Commission Regulation (EU) No 900/2014 of 15 July 2014 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (Text with EEA relevance)

COMMISSION REGULATION (EU) No 900/2014

of 15 July 2014

amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC[(1)], and in particular Article 13(2) thereof,

Whereas:

(1) Commission Regulation (EC) No 440/2008[(2)] contains the test methods for the purposes of the determination of the physico-chemical properties, toxicity and eco-toxicity of chemicals to be applied for the purposes of Regulation (EC) No 1907/2006.

(2) It is necessary to update Regulation (EC) No 440/2008 to include with priority new and updated test methods recently adopted by the OECD in order to take into account technical progress, and to ensure the reduction of the number of animals to be used for experimental purposes, in accordance with Directive 2010/63/EU of the European Parliament and of the Council[(3)]. Stakeholders have been consulted on this draft.

(3) This adaptation to technical progress contains six new test methods for the determination of toxicity and other health effects including a developmental neurotoxicity study, an extended one-generation reproductive toxicity study, an transgenic rodent *in vivo* gene mutation assay, an *in vitro* test to assess effects on the synthesis of steroid hormones, as well as two *in vivo* methods to assess oestrogenic and (anti)androgenic effects.

(4) Regulation (EC) No 440/2008 should therefore be amended accordingly.

(5) The measures provided for in this Regulation are in accordance with the opinion of the Committee established under Article 133 of Regulation (EC) No 1907/2006,

HAS ADOPTED THIS REGULATION:

Article 1

The Annex to Regulation (EC) No 440/2008 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the third day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 15 July 2014.

For the Commission

The President

José Manuel BARROSO

***Changes to legislation:** There are currently no known outstanding effects for the Commission Regulation (EU) No 900/2014. (See end of Document for details)*

ANNEX

The Annex to Regulation (EC) No 440/2008 is amended as follows:

Chapters B.53, B.54, B.55, B.56, B.57 and B.58 are inserted:

B.53 **DEVELOPMENTAL NEUROTOXICITY STUDY**

INTRODUCTION

1. This test method is equivalent to OECD Test Guideline (TG) 426 (2007). In Copenhagen in June 1995, an OECD Working Group on Reproduction and Developmental Toxicity discussed the need to update existing OECD test guidelines for reproduction and developmental toxicity, and the development of new guidelines for endpoints not yet covered (1). The working group recommended that a test guideline for developmental neurotoxicity should be written based on a US EPA guideline, which has since been revised (2). In June 1996, a second consultation meeting was held in Copenhagen to provide the Secretariat with guidance on the outline of a new test guideline on developmental neurotoxicity, including the major elements, e.g. details concerning choice of animal species, dosing period, testing period, endpoints to be assessed, and criteria for evaluating results. A US neurotoxicity risk assessment guideline was published in 1998 (3). An OECD Expert Consultation Meeting and an ILSI Risk Science Institute Workshop were held back-to-back in October 2000 and an expert consultation meeting was held in Tokyo 2005. These meetings were held to discuss the scientific and technical issues related to the current test guideline and the recommendations from the meetings (4)(5)(6)(7) were considered in the development of this test method. Additional information on the conduct, interpretation and terminology used for this test method can be found in OECD Guidance Documents No 43 on "Reproductive Toxicity Testing and Assessment" (8) and No 20 on "Neurotoxicity Testing" (9).

INITIAL CONSIDERATIONS

2. A number of chemicals is known to produce developmental neurotoxic effects in humans and other species (10)(11)(12)(13). Determination of the potential for developmental neurotoxicity may be needed to assess and evaluate the toxic characteristics of a chemical. Developmental neurotoxicity studies are designed to provide data, including dose-response characterisations, on the potential functional and morphological effects on the developing nervous system of the offspring that may arise from exposure in utero and during early life.

3. A developmental neurotoxicity study can be conducted as a separate study, incorporated into a reproductive toxicity and/or adult neurotoxicity study (e.g. test methods B.34 (14), B.35 (15), B.43 (16)), or added onto a prenatal developmental toxicity study (e.g. test method B.31 (17)). When the developmental neurotoxicity study is incorporated within or attached to another study, it is imperative to preserve the integrity of both study types. All testing should comply with applicable legislation or government and institutional guidelines for the use of laboratory animals in research (e.g. 18).

4. The testing laboratory should consider all available information on the test chemical prior to conducting the study. Such information will include the identity and structure of the chemical; its physico-chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the chemical; toxicological data on structurally related chemicals; and the anticipated use(s) of the chemical. This information is necessary to satisfy all concerned that the test is relevant for the protection of human health, and will help in the selection of an appropriate starting dose.

PRINCIPLE OF THE TEST

5. The test chemical is administered to animals during gestation and lactation. Dams are tested to assess effects in pregnant and lactating females and may also provide comparative information (dams versus offspring). Offspring are randomly selected from within litters for

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neurotoxicity evaluation. The evaluation consists of observations to detect gross neurologic and behavioural abnormalities, including the assessment of physical development, behavioural ontogeny, motor activity, motor and sensory function, and learning and memory; and the evaluation of brain weights and neuropathology during postnatal development and adulthood.

6. When the test method is conducted as a separate study, additional available animals in each group could be used for specific neurobehavioral, neuropathological, neurochemical or electrophysiological procedures that may supplement the data obtained from the examinations recommended by this test method (16)(19)(20)(21). The supplemental procedures can be particularly useful when empirical observation, anticipated effects, or mechanism/mode-of-action indicate a specific type of neurotoxicity. These supplemental procedures may be used in the dams as well as in the pups. In addition, *ex vivo* or *in vitro* procedures may also be used, as long as these procedures do not alter the integrity of the *in vivo* procedures.

