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The challenge of reproductive and developmental toxicology under REACH

Anthony R. Scialli *

Sciences International, Inc., 1800 Diagonal Road Suite 500, Alexandria, VA 22314-2808, USA

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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.

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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

* Fax: +1 703 684 2223.

E-mail address: ascialli@sciences.com

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.

Indeed, under REACH, data gaps are not to be filled by vertebrate animal testing without permission. Part of the registration process includes the proposal of experimental animal testing if a case can be made that alternative tests are not available to fill a data gap.

The use of alternative tests in reproductive and developmental toxicology is problematic, however. The reproductive and developmental data requirements of REACH mention alternative tests at the 10 metric ton/year band (Table 1) but specify that alternative tests will only obviate the need for whole-animal test results if the alternative tests lead to the conclusion that the substance is toxic to reproduction or development. The Technical Guidance Document on information requirements under REACH reinforces this restricted use of alternative tests by indicating that there are no available structural alerts for reproductive toxicity, making quantitative structure–activity relationship analysis unlikely to be informative, and that *in vitro* tests when negative cannot be interpreted as indicating the absence of a reproductive hazard (pages 328–329 of ECB, 2007b). It appears, then, that unless a registrant wishes to conclude that a substance is toxic to reproduction or development, vertebrate animal testing is necessary. It is possible, however, that grouping or read-across will be an acceptable way to avoid vertebrate animal testing. This possibility will be discussed in Section 4.

3. The cost of compliance

Estimates of the cost for filling data gaps for the EINECS substances vary widely depending on the assumptions made about the number and kinds of gaps in this large group of chemicals and the tonnage band. A study performed for the European Chemicals Bureau (Risk and Policy Analysts Limited, 2002) calculated costs between €18,850 (\$28,034) for a substance brought into the European Union at 1 metric ton/year and for which substantial data already exist and €683,400 (\$1,016,360) for a substance brought in at >1000 metric tons/year for which all testing remains to be performed. The European Union White paper that introduced the REACH concept estimated that the base set of tests required for all substances would cost €85,000 (\$126,400) per substance and that the highest level of testing (i.e., >1000 tons) would cost €325,000 (\$483,343) per substance. Another estimate of “average test needs” for substances was €7700 (\$11,450) at 1 metric ton/year and €236,700 (\$352,000) at 1000 metric tons/year (Angerer et al., 2008).

Reproductive and developmental toxicity testing are estimated to account for 54% of the testing costs associated with REACH compliance (Piersma, 2006). The highest costs are associated with two-generation reproductive toxicity testing, which costs in the range of \$500,000–\$750,000 for tests using rats. Developmental toxicity tests are comparatively inexpensive at about one-tenth the cost of two-generation studies. Far more vertebrate animals are used in reproductive and developmental toxicity testing than in other testing: 560 for a screening test, 150 for a developmental toxicity test, and 3200 for a two-generation reproductive toxicity test (Höfer et al., 2004). Assuming 20,000 chemicals at the ≥ 1 ton/year level, 4600 chemicals at ≥ 10 tons/year, 2900 at 100 tons/year, and 2600 at 1000 tons/year, REACH could require use of almost 22 million experimental animals for reproductive and developmental toxicity testing, compared to fewer than 4 million experimental animals for all other test endpoints combined (Höfer et al., 2004).

4. Alternatives to whole-animal testing

As discussed in Section 2.2, REACH calls for the use of vertebrate animal testing only as a last resort and specifically recommends

the use of quantitative structure–activity relationship analysis, *in vitro* testing, and grouping or read-across as alternatives.

4.1. Quantitative structure–activity relationship and related analyses

The belief that biological activity can be determined from chemical structure has led to the development of expert systems that attempt to predict toxicological endpoints based on structure and training sets of data. These systems have been evaluated for regulatory use in filling data gaps for acute and chronic toxicity, skin and eye irritation, corrosion, and mutagenicity (Cronin et al., 2003).

According to the draft Technical Guidance Document for REACH (ECB, 2007a), results of quantitative structure–activity relationship analysis can be used when the results of the model have been validated, the substance falls within the applicability domain of the model, the results are adequate for classification and labeling and/or risk assessment, and adequate and reliable documentation of the method is provided. The technical guidance document characterizes the extent to which valid models are available as variable and evolving. Reference is made to the OECD Guidance Document for criteria on the evaluation and validation of these models (OECD, 2007).

