OECD/OCDE 414
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OECD GUIDELINE FOR TESTING OF CHEMICALS

Prenatal developmental toxicity study

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress. The original version of the this Test Guideline (TG 414) was published in 1981 and revised in 2001 based on the output of an OECD Expert Group on Reproductive and Developmental Toxicity Testing (1). The TG 414 was updated again in 2018 to add additional endpoints to increase the possibility of detecting endocrine disrupting chemicals.

2. The selected additional endocrine disrupter relevant endpoints (AGD in fetuses and thyroid hormones in dams) were included in TG 414 following a feasibility study addressing scientific and technical concerns regarding inclusion of additional endpoints in the test method (2). The 2018 update is to include rat-specific requirements in the TG 414; thus applies to rats and not to rabbits.

INITIAL CONSIDERATIONS

3. This Guideline for developmental toxicity testing is designed to provide general information concerning the effects of prenatal exposure on the pregnant test animal and on the developing organism; this may include assessment of maternal effects as well as death, structural abnormalities, or altered growth in the fetus. Functional deficits, although an important part of development, are not a part of this Guideline. They may be tested for in a separate study or as an adjunct to this study using the Guideline for developmental neurotoxicity. For information on testing for functional deficiencies and other postnatal effects, the Guidelines 416, 421/422, 426 and 443 (3-7) should be consulted.

4. This Guideline may require specific adaptation in individual cases on the basis of specific knowledge on e.g. physicochemical or toxicological properties of the test chemical. Such adaptation is acceptable, when convincing scientific evidence suggests that the adaptation will lead to a more informative test. In such a case, this scientific evidence should be carefully documented in the study report. In conducting the study, the guiding principles and considerations outlined in the OECD Guidance Document 19 on euthanizing for humane reasons should be followed (8).

Definitions used are given in Annex A.

In accordance with the decision of the Council on a delegation of authority to amend Annex I of the decision of the council on the Mutual Acceptance of Data in the assessment of chemicals [C(2018)49], this Guideline was amended by the OECD's Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology by written procedure on 25 June 2018..

PRINCIPLE OF THE TEST

5. Normally, the test chemical is administered to pregnant animals at least from implantation to one day prior to the day of scheduled humane killing, which should be as close as possible to the normal day of delivery without risking loss of data resulting from early delivery. The Guideline is not intended to examine solely the period of organogenesis, (e.g. days 5-15 in the rodent, and days 6-18 in the rabbit) but also effects from preimplantation, when appropriate, through the entire period of gestation to the day of caesarean section. Shortly before caesarean section, the females are killed, the uterine contents are examined, and the fetuses are evaluated for soft tissue and skeletal changes.

PREPARATION FOR THE TEST

Selection of animal species

6. It is recommended that testing be performed in the most relevant species, and that laboratory species and strains which are commonly used in prenatal developmental toxicity testing be employed. The preferred rodent species is the rat and the preferred non-rodent species is the rabbit. Justification should be provided if another species is used.

Housing and feeding conditions

7. The temperature in the experimental animal room should be (22 ± 3)°C for rodents and (18 ± 3)°C rabbits. 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. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

8. Care should be taken to avoid diets or animal bedding that may contain unacceptably high levels of hormonally active substances prone or likely to interfere with the interpretation of the study results (e.g., phytoestrogens). High levels of phytooestrogens in laboratory diets have been known to increase uterine weights in rodents. As a guide, dietary levels of phytooestrogens should not exceed 350 µg of genistein equivalents/gram of rodent laboratory diet.

9. Mating procedures should be carried out in cages suitable for the purpose. While individual housing of mated rodents is preferred, group housing in small numbers is also acceptable. Mated females should be provided with nesting material at the end of the pregnancy. In the case of rabbits, animals should be housed individually.

Preparation of the animals

10. Healthy animals, which have been acclimated to laboratory conditions for at least five days and have not been subjected to previous experimental procedures, should be used. The test animals should be characterised as to species, strain, source, sex, weight and/or age. The animals of all test groups should, as nearly as practicable, be of uniform weight and age. Young adult nulliparous female animals should be used at each dose level. The females should be mated with males of the same species and strain, and the mating of siblings should be avoided. For rodents day 0 of gestation is the day on which a vaginal plug and/or sperm are observed; for rabbits day 0 is usually the day of coitus or of artificial insemination, if this technique is used. Cages should be arranged in such a way that possible effects due to cage placement are minimised. Each animal should be assigned a unique identification number.

Mated females should be assigned in an unbiased manner to the control and treatment groups, and if the females are mated in batches, the animals in each batch should be evenly distributed across the groups. Similarly, females inseminated by the same male should be evenly distributed across the groups.