PREPARATIONS FOR THE TEST

Selection of animal species

7. The preferred test species is the rat; other species can be used when appropriate. Note, however, the gestational and postnatal days specified in this test method are specific to commonly used strains of rats, and comparable days should be selected if a different species or unusual strain is used. The use of another species should be justified based on toxicological, pharmacokinetic, and/or other data. Justification should include availability of species-specific postnatal neurobehavioral and neuropathological assessments. If there was an earlier test that raised concerns, the species/strain that raised a concern should be considered. Because of the differing performance attributes of different rat strains, there should be evidence that the strain selected for use has adequate fecundity and responsiveness. The reliability and sensitivity of other species to detect developmental neurotoxicity should be documented.

Housing and feeding conditions

8. The temperature in the experimental animal room should be 22 ± 3 °C. Although the relative humidity should be at least 30 % and preferably not exceed 70 % other than during room cleaning, the aim should be 50-60 %. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. It is also possible to reverse the light cycle prior to mating and for the duration of the study, in order to perform the assessments of functional and behavioural endpoints during the dark period (under red light), i.e. during the time the animals are normally active (22). Any changes in the light-dark cycle should include adequate acclimation time to allow animals to adapt to the new cycle. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. The type of food and water should be reported and both should be analysed for contaminants.

9. Animals may be housed individually or be caged in small groups of the same sex. Mating procedures should be carried out in cages suitable for the purpose. After evidence of copulation or no later than day 15 of pregnancy, mated animals should be caged separately in delivery or maternity cages. Cages should be arranged in such a way that possible effects due to cage placement are minimised. Mated females should be provided with appropriate and defined nesting materials when parturition is near. It is well known that inappropriate handling or stress during pregnancy can result in adverse outcomes, including prenatal loss and altered foetal and postnatal development. To guard against foetal loss from factors which are not treatment-related, animals should be carefully handled during pregnancy, and stress from outside factors such as excessive outside noise should be avoided.

Preparation of the animals

10. Healthy animals should be used, which have been acclimated to laboratory conditions and have not been subjected to previous experimental procedures, unless the study is incorporated in another study (see paragraph 3). The test animals should be characterised as to

species, strain, source, sex, weight and age. Each animal should be assigned and marked with a unique identification number. The animals of all test groups should, as nearly as practicable, be of uniform weight and age, and should be within the normal range of the species and strain under study. Young adult nulliparous female animals should be used at each dose level. Siblings should not be mated, and care should be taken to ensure this. Gestation Day (GD) 0 is the day on which a vaginal plug and/or sperm are observed. Adequate acclimation time (e.g. 2-3 days) should be allowed when purchasing time-pregnant animals from a supplier. Mated females should be assigned in an unbiased way to the control and treatment groups, and as far as possible, they should be evenly distributed among the groups (e.g. a stratified random procedure is recommended to provide even distribution among all groups, such as that based on body weight). Females inseminated by the same male should be equalised across groups.

PROCEDURE

Number and sex of animals

11. Each test and control group should contain a sufficient number of pregnant females to be exposed to the test chemical to ensure that an adequate number of offspring are produced for neurotoxicity evaluation. A total of 20 litters are recommended at each dose level. Replicate and staggered-group dosing designs are allowed if total numbers of litters per group are achieved, and appropriate statistical models are used to account for replicates.

12. On or before postnatal day (PND) 4 (day of delivery is PND 0), the size of each litter should be adjusted by eliminating extra pups by random selection to yield a uniform litter size for all litters (23). The litter size should not exceed the average litter size for the strain of rodents used (8-12). The litter should have, as nearly as possible, equal numbers of male and female pups. Selective elimination of pups, e.g. based upon body weight, is not appropriate. After standardisation of litters (culling) and prior to further testing of functional endpoints, individual pups that are scheduled for pre-weaning or post-weaning testing should be identified uniquely, using any suitable humane method for pup identification (e.g. 24).

Assignment of animals for functional and behavioural tests, brain weights, and neuropathological evaluations

13. The test method allows various approaches with respect to the assignment of animals exposed *in utero* and through lactation to functional and behavioural tests, sexual maturation, brain weight determination, and neuropathological evaluation (25). Other tests of neurobehavioral function (e.g. social behaviour), neurochemistry or neuropathology can be added on a case-by-case basis, as long as the integrity of the original required tests are not compromised.

14. Pups are selected from each dose group and assigned for endpoint assessments on or after PND 4. Selection of pups should be performed so that to the extent possible both sexes from each litter in each dose group are equally represented in all tests. For motor activity testing the same pair of male and female pups should be tested at all pre-weaning ages (see paragraph 35). For all other tests the same or separate pairs of male and female animals may be assigned to different behavioural tests. Different pups may need to be assigned to weanling versus adult tests of cognitive function in order to avoid confounding the effects of age and prior training on these measurements (26)(27). At weaning (PND 21), pups not selected for testing can be disposed of humanely. Any alterations in pup assignments should be reported. The statistical unit of measure should be the litter (or dam) and not the pup.

15. There are different ways to assign pups to the pre-weaning and post-weaning examinations, cognitive tests, pathological examinations, etc., (see Figure 1 for general design and Appendix 1 for examples of assignment). Recommended minimum numbers of animals in each dose group for pre-weaning and post-weaning examinations are as follows: