Substances of Very High Concern

Acrylamide
Alkanes, C10-13, chloro (short chain chlorinated paraffins)
Aluminosilicate refractory ceramic fibers
Ammonium dichromate
Anthracene
Anthracene oil
Anthracene oil, anthracene paste
Anthracene oil, anthracene paste, anthracene fraction
Anthracene oil, anthracene paste, distn. lights
Anthracene oil, anthracene-low
1,2-Benzenedicarboxylic acid
Benzyl butyl phthalate (BBP)
Bis(2-ethylhexyl)phthalate (DEHP)
Bis(tributyltin)oxide (TBTO)
Boric acid
5-tert-Butyl-2,4,6-trinitro-m-xylene (musk xylene)
Chromic acid
Oligomers of chromic acid and dichromic acid
Dichromic acid
Chromium trioxide
Cobalt dichloride
Cobalt(II) carbonate
Cobalt(II) diacetate
Cobalt(II) dinitrate
Cobalt(II) sulfate
DHNUP (di-C7-11 branched and linear alkyl esters)
4,4′-Diaminodiphenylmethane (MDA)
Diarsenic pentoxide
Diarsenic trioxide
Dibutyl phthalate (DBP)
DIHP (di-C6-8-branched alkyl esters, C7-rich)
Diisobutyl phthalate
2,4-Dinitrotoluene
Disodium tetraborate, anhydrous
2-Ethoxyethylacetate
2-Ethoxyethanol
Hexabromocyclododecane (HBCDD) and all major diastereoisomers
Hydrazine
Lead chromate
Lead chromate molybdate sulfate red (C.I. Pigment Red 104)
Lead hydrogen arsenate
Lead sulfochromate yellow (C.I. Pigment Yellow 34)
2-Methoxyethanol
1-Methyl-2-pyrrolidone
Pitch, coal tar, high temp
Potassium chromate
Potassium dichromate
Sodium chromate
Sodium dichromate
Strontium chromate
Tetraboron disodium heptaoxide, hydrate
Trichloroethylene
1,2,3-Trichloropropane
Triethyl arsenate
Tris(2-chloroethyl)phosphate
Zirconia aluminosilicate refractory ceramic fibers

Abbreviation: REACH, Registration, Evaluation, Authorization, and Restriction of Chemicals.
Source: From Ref. 13.

result in the use of almost 22 million animals for reproductive and developmental toxicity testing requirements and fewer than 4 million animals for all other required test protocols combined (11).

In spite of the appearance of REACH as requiring substantial experimental animal testing, the law specifically discourages the use of vertebrate animals for testing. Article 13 of REACH says, "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 says, "In order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort."

When a registrant or SIEF identifies a gap in the database on a substance, that gap cannot be filled using a vertebrate animal test without prior authorization from EChA. In theory, EChA will deny authorization unless alternative methods cannot fill the data gap.

There is a special problem with the use of alternative tests in reproductive and developmental toxicology, because EChA believes that these tests are too limited to replace whole animal testing. The Guidance Document covering this subject says:

> At the present time in vitro approaches have many limitations, for example, the lack of capacity for biotransformation of the test substance. . . Consequently, no firm recommendations can be made for the exclusive use of in vitro methods in a testing strategy for reproductive toxicity. The combination of assays in a tiered and/or battery approach may improve predictivity, but the in vivo situation remains more than the sum of the areas modeled by a series of in vitro assays. . . Therefore, a negative result for a substance with no supporting information cannot be interpreted with confidence as demonstrating the absence of a reproductive hazard (10).

This attitude presumably applies also to alternative tests such as zebrafish, although it is possible that zebrafish will fall under the prohibition against vertebrate animal testing. The Guidance Document also says that there are no available structural alerts for reproductive toxicity, so quantitative structure–activity relationship analysis may not be informative. Therefore, of the three general ways to avoid whole vertebrate animal testing (in vitro testing, structure–activity analysis, and read-across), only read-across appears to be a viable possibility for filling gaps in reproductive and developmental toxicity testing.

Read-across is based on inferring the toxicologic properties of a chemical based on the properties of related chemicals. For example, if a chemical nucleus with carbon side chains of 3, 5, and 6 carbons in length are known to share a particular toxicologic property, it might be reasonable to infer that the chemical with a 4-carbon side chain will share the same property. It is not clear to what extent read-across assumptions can be applied to reproductive and developmental toxicology, although in some cases registrants have included such assumptions in their registration dossiers.

HOW SUCCESSFUL WILL REACH BE?

It is too early to evaluate whether REACH will accomplish its goals and to what extent differences between competitors within an SIEF can be overcome. By the November 30, 2010 deadline, 24,675 registration dossiers had been submitted on 4300 substances (12). Of these 4300 substances, 3400 were existing EINECS substances. Most of the registrants were large companies, which presumably constituted or dominated the SIEFs. As the deadlines arrive for substances in the lower tonnage bands, it is expected that smaller companies will comprise a larger proportion of the registrants. It is not known to what extent these smaller companies will have the resources to complete the required registrations and to what extent their competitive stances will give way to the requirements of the law.

The goal of REACH is arguably the creation of a safer environment for people within the European Union. It is not clear that there is a metric by which to measure the success of this complex and costly registration scheme in improving the public health. It can be expected that as the submission and review of registration dossiers continues, there will be further clarification of the scientific and policy issues raised by REACH.

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