PROCEDURE

Number and sex of animals

11. Each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy. Groups with fewer than 16 animals with implantation sites may be inappropriate. Maternal mortality does not necessarily invalidate the study providing it does not exceed approximately 10 percent.

Preparation of doses

12. If a vehicle or other additive is used to facilitate dosing, consideration should be given to the following characteristics: effects on the absorption, distribution, metabolism, and retention or excretion of the test chemical; effects on the chemical properties of the test chemical which may alter its toxic characteristics; and effects on the food or water consumption or the nutritional status of the animals. The vehicle should neither be developmentally toxic nor have effects on reproduction.

Dosage

13. Normally, the test chemical should be administered daily from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section. If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled humane killing. It is well known that inappropriate handling or stress during pregnancy can result in prenatal loss. To guard against fetal loss from factors which are not treatment-related, unnecessary handling of pregnant animals as well as stress from outside factors such as noise should be avoided.

14. At least three dose levels and a concurrent control should be used. Healthy animals should be assigned in an unbiased manner to the control and treatment groups. The dose levels should be spaced to produce a gradation of toxic effects. Unless limited by the physical/chemical nature or biological properties of the test chemical, the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. At least one intermediate dose level should produce minimal observable toxic effects. The lowest dose level should not produce any evidence of either maternal or developmental toxicity. A descending sequence of dose levels should be selected with a view to demonstrating any dosage-related response and no-observed-adverse-effect level (NOAEL) or doses near the limit of detection that would allow the determination of a benchmark dose. Two- to four-fold intervals are frequently optimal for setting the descending dose levels, and the addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages. Although establishment of a maternal NOAEL is the goal, studies which do not establish such a level may also be acceptable (9).

15. Dose levels should be selected taking into account any existing toxicity data as well as additional information on metabolism and toxicokinetics of the test chemical or related

materials. This information will also assist in demonstrating the adequacy of the dosing regimen.

16. A concurrent control group should be used, and it should follow the same dosing regimen than the treated groups. This group should be a sham-treated control group or a vehicle-control group if a vehicle is used in administering the test chemical. All groups should be administered the same volume of either test chemical or vehicle. Animals in the control group(s) should be handled in an identical manner to test group animals. Vehicle control groups should receive the vehicle in the highest amount used (as in the lowest treatment group).

Limit test

17. If a test at one dose level of at least 1000 mg/kg body weight/day by oral administration, using the procedures described for this study, produces no observable toxicity and if an effect would not be expected based upon existing data (e.g., from structurally and/or metabolically related compounds), then a full study using three dose levels may not be considered necessary. Expected human exposure may indicate the need for a higher oral dose level to be used in the limit test. For other types of administration, such as inhalation or dermal application, the physical chemical properties of the test chemical often may indicate the maximum attainable level of exposure (for example, dermal application should not cause severe localised toxicity).

Administration of doses

18. The test chemical or vehicle is usually administered orally by intubation. If another route of administration is used, the tester should provide justification and reasoning for its selection, and appropriate modifications may be necessary (10-12). The test chemical should be administered at approximately the same time each day.

19. The dose to each animal should normally be based on the most recent individual body weight determination. However, caution should be exercised when adjusting the dose during the last trimester of pregnancy. Existing data should be used for dose selection to prevent excess maternal toxicity. However, if excess toxicity is noted in the treated dams, those animals should be humanely killed. If several pregnant animals show signs of excess toxicity, consideration should be given to terminating that dose group. When the test chemical is administered by gavage, this should preferably be given as a single dose to the animals using a stomach tube or a suitable intubation canula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1 ml/100 g body weight, except in the case of aqueous solutions where 2 ml/100 g body weight may be used. When corn oil is used as a vehicle, the volume should not exceed 0.4 ml/100 g body weight. Variability in test volume should be minimised by adjusting the concentrations to ensure a constant volume across all dose levels.

Observations of the dams

20. Clinical observations should be made and recorded at least once a day, preferably at the same time(s) each day taking into consideration the peak period of anticipated effects after dosing. The condition of the animals should be recorded including mortality, moribundity, pertinent behavioural changes, and all signs of overt toxicity.

Body weight and food consumption

21. Animals should be weighed on day 0 or no later than day 3 if time-mated animals are supplied by an outside breeder, on the first day of dosing, at least every 3 days during the dosing period and on the day of scheduled humane killing. Body weight measures from pregnant and non-pregnant females should not be combined.

22. Food consumption should be recorded at three-day intervals and should coincide with days of body weight determination.

