Commentary

Reproductive and developmental toxicity testing: Examination of the extended one-generation reproductive toxicity study guideline

Shakil A. Saghir*, Michael A. Dorato
Smithers Avanza Toxicology Services, LLC, 11B Firstfield Road, Gaithersburg, MD 20878, USA

Abstract

An important aspect of safety assessment of chemicals (industrial and agricultural chemicals and pharmaceuticals) is determining their potential reproductive and developmental toxicity. A number of guidelines have outlined a series of separate reproductive and developmental toxicity studies from fertilization through adulthood and in some cases to second generation. The Extended One-Generation Reproductive Toxicity Study (EOGRTS) is the most recent and comprehensive guideline in this series. EOGRTS design makes toxicity testing progressive, comprehensive, and efficient by assessing key endpoints across multiple life-stages at relevant doses using a minimum number of animals, combining studies/evaluations and proposing tiered-testing approaches based on outcomes. EOGRTS determines toxicity during preconception, development of embryo/fetus and newborn, adolescence, and adults, with specific emphasis on the nervous, immunological, and endocrine systems, EOGRTS also assesses maternal and paternal toxicity. However, EOGRTS guideline is complex, criteria for selecting doses is unclear, and monitoring systemic dose during the course of the study for better interpretation and human relevance is not clear. This paper discusses potential simplification of EOGRTS, suggests procedures for relevant dose selection and monitors systemic dose at multiple life-stages for better interpretation of data and human relevance.

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Background

In order to provide a background for discussion of the EOGRTS guidance, the readers should be aware of several other guideline studies routinely conducted, primarily in rats, to determine immediate and latent reproductive effects of chemical exposure. Assessment of toxicity to reproduction includes possible effects of chemicals on fertility, embryonic and fetal development, peri- and postnatal development, and maternal function. Traditionally, separate reproductive/developmental toxicity studies are conducted to evaluate these effects. Guidelines OECD 414 and OPPTS 870.3350 determine effects of chemicals on embryo-fetal development/death, altered growth and structural changes (ICH, 2005; OECD, 1995; USEPA, 2000a). Effects of chemicals on maternal behavior, length of gestation, dystocia, number and sex of pups, live births, runts, presence of gross abnormalities, and abnormal behavior in pups are determined in guidelines OECD 415 (screening test) and OPPTS 870.3550 (ICH, 2005; OECD, 1995; USEPA, 2000a). General and reproductive/developmental toxicity endpoints are combined in OECD 422 (screening test) and OPPTS 870.3650 guidelines (OECD, 1996; USEPA, 2000b).

Guidelines OECD 415 and 416 determine effects of chemicals on reproduction in one- and two-generation studies, respectively (OECD, 1983, 2001b; USEPA, 1998b). The two-generation study (OECD 416; OPPTS 870.3800) is considered the most comprehensive design to assess reproductive toxicity (Carney and Sattivari, 2013) and the effects of chemicals on the reproductive performance of the F1 parents. The two-generation study assesses effects of chemicals on reproductive parameters listed for OECD 421 in P and F1 generations as well as the presence of gross abnormalities and abnormal behavior in F1 and F2 animals. The NTP's modified one-generation study design determines effects of chemicals on animals from gestation through weaning of F2 animals (Foster, 2014); however, no formal guideline document exists. The difference between the NTP design and other approved guidelines include retention of multiple pups per litter rather than 1 pup/sex/litter/dose group and premating treatment of males for a full 10
weeks. However, both ICH and OECD guidelines indicate that a full 10-week premating period is often not needed, especially when other general toxicity studies (e.g., existing subchronic studies) indicate a lack of toxicity to the testes or uterus.