Although the general discussion of quantitative structure–activity relationship analysis in the REACH guidance document suggests that these models may replace vertebrate animal testing, the specific discussion on reproductive toxicity states, “There are a large number of potential targets/mechanisms associated with reproductive toxicity that, on the basis of current knowledge, cannot be adequately covered by a battery of QSAR models.” The guidance goes on to indicate that a positive result from a validated model could provide an alert for additional testing, but that such a result would not by itself be adequate for classification. A negative result would not be considered informative without “supporting evidence.” The guidance does not further define supporting evidence, nor does it give examples of what might be considered acceptable supporting evidence.

It appears, then, at this time that quantitative structure–activity relationship analysis does not offer a way to reduce experimental animal testing and cost in reproductive and developmental toxicity testing under REACH.

4.2. *In vitro* testing

There are three *in vitro* tests that are considered to have been validated according to the European Centre for the Validation of Alternative Methods (ECVAM), including the embryonic stem cell test, limb bud micromass culture, and whole post-implantation embryo culture (Genschow et al., 2004; Piersma et al., 2004; Spielmann et al., 2004). There are also a number of other *in vitro* tests that have been presented in the literature although not validated by ECVAM.

The embryonic stem cell test uses a permanent line of mouse cells that are maintained in a de-differentiated state by leukemia inhibiting factor. On removal of leukemia inhibiting factor, the embryonic cells differentiate into embryoid bodies, ultimately producing beating cardiomyocytes, identifiable using light microscopy. The test uses a comparison of the concentration of test chemical at which differentiation is inhibited with the concentration at which growth and viability of cultured fibroblasts is reduced. Decreased growth and viability of the embryonic cells is also taken into consideration. A chemical that inhibits embryoid body differentiation at concentrations much lower than those toxic to adult fibroblasts would be considered to have strong embryotoxic potential. The test distinguishes test chemicals as not embryotoxic, weakly embryotoxic, or strongly embryotoxic using a series

of formulas developed using discriminant analysis. Because this test uses established cell lines, the use of additional vertebrate animals is avoided.

The limb bud micromass culture system uses rat limb buds harvested on gestation day 14. Under culture conditions, mesenchymal cells in the limb bud will differentiate into chondrocytes, identifiable on light microscopy using Alcian blue staining. A decrease in the number of chondrocyte islands and/or the number of cells within an island is an indication of impaired differentiation. The concentration of test chemical impairing differentiation is compared to the concentration at which overall cell number and viability are decreased. A substance that decreases chondrocytes without an effect on overall cell number and viability would be considered to have selective effects on embryonic development.

The whole embryo culture uses explanted rat embryos at the 1–5 somite stage. Embryos are cultured for 48 h during which time substantial differentiation occurs. The concentration of test chemical causing malformations is determined. In some iterations of this test, the concentration causing malformations is compared to the concentration inhibiting growth and development in cultured fibroblasts, analogous to the embryonic stem cell test. A substance that causes malformations at a concentration that is not toxic to fibroblasts would be considered to have selective effects on embryonic development.

These tests may be considered screening tests rather than replacements for whole-animal toxicity tests. The Technical Guidance Document for REACH states that negative *in vitro* tests cannot be interpreted with confidence as showing an absence of hazard without supporting information, again without defining or characterizing what such supporting information might be (ECB, 2007b). Positive results are characterized as possibly providing justification for further testing.

In vitro developmental toxicity tests are considered by their proponents to be most useful for screening (Spielmann, 2005). Screening refers to a process by which chemicals with the greatest likelihood of producing developmental toxicity are separated from chemicals with less likelihood of producing developmental toxicity. Screening could be used, for example, by a manufacturer to decide which of a series of related chemicals to select for further development and possible commercialization or as a means of prioritizing a group of chemicals for additional testing in whole-animal studies. Under REACH, this type of prioritization does not appear relevant inasmuch as *all* substances manufactured or imported above 10 metric tons/year are designated for reproductive toxicity testing. It does not appear, then, that *in vitro* tests offer an opportunity to decrease cost and experimental animal utilization under REACH.

4.3. Grouping or read-across

Grouping of substances refers to making inferences about toxicological properties of substances based on similarities to other

well-studied structurally related substances. The term “read-across” is based on the concept that structurally related compounds can be listed in a table with their properties. Empty cells (data gaps) in the table can be filled by reading across and interpolating or extrapolating the missing data values (Fig. 1). The grouping of substances for read-across may be facilitated using structure–activity relationship models or *in vitro* testing.