Post-mortem examination

23. Females should be humanely killed one day prior to the expected day of delivery. Females showing signs of abortion or premature delivery prior to scheduled humane killing should be killed and subjected to a thorough macroscopic examination.

24. At the time of termination or death during the study, the dam should be examined macroscopically for any structural abnormalities. The weight of the thyroid gland and histopathological assessment of the thyroid gland should be taken from every dam to observe pathological changes. Evaluation of the dams during caesarean section and subsequent fetal analyses should be conducted preferably without knowledge of treatment group in order to minimise bias. Effort will be taken to avoid sampling bias by randomising sample collection across treatment groups (i.e. avoiding collection of all of one dose group followed by the next dose group, and so on).

Examination of uterine contents

25. Immediately after termination or as soon as possible after death, the uteri should be removed and the pregnancy status of the animals ascertained. Uteri that appear non-gravid should be further examined (e.g. by ammonium sulphide staining for rodents and Salewski staining or a suitable alternative method for rabbits) to confirm the non-pregnant status (13).

26. Gravid uteri including the cervix should be weighed. Gravid uterine weights should not be obtained from animals found dead during the study.

27. The number of corpora lutea should be determined for pregnant animals.

28. The uterine contents should be examined for numbers of embryonic or fetal deaths and viable fetuses. The degree of resorption should be described (early, late) in order to estimate the relative time of death of the conceptus (see Annex for definitions).

Examination of fetuses

29. The sex and body weight of each fetus should be determined. The anogenital distance (AGD) should be measured in all live rodent fetuses.

30. Each fetus should be examined for external alterations (14).

31. Fetuses should be examined for skeletal and soft tissue alterations (e.g. variations and malformations or anomalies) (15-32). Categorisation of fetal alterations is preferable but not required. When categorisation is done, the criteria for defining each category should be clearly stated. Particular attention should be paid to the reproductive tract which should be examined for signs of altered development. External fetal sex (as determined by gross examination) should be compared with internal (gonadal) sex in all fetuses (examined for both skeletal and soft tissue malformations). In addition, indication of incomplete testicular descent/cryptorchidism should be noted in male fetuses.

32. For rodents, approximately one-half of each litter should be prepared and examined for skeletal alterations. The remainder should be prepared and examined for soft tissue alterations, using accepted or appropriate serial sectioning methods or careful gross dissection techniques.

33. For non-rodents, e.g. rabbits, all fetuses should be examined for both soft tissue and skeletal alterations. The bodies of these fetuses are evaluated by careful dissection for soft tissue alterations, which may include procedures to further evaluate internal cardiac structure (33). The heads of one-half of the fetuses examined in this manner should be removed and processed for evaluation of soft tissue alterations (including eyes, brain, nasal passages and tongue), using standard serial sectioning methods (34) or an equally sensitive method. The bodies of these fetuses and the remaining intact fetuses should be processed and examined for skeletal alterations, utilising the same methods as described for rodents.

Blood sample collection (rats)

34. All blood samples should be stored under appropriate conditions. Blood samples should be collected as follows:

- From all dams at termination for mandatory assessment of thyroid hormones T4, T3 and thyroid stimulating hormone (TSH) within a short timeframe (e.g. two hours) on the morning of the day of necropsy. Effort should be made to avoid sampling bias by randomising blood collection across treatment groups. Blood samples from non-pregnant females should not be pooled with pregnant dams.
- As an option, other hormones may be measured if relevant.
- For quality control it is proposed that historical control data are collected and coefficients of variation are calculated for analytes, especially for the parameters linked with endocrine system function. These data can be used for comparison purposes when subsequent studies are evaluated.

DATA AND REPORTING

Data

35. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals at the start of the test, the number of animals found dead during the test or killed for humane reasons, the time of any death or humane kill, the number of pregnant females, the number of animals showing signs of toxicity, a description of the signs of toxicity observed(including time of onset, duration and severity of any toxic effects), the types of histopathological changes (thyroid gland), the types of fetal observations and all relevant litter data..

36. Numerical results should be evaluated by an appropriate statistical method using the litter as the unit for data analysis. A generally accepted statistical method or new advanced statistical method should be used; the statistical methods should be selected as part of the design of the study. Data from animals that do not survive to the scheduled humane killing should also be reported. These data may be included in group means where relevant. Relevance of the data from such an animal, and therefore inclusion or exclusion from any group mean(s), should be judged on an individual basis.