2. Current guidelines and modified approach

Most of the above described individual guidelines evaluate toxicity of chemicals to only parts of the reproductive and developmental stages with the exception the two-generation reproductive toxicity study. These guideline studies have not been updated to reflect advancements in the assessment of developmental and reproductive toxicity. For example, researchers now like to combine multiple reproductive and developmental toxicity studies into a single study and determine systemic exposure during dose range-finding or other general toxicity studies for the selection of appropriate doses (Chapman et al., 2013; Dorato et al., 2014; Marty et al., 2013; Saghir et al., 2013). Although the two-generation toxicity study is considered “the gold standard” for the assessment of reproductive toxicity, it is complex in design, high in the utilization in animals (~2600 animals for study in rats) and with debatable value of the F2 generation (Janer et al., 2007a, 2007b; Moore et al., 2009; Piersma et al., 2011; Rorije et al., 2011). The two-generation toxicity study is also not designed to evaluate developmental neurotoxicity (DNT) or developmental immunotoxicity (DIT) endpoints, which require standalone studies using an additional 1280 animals.
2.1. Extended one-generation reproductive toxicity study (EOGRTS)

To make the toxicity testing across life stages state-of-the-art, the International Life Sciences Institute/Health and Environmental Science Institute (ILSI/HESI) Agricultural Chemicals Safety Assessment (ACSA) Technical Committee was charged with proposing an improved testing paradigm to assess potential effects of chemicals across life stages by incorporating the current understanding of developmental and reproductive toxicity (ILSI/HESI, 2001; Cooper et al., 2006). The committee identified key toxicity profiles across life stages beyond developmental and reproductive phases, combined studies/evaluations of endpoints across multiple life stages, and proposed a tiered testing approach for flexibility based on the needs and available data. The committee considered approaches to assess the potential of chemicals to cause adverse effects on reproduction, developmental life stages, and in the elderly. The life stage toxicity was defined as the potential adverse effects of chemicals on preconception, development (embryo/fetal and newborn/pre-weaning life stages), adolescence, and adults of all ages for reproductive and developmental toxicity, any special sensitivity with respect to general toxicity and specific effects on the nervous, immunological, and endocrine systems at critical life stages.

Fig. 3. Change in systemic dose of a herbicide (2,4-D) at different life stages of rats (modified from Saghir et al., 2013).

Fig. 4. Graphic depiction of the improvements to EOGRTS (OECD 443) design with scheme for the determination of systemic dose at different life-stage (reproductive/developmental landmarks) during study using core study animals.
The study starts with exposing a sufficient number of adult male and female rats (to achieve 20 litters/dose) to the test chemical for two weeks prior to mating through weaning. Both parents are then sacrificed on study day (SD) 71 and evaluated while pups are continuously dosed with the test chemical until their scheduled sacrifice after evaluation for possible toxicological effects (Fig. 1). Groups of pups are evaluated for developmental neurotoxicity and at sexual maturity for reproductive, immuno, neuro, and general toxicity, and bred, when triggered, to produce F2 litters. The trigger to generate F2 animals in EOGRTS is based on developmental landmarks (e.g., anogenital distance, nipple retention, puberty onset) in F1 animals. In addition to the enhanced interpretative value, the EOGRTS protocol also retains multiple pups per litter, similar to the NTP study design and in contrast to retaining 1 pup/sex/dose in conventional two-generation reproductive toxicity study protocols (Marty et al., 2013). Therefore, it is not clear how the NTP design offers additional advantage as mentioned by Foster (2014). Feasibility/validation of EOGRTS was achieved in four studies conducted for 2,4- dichlorophenoxyacetic acid (2,4-D) methimazole, vinclozolin, and lead acetate (Fegert et al., 2012; Martin et al., 2009; Piersma et al., 2011; Rorije et al., 2014). Feasibility/validation of EOGRTS was achieved in four studies conducted for 2,4- dichlorophenoxyacetic acid (2,4-D) methimazole, vinclozolin, and lead acetate (Fegert et al., 2012; Martin et al., 2009; Piersma et al., 2011; Rorije et al., 2014).