An example of how read-across might be used for reproductive and developmental toxicology end points for phthalic acid diesters has been published (Fabjan et al., 2006). In this exercise, 10 phthalic acid diesters (Table 2) were selected based on the availability of data and similarity of structure (Fig. 2). Some of these agents (e.g., di(2-ethylhexyl) phthalate, dibutyl phthalate, butyl benzyl phthalate) produce testicular toxicity after exposure of late fetal or neonatal male rats, resulting in decreased testosterone synthesis and consequent abnormalities of the male genital tract at exposure levels below those causing generalized toxicity. Evaluation of available literature showed that an alkyl side chain consisting of 4–6 carbons was associated with the greatest potency in producing this testicular toxicity. Smaller or larger numbers of carbons in the alkyl side chain resulted in compounds that were active only at much higher exposure levels if at all.

From the evaluation of the grouped phthalic acid diesters, toxicity to the developing testis would be expected after exposure to di-*n*-pentyl and di-*n*-hexyl phthalate, for which there are fewer

Table 2
Phthalic acid esters selected for grouping^a

Substance name	Alkyl side chain
Diethyl phthalate	2-carbon, linear
Dibutyl phthalate	4-carbon, linear
Benzyl butyl phthalate	4-carbon, linear
Di- <i>n</i> -hexyl phthalate	6-carbon, linear
Di(2-ethylhexyl) phthalate	6-carbon, branched
Di- <i>n</i> -octyl phthalate	8-carbon, linear
Diisononyl phthalate	9-carbon, branched
Diisodecyl phthalate	10-carbon, branched
Di(2-propylheptyl) phthalate	10-carbon, branched
1,2-Benzenecarboxylic acid branched and linear alkyl esters	8–10-carbon, branched and linear

^a From Fabjan et al., 2006.

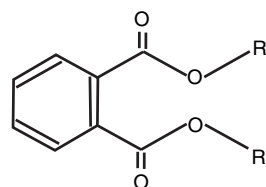


Fig. 2. Generic structure of the phthalic acid esters. For the compounds listed in Table 2, except butyl benzyl phthalate, R1 = R2 = a linear or branched alkyl group.

	Substance A	Substance B	Substance C	Substance D
Property				
1	36	48		72
2	180	240	300	360
3	1.2		2.0	2.4
4	5.0	7.5	12.5	22.5

Fig. 1. Filling data gaps using grouping or read-across. An examination of the data for related compounds suggests that property 1 for substance C is 60 and property 3 for substance B is 1.6, based on the mathematical progressions within rows.

data than for some of the other agents in Table 2. Using this read-across approach, it might be possible to avoid additional whole-animal tests in the REACH registration of phthalic acid diesters containing alkyl side chains of 4–6 carbons. The question arises whether it would be similarly possible to avoid whole-animal testing for phthalic acid diesters with longer alkyl side chains. This question is discussed in Section 5.

5. Classification and labeling

REACH includes requirements for the classification and labeling of substances derived from existing European Union requirements (European Commission, 1992). Classification abbreviations relevant to reproductive and developmental toxicology are summarized in Table 3. This scheme is augmented by “categories” of reproductive and developmental toxicity. Category 1 applies to substances that are known based on epidemiology studies to produce reproductive or developmental toxicity in humans. Category 2 applies to substances for which data showing reproductive or developmental toxicity were obtained in well-conducted experimental animal studies using an appropriate route of administration and showing reproductive or developmental toxicity that appears not to be due to generalized toxicity, poor animal husbandry, intercurrent infection, or nutritional deficiency. Category 3 applies to substances for which data come from experimental animal studies with design deficiencies or where the adverse reproductive or developmental effects may be due to generalized toxicity. Category 3 can also be used for substances that cause small changes in the incidence of spontaneous abnormalities or in postnatal developmental assessments.

This classification system will be replaced by a Globally Harmonized System that has been developed by the United Nations Economic Commission (UNECE, 2003). The Globally Harmonized System for reproductive and developmental toxicity will be similar to the current system, with Category 1 and 2 being renamed Category 1A and B and Category 3 being renamed Category 2.