Evaluation of Results

37. The findings of the Prenatal Developmental Toxicity Study should be evaluated in the light of the observed effects. The evaluation will include the following information:

- maternal and fetal test results, including an evaluation of the relationship, or lack thereof, between the exposure of the animals to the test chemical and the incidence and severity of all findings;
- criteria used for categorising fetal external, soft tissue, and skeletal alterations if categorisation has been done;
- historical control data to enhance interpretation of study results, as appropriate;
- (raw) numbers used in calculating all percentages or indices;
- adequate statistical analysis of the study findings, including sufficient information on the method of analysis, so that an independent reviewer/statistician can re-evaluate and reconstruct the analysis.

38. In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test chemical should be considered.

Test report

39. The test report or study records should include the following specific information:

- Test chemical:
  - chemical identification, such as IUPAC or CAS name, CAS number, SMILES or InChI code, structural formula, purity, chemical identity of impurities as appropriate and practically feasible, etc.
  - source, lot number, limit date for use, if available;
  - stability of the test chemical, if known;
  - homogeneity of the test chemical, if known;
- Mono-constituent substance:
  - physical appearance, water solubility, and additional relevant physicochemical properties;
- Multi-constituent substance, UVCBs and mixtures:
  - characterised as far as possible by chemical identity (see above), quantitative occurrence and relevant physicochemical properties of the constituents.
- Vehicle (if appropriate):
  - justification for choice of vehicle, if other than water.
- Test animals:
  - species and strain used;
  - number and age of animals;
  - source, housing conditions, diet, etc.;
  - individual weights of animals at the start of the test.
- Test conditions:
  - rationale for dose level selection;
  - details of test chemical formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation;
  - details of the administration of the test chemical;
  - conversion from diet/drinking water test chemical concentration (ppm) to the actual dose (mg/kg body weight/day), if applicable;
  - environmental conditions;

- details of food and water quality.

Results:

- Maternal toxic response data by dose, including but not limited to:
  - the number of animals at the start of the test, the number of animals surviving, the number pregnant, and the number aborting, number of animals delivering early;
  - day of death during the study or whether animals survived to termination;
  - data from animals that do not survive to the scheduled humane kill should be reported but not included in the inter-group statistical comparisons;
  - day of observation of each abnormal clinical sign and its subsequent course;
  - body weight, body weight change and gravid uterine weight, including, optionally, body weight change corrected for gravid uterine weight;
  - food consumption and, if measured, water consumption;
  - From rats dams, thyroid hormones T4, T3 and thyroid-stimulating hormone (TSH), and other hormone levels (if measured), details included on the hormone kit or antibody used to hormones, historical control data for the laboratory (means and standard deviations), along the limit of detection/limit of quantification
  - necropsy findings, including uterine weight;
  - NOAEL values for maternal and developmental effects should be reported.
- Developmental endpoints by dose for litters with implants, including:
  - number of corpora lutea;
  - number of implantations, number and percent of live and dead fetuses and resorptions;
  - number and percent of pre- and post-implantation losses.
- Developmental endpoints by dose for litters with live fetuses, including:
  - number and percent of live offspring;
  - sex ratio;
  - fetal body weight, preferably by sex and with sexes combined;
  - Anogenital distance of all rodent fetuses (statistically evaluated by sex/gender and related to weight)
  - external, soft tissue, and skeletal malformations and other relevant alterations;
  - criteria for categorisation if appropriate;
  - total number and percent of fetuses and litters with any external, soft tissue, or skeletal alteration, as well as the types and incidences of individual anomalies and other relevant alterations (including indication of incomplete testicular descent/cryptorchidism should be noted in male fetuses.).
- Discussion of results.
- Conclusions.

Interpretation of Results

40. A prenatal developmental toxicity study will provide information on the effects of repeated oral exposure to a substance during pregnancy. The results of the study should be interpreted in conjunction with the findings of sub-chronic, reproduction, toxicokinetic and other studies. Since emphasis is placed on both general toxicity and developmental toxicity endpoints, the results of the study will allow for the discrimination between developmental effects occurring in the absence of general toxicity and those which are only expressed at

levels that are also toxic to the maternal animal (35). OECD Guidance Document 43 should be consulted for aid in the interpretation of reproduction and developmental results (36). OECD Guidance Document 106 on Histologic Evaluation of Endocrine and Reproductive Tests in Rodents (37) provides information on the preparation and evaluation of (endocrine) organs and may be helpful for TG 414 studies.

Literature

(1) Organisation for Economic Co-operation and Development (1995). Report of the OECD *Ad Hoc* Working Group on Reproduction and Developmental Toxicity. Copenhagen, Denmark, (13th-14th June 1995).

(2) OECD (2018). Feasibility Study for Minor Enhancements of TG 414 with ED Relevant