Table 1
Outline of EOGRTS for parents including systemic dose determination.

| Prior to and during cohabitation | Group by sex |
|----------------------------------|--------------|
| Housing prior to cohabitation    | 1:1 male/female |
| Housing during cohabitation     | ≥ twice daily |
| Clinical observations (CO)      | SD1 and weekly |
| PE                              | SD1 and weekly |
| BW                              |Weekly |
| FC                              | Daily for 2 weeks |
| Vaginal smear                   | SD 14 or 28, test chemical concentration at SS (2 or 4 weeks) |
| Blood                           | At sacrifice, SD 71 or SS, test chemical concentration |
| Males following cohabitationa   | At sacrifice, SD 71, using one testis/epididymis/vas deferens |
| Blood                           | Urinalysis, Hematology, Clinical Path, T4, TSH |
| Tissues                         | Histopathology |
| During gestation                | Dam |
| Housing                         | Same as above |
| CO, PE, BW, FC                  | Every 2 days |
| BW                              | SD 14 or 17, test chemical concentration |
| Blood                           | Dam for test chemical concentration |
| At birth                        | |
| Blood                           | |
| During lactation                | |
| Housing                         | |
| CO, PE, BW                      | |
| BW                              | |
| Blood                           | |
| Milkf                          | |
| At terminationb                 | |
| Urine                           | |
| Blood                           | |
| Tissues                         | |
| Uteri                           | |
| Vaginal smears                  | |
| BW, body weight; CO, clinical observation; FC, food consumption; GD, gestational day; LD, lactational day; PE, physical examination; SD, study day; SS, steady-state; T4, thyroxin; TSH, thyroid stimulating hormone. |
| Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration. |
| Bold italic texts are proposed additions to improve the current EOGRTS design. |
| a Randomly selected 4♂ and 4♀ for systemic dose determination (one ≤100 µl blood, see text for time). |
| b As outlined in the protocol for hematology, clinical biochemistry, T4, TSH, urinalysis, gross pathology and tissues for histopathology. |
| c Ideally same randomly-selected parental male rats for systemic dose determination (one ≤100 µl blood, see text for detail). |
| d Ideally same randomly selected dams for systemic dose determination (one ≤100 µl blood, see text for time). |
| e For placental transfer, collect ≤100 µl blood from dam and her pups (or placenta), see text for detail. |
| f For lactational transfer, collect ≤100 µl milk likely from extra or dose-range-finding animals (see text for detail). |
| g From randomly-selected 4–5 ♀ and 9♂ per dose group. |

stages. Additionally, they emphasized using doses that are relevant to realistic human exposures while maintaining adequate power to detect toxicity utilizing a systemic dose in a minimum number of animals (see Cooper et al., 2006; Marty et al., 2013; Saghir, 2015; Saghir et al., 2012, 2013).

The ILSI/HESI-ACSA proposed study design (Cooper et al., 2006) became the basis for the OECD 443 EOGRTS guideline (OECD, 2012). The study starts with exposing a sufficient number of adult male and female rats (to achieve 20 litters/dose) to the test chemical for two weeks prior to mating through weaning. Both parents are then sacrificed on study day (SD) 71 and evaluated while pups are continuously dosed with the test chemical until their scheduled sacrifice after evaluation for possible toxicological effects (Fig. 1). Groups of pups are evaluated for developmental neurotoxicity and at sexual maturity for reproductive, immuno, neuro, and general toxicity, and bred, when triggered, to produce F2 litters. The trigger to generate F2 animals in EOGRTS is based on developmental landmarks (e.g., anogenital distance, nipple retention, puberty onset) in F1 animals. In addition to the enhanced interpretative value, the EOGRTS protocol also retains multiple pups per litter, similar to the NTP study design and in contrast to retaining 1 pup/sex/dose in conventional two-generation reproductive toxicity study protocols (Marty et al., 2013). Therefore, it is not clear how the NTP design offers additional advantage as mentioned by Foster (2014). Feasibility/validation of EOGRTS was achieved in four studies conducted for 2,4- dichlorophenoxyacetic acid (2,4-D) methimazole, vinclozolin, and lead acetate (Fegert et al., 2012; Martin et al., 2009; Piersma et al., 2011; Rorije et al., 2014).