The classification scheme has important implications under REACH, which is expected to define chemicals as Substances of Very High Concern (SVHC) based on several criteria among which are R60 or R61 classifications (Category 1 or 2 reproductive or developmental toxicity). SVHCs will require authorization for marketing under REACH. The authorization will entail a showing that the substance cannot be replaced by a less toxic substance and that its presence in the market can be justified by benefits to the public. The authorization process may lead to restriction, meaning that a substance may not be marketed or that it may only be marketed under certain conditions. In addition, manufacturers or importers

Table 3
Reproductive and Developmental Hazard Labels in Europe

Code	Designation	Criteria
R60	May impair fertility	Substance impairs fertility or should be regarded as if it impairs fertility in humans (Category 1 and 2)
R61	May cause harm to the unborn child	Substance causes developmental toxicity or should be regarded as if it causes developmental toxicity in humans (Category 1 and 2)
R62	Possible risk of impaired fertility	Substance causes concern for human fertility (Category 3)
R63	Possible risk of harm to the unborn child	Substance causes concern for humans owing to possible developmental toxic effects (Category 3)
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

From Commission Directive 92/32/EEC (European Commission 1992).

of articles (basically, finished products) containing an SVHC at levels above 0.1% will be required to notify the European Chemicals Agency if the amount of the SVHC manufactured or imported is ≥ 1 metric ton/year.

The classification of a substance as a Category 2 (Globally Harmonized System 1B) reproductive or developmental toxicant can be made without regard to the exposure level at which an adverse effect occurred in an experimental animal model, so long as the adverse effect did not appear to be due to excessive generalized toxicity. If reproductive or developmental toxicity occurred at an exposure level in the experimental animal model that was implausibly high compared to anticipated human exposure levels, that information might be used as part of the authorization process, but authorization (or notification in the case of an article) would be triggered nonetheless. The nonrefundable authorization application fee is expected to be €25,000–50,000, regardless of the outcome of the authorization decision.

The categorization of a substance as a reproductive or developmental toxicant irrespective of exposure level is toxicologically unsound, inasmuch as all substances will demonstrate adverse effects on reproduction and development at some exposure level, if only secondary to parental morbidity or mortality. The categorization and labeling scheme does not provide information on the actual risk of the anticipated chemical exposure. The risk of rating all substances as reproductive or developmental toxicants is mitigated somewhat by the exclusion of effects likely due to general toxicity. In addition, there is language in the Directive that set up the categories and there is language in REACH suggesting that a 1000 mg/kg body weight threshold (by a route relevant to human exposure) might be used as a testing maximum. In other words, if a substance does not produce reproductive or developmental toxicity at 1000 mg/kg body weight, it need not be tested at higher dose levels and can be assumed to be without important reproductive or developmental effects. This “limit dose” does not address the substance for which toxicity occurs at 1000 mg/kg body weight but for which human exposure is to 1 μ g/kg body weight. At present, such a substance may be considered an SVHC and may be subject to authorization and possible restriction under REACH.

Grouping or read-across can be used to estimate effect levels of substances based on effect levels obtained for well-studied substances in the same group. In the phthalic acid diester grouping exercise discussed in Section 4.3, compounds with 7 or more carbons in the alkyl side chain produced testicular toxicity at exposure levels an order of magnitude or more higher than compounds with 4–6-carbon side chains. In some instances, the no effect levels for the longer chain compounds were above the 1000 mg/kg body weight threshold. In other cases, the existing data did not include dose levels that high. Compounds with alkyl side chain lengths close to 4–6 carbons would need further information or testing before the lack of need for a reproductive hazard label could be argued. It is possible that with additional support from in vitro studies and quantitative structure–activity relationship models, a convincing case could be made that the longer chain compounds could be registered without being designated as toxic to reproduction or development and without additional whole-animal testing.

6. Integrated testing strategy

The Technical Guidance Document for REACH (ECB, 2007b) describes a strategy to be used before deciding whether any testing for reproductive or developmental toxicity is needed. This strategy, which is recommended for all substances in the ≥ 10 metric ton/year band, is summarized in Fig. 3. Cost savings and a reduction in vertebrate animal usage can be achieved by staying out of the