Although, the EOGRTS approach provides advantage by combining evaluations, adding DNT and DIT parameters and decreasing animal use, it is not without criticism. Even though Schiffelers et al. (2015) raised concern about the acceptance of the current EOGRTS protocol in the Europe without amendments due to criticism, the European Commission has recently adopted the EOGRTS (EC, 2015). However, the Commission has left an option for the European Chemicals Agency (ECHA) to request performance of the F2 generation when justified (EC, 2015). In addition to the debate on the limited added value of the second generation (Jäner et al., 2007a, 2007b; Martin et al., 2009; Piersma et al., 2011; Rorije et al., 2015).
et al., 2011), the organization of the OECD 443 guideline is perceived to be difficult to follow. The criteria for selecting the highest dose is unclear (even though it recommends using toxicokinetic data generated in dose-range-finding or other earlier studies). In addition, procedures for monitoring the systemic dose, for better interpretation and human relevance of the animal data, are not included. Saghir et al. (2013), on the other hand, offered criteria that can effectively guide dose selection and provide a direct example of the strategy for practical implementation of EOGRTS protocols. This paper examines ways to monitor systemic dose during the course of EOGRTS using core study animals and to select appropriate doses within dose-proportional range that are relevant to actual human exposure (Saghir, 2015; Saghir et al., 2012, 2013).

3. Role of kinetics in dose selection and incorporation into EOGRTS

Safety assessment of chemicals should focus on doses in animals that are relevant to human exposure while adequate to detect toxicity. One of the ways to determine the top dose for EOGRTS is to determine systemic dose proportionality and select the top dose based on the kinetically-derived maximum dose (KMD) at or slightly above the point of departure (POD) from dose proportionality (Marty et al., 2013; Saghir, 2015; Saghir et al., 2012, 2013). The POD from dose proportionality can be determined in a dose range-finding developmental study or in other repeat-dose toxicity studies as described by Saghir et al. (2012) and Saghir (2015). An effect observed in animals at the non-proportional systemic dose may not be relevant to the assessment of actual human risk; especially when the actual human exposure is many orders of magnitude lower than those used in animal studies. Additionally, it is recommended to have some kinetic information of chemicals in the test animal species along with likely human exposure estimates for appropriate margin of exposure before the initiation of reproductive toxicity studies with collection of further kinetic information in pregnant and lactating animals and in pups (LH/AHESI, 2001; Cooper et al., 2006; Saghir et al., 2013). An example is given in Fig. 2 where the top dose for 2,4-D EOGRTS was selected based on KMD at slightly above the POD from proportionality of the systemic dose; the dose selected was half of the maximum tolerated dose and still several orders of magnitude higher than the expected human exposure (see Marty et al., 2013; Saghir et al., 2013 for detail). Determining systemic dose during the course of a reproductive/developmental study is also helpful in understanding the exposure at different life-stages (Fig. 3) for better interpretation of the human relevance of the results in test animals (Fegert et al., 2012; Marty et al., 2013; Saghir et al., 2013). In dietary exposure studies, the importance of adequately adjusting doses during different life-stages is emphasized in Fig. 3. Failure to adjust dietary concentrations can result in dramatically different systemic doses of test chemicals reflective of differences in bodyweight to food intake ratio, skewing the resulting risk assessment. In order to accomplish the determination of systemic dose at various developmental life stages, an approach for a single blood collection (≥10 μl) at reproductive/developmental landmarks during the course of the study is proposed in Fig. 4 and Tables 1–4. Cord/pup along with maternal blood may be collected from animals used in the dose-range-finding (DRF) study or from dedicated groups in the main study as outlined in Fig. 4, pooled for each litter/dose group to achieve the minimum volume required for analysis. For the collection of blood from PND 4 pups, use of culled and extra animals in Cohort 3 of EOGRTS is recommended. Blood from each litter may be pooled once before parturition, if warranted, in a few designated animals in the DRF or main study. Similarly, milk can be obtained from

| Table 2 Outline of EOGRTS for F1 including systemic dose determination. |
|---------------------------------------------------------------|
| **Blood samples**<sup>a</sup> | **Culling** | **Food consumption** |
| At birth | Unique litter and group identification on PND 0 or 1 | From culler/litter/group for T4, TSH and test chemical concentration |
| Culling | Reduce to 5 males and 5 females per litter on PND 4 | Males PND 4 |
| Blood samples<sup>b</sup> | From culled/litter/group for T4, TSH and test chemical concentration | All culled pups on PND 4 |
| Angiogenital Distance | Litters with respective mothers | Litters with respective mothers |
| Gross necropsy | Male pups PND 12 or 13 | Small groups of same sex and treatment |
| Housing until weaning | ≥ twice daily | Weekly at the time of weighing |
| Nipple assessment | At weaning and weekly thereafter<sup>c</sup> | PND 4, 7, 14, 21 (at weaning) |
| Housing after weaning | Weekly following assigning to cohorts | At weaning and weekly thereafter<sup>c</sup> |
| Clinical observations | PND 22 for T4, TSH and test chemical concentration | At weaning and weekly thereafter<sup>c</sup> |
| Physical examination | Gross necropsy of pups not selected for cohort on PND 22 | At weaning and weekly thereafter<sup>c</sup> |
| BW before weaning | Brain, spleen, thymus, mammary gland, target tissues on PND 22 | Brain, spleen, thymus, mammary gland, target tissues on PND 22 |
| BW after weaning | PND 14, 56, between 76 and 89, selected animals for test chemical concentration | PND 14, 56, between 76 and 89, selected animals for test chemical concentration |
| Food consumption | Evaluated daily starting before the expected day in all selected animals | Evaluated daily starting before the expected day in all selected animals |
| Blood samples<sup>d</sup> | BW, body weight; PND, postnatal day; T4, thyroxin; TSH, thyroid stimulating hormones. | |
The EOGRTS may also eliminate the need to separately study in a non-rodent (likely rabbit) species or to only those listed in OPPTS and ICH guidelines, ideally to only one additional separate developmental and reproductive toxicity study across life-stage, has the potential to reduce the needs to conduct proposed evaluations, especially systemic dose determination design needed significantly.

**Table 4**

Outline of FOB for EOGRTS for F1 cohort 2 and 3.

| Cohort 2A: neurobehavioral testing and neurohistopathology assessment as adults |
| --- |
| Number | 10/sex/group (1 male or 1 female per litter per group) |
| CO, PE, BW, FC | See table outlining general considerations for F1 animals |
| Auditory startle test | PND 24 ± 1 |
| FOB | Between PND 63 and PND 75 |
| Motor activity | Between PND 63 and PND 75 |
| **Termination after PND 75 and before PND 90** |
| **Blood** | For test chemical concentration |
| Tissues | Brain weight and full neurohistopathology - perfusion fixation |
| Brain (examination) | Multiple section from different regions of the brain |
| **Cohort 2B: neurohistopathology assessment at weaning (PND 21 or PND 22)** |
| Number | 10/sex/group (1 male or 1 female per litter per group) |
| CO, PE, BW, FC | See table outlining general considerations for F1 animals |
| Termination PND 21 or PND 22 | Brain weight and full neurohistopathology - perfusion fixation (optional) |
| Tissues | Multiple section from different regions of the brain |
| Brain (examination) | For test chemical concentration |
| **Cohort 3: developmental immunotoxicity assessment** |
| Number | 10/sex/group (1 male or 1 female per litter per group) |
| CO, PE, BW, FC | See table outlining general considerations for F1 animals |
| Termination PND 56 ± 3 | Blood Assays |
| For test chemical concentration | TDAR |

BW, body weight; CO, clinical observation; FC, food consumption; FOB, functional observation battery; PE, physical examination; PND, postnatal day; TDAR, T-cell-dependent antibody response.