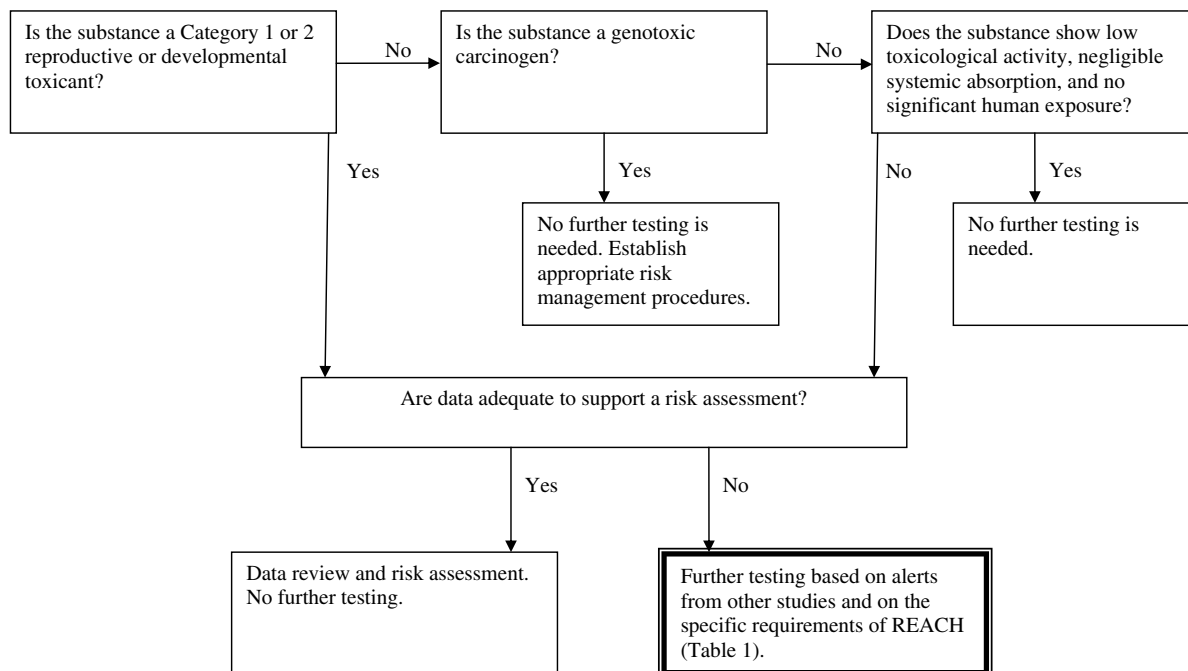


Fig. 3. Flow chart for considering whether a substance requires additional testing under REACH for reproductive or developmental toxicity. Drawn after ECB, 2007b.

double-outlined box in the lower right-hand corner of the figure. There are three kinds of substances that can avoid getting to that box:

- Substances demonstrating genotoxic carcinogenicity or reproductive/developmental toxicity. These substances will likely be considered SVHCs and will require authorization for marketing in the European Union. If these substances are authorized, a risk management plan will be required.
- Substances that have little activity in any toxicological tests have little systemic absorption, and for which there is no appreciable human exposure. All three conditions must be satisfied.
- Substances for which there are adequate existing data to support a risk assessment. Although the requirements listed in Table 1 might appear to require additional vertebrate animal testing, REACH has provisions for such testing to be avoided using *in vitro* testing, quantitative structure–activity relationship analysis, and grouping (read-across). It appears that grouping, perhaps supported by *in vitro* data and by structure–activity relationship analyses, offers the best opportunity to avoid additional reproductive and developmental toxicity testing in vertebrate animals.

7. Conclusions

The reproductive and developmental toxicology data requirements of REACH can potentially impose a high demand for financial and experimental animal resources. All chemicals manufactured or imported into the European Union at ≥ 10 metric tons/year require experimental animal test data. REACH introduces the possibility that vertebrate animal testing can be avoided by the use of *in vitro* testing, quantitative structure–activity relationship analysis, and grouping or read-across. *In vitro* testing and quantitative structure–activity relationship analysis are not considered adequate replacements for reproductive and developmental toxicity testing in intact animals, although these tests may be useful for prioritizing chemicals for further testing and may provide supplemental information.

Grouping offers an opportunity for estimating the reproductive and developmental toxicity characteristics of a compound when other compounds in the group have a sufficient amount of data and when there is convincing evidence that the members of the group have similar toxicological properties. Grouping may be an effective way to avoid performing additional vertebrate animal testing for some substances.

The classification scheme used under REACH is expected to label a substance as a substance of very high concern (SVHC) if it is known to produce reproductive or developmental toxicity in humans or if it produces reproductive or developmental toxicity in experimental animals in the absence of excessive generalized toxicity and in the absence of deficiencies in study design or performance. The exposure level at which a substance produces reproductive or developmental toxicity does not appear to be a consideration in the planned designation of SVHCs. This designation has unfavorable implications for the ability of the substance or articles containing the substance to be marketed in the European Union.

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