**Bold italic texts are proposed additions to improve the current EOGRTS design.**

Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration.

**Table 5**

Outline of FOB for EOGRTS.

| Home Cage & open field | Manipulative | Physiologic |
| --- | --- | --- |
| Involuntary Clonic & Tonic | Ease of removal | Temperature |
| Palpebral Closure | Ease of handling | Body weight |
| Piloerection | Muscle Tone | Pupil response |
| Salivation | Approach Response | Pupil size |
| Lactation | Touch Response | |
| Vocalizations | Auditory Response | |
| Rearing | Tail Pinch Response | |
| Gaits Abnormalities | Righting Response | |
| Arousal | Landing Foot Splay | |
| Stereotypy | Forelimb Grip Strength | |
| Bizarre Behavior | Hindlimb Grip Strength | |
| Stains | | |
| Respiratory Abnormalities | | |

question and the need for the data. Although EOGRTS is a complex design needed significant resources, when conducted with the proposed evaluations, especially systemic dose determination across life-stage, has the potential to reduce the needs to conduct several additional separate developmental and reproductive toxicity studies. EOGRTS, when designed properly, may reduce the needs for studies such as OECD 414, 415, 416, 421, and 422 or those listed in OPPTS and ICH guidelines, ideally to only one additional study in a non-rodent (likely rabbit) species or to only those needing to determine fetal abnormalities that cannot be assessed in EOGRTS. The EOGRTS may also eliminate the need to separately conduct DNT and/or DIT studies. Therefore, in our opinion, EOGRTS with the proposed modifications, or a variant of it based on the properties of the test chemicals and issues at hand, will accomplish an overall reduction in the use of resources including the number of animals used in a series of studies conducted to assess the developmental and reproductive toxicity (and possible mode-of-action studies) of test chemicals by consolidating them into one large multipurpose study. It is agreed that the benefits of consolidating developmental and reproductive toxicity studies into one large multipurpose study must be evaluated carefully in relation to the questions needing answers.

**Acknowledgment**

The authors acknowledge the late Ms. Barbara Neal for her involvement in the early implementation of the EOGRT study approach for 2,4-D. Her insights and willingness to share generated and helped advance many of the concepts presented in this paper.

**Transparency document**

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2016.03.023

**References**

Carney, E.W., Sattivari, R., 2013. Predictive toxicology: biological assay platforms. In: Faqi, A.S. (Ed.), A Comprehensive Guide to Toxicology in Preclinical Drug Development. Academic Press, Waltham, MA, pp. 777–806.

Chapman, K.L., Holzgrewe, H., Black, L.E., Brown, M., Chellman, G., Copeman, C., Couch, J., Creton, S., Gheen, S., Hberman, A., Kinter, L.B., Madden, S., Mattis, C., Stemple, H.A., Wilson, S., 2013. Pharmaceutical toxicology: designing studies to
reduce animal use, while maximizing human translation. Regul. Toxicol. Pharmacol. 66, 88–103.

Cooper, R.L., Lamb, J.C., Barlow, S.M., Bentley, K., Brady, A.M., Doerrner, N.G., Eisenbrandt, D.L., Fenner-Crisp, P.A., Hines, R.N., Irvine, L.F., Kimmel, C.A., Koeter, H., Li, A.A., Makris, S.L., Sheets, L.P., Speijers, G., Whitby, K.E., 2006. A tiered approach to life stages testing for agricultural chemical safety assessment. Crit. Rev. Toxicol. 36, 93–98.

Dorato, M.A., McMillian, C.L., Williams, T.M., 2014. The toxicologic assessment of pharmaceutical and biotechnology products. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology, sixth ed. Taylor and Francis, NY, pp. 344–345.

(European Commission) EC, 2015. Commission Regulation (EU) 2015/282 of 20 February 2015 Amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as Regards the Extended One-generation Reproductive Toxicity Study. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=uriserv:OJ.L_.2015.050.01.0001.01.ENG.

Fegert, I., Billington, R., Botham, P., Carney, E., FitzGerald, R.E., Hanley, T., Lewis, R., Marty, M.S., Schneider, S., Sheets, L.P., Stahl, B., van Ravenzwaay, B., 2012. Feasibility of the extended one-generation reproductive toxicity study (OECD 443). Repro. Toxicol. 34, 331–339.

Foster, P.M.D., 2014. Regulatory forum opinion piece: new testing paradigms for reproductive and developmental toxicity — the NTP modified one generation study and OECD 443. Toxicol. Pathol. 42, 1165–1167.

( international Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) ICH, 2003. ICH Harmonized Tripartite Guideline: Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility 55/R2 (Geneva, Switzerland).

( International Life Sciences Institute/Health and Environmental Science Institute) ILSI/HESI, 2001. Developing Strategies for Agricultural Chemical Safety Evaluation: a Report from April 22–23, 2001 Workshop, September 2001, Washington, DC.

Janer, G., Hakkert, B.C., Slob, W., Vermeire, T., Piersma, A.H., 2007a. A retrospective analysis of the two-generation study: what is the added value of the second generation? Reprod. Toxicol. 24, 97–102.

Janer, G., Hakkert, B.C., Piersma, A.H., Vermeire, T., Slob, W., 2007b. A retrospective analysis of the added value of the rat two-generation reproductive toxicity study versus the rat subchronic toxicity study. Reprod. Toxicol. 24, 103–113.

Martin, M.T., Mendez, E., Corum, D.G., Judson, R.S., Kavlock, R.J., Rotroff, D.M., Dix, D.J., 2009. Profiling the reproductive toxicity of chemicals from multi-generation studies in the toxicity reference database. Toxicol. Sci. 110, 181–190.

Marty, M.S., Neal, B.H., Zablotsky, C.L., Vano, B.L., Andrus, A.K., Woolhiser, M.R., Boverhof, D.R., Saghir, S.A., Peralta, A.W., Passage, J.K., Peralta, A.W., Neal, B.H., Hammond, L., Bus, J.S., 2013. Life-stage, sex-, and dose-dependent dietary toxicokinetics and relationship to toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) in rats: implications for toxicity test dose selection, design, and interpretation. Toxicol. Sci. 136, 294–307.

Saghir, S.A., Mentorala, A.L., Bartels, M.J., Day, S.J., Hansen, S.C., Sushinsky, J.M., Bus, J.S., 2006. Strategies to assess systemic exposure of chemicals in subchronic/chronic diet and drinking water studies. Toxicol. Appl. Pharmacol. 211, 245–260.

Schneider, S., Kaufmann, W., Straus, V., van Ravenzwaay, B., 2011. Vincolozolin: a feasibility and sensitivity study of the ILSI-HESI F1-extended one-generation rat reproduction protocol. Regul. Toxicol. Pharmacol. 59, 91–106.

Schaffers, M.-J.W.A., Blaauboer, B.J., Bakker, W.E., Hendriksen, C.F., Krul, C., 2015. Regulatory acceptance and use of the extended one generation reproductive toxicity study within Europe. Regul. Toxicol. Pharmacol. 71, 114–124.

( U.S. Environmental Protection Agency) USEPA, 1998a. Health Effects Test Guidelines, OPPTS 870.3700, Prenatal Developmental Toxicity Study, August 1998.

( U.S. Environmental Protection Agency) USEPA, 1998b. Health Effects Test Guidelines, OPPTS 870.3650, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, July 2000.

( U.S. Environmental Protection Agency) USEPA, 2000a. Health Effects Test Guidelines, OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264070844-en.

( U.S. Environmental Protection Agency) USEPA, 2000b. Health Effects Test Guidelines, OPPTS 870.3650, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, July 2000.

Wright, J., Maslje, P., Kubaszyk, R., Esdaile, D.J., Hanley, T.R., Minnema, D., Lewis, R., 2011. Peripheral neuropathy in an extended one generation reproductive toxicity test with lead acetate. Toxicologist 26, 110–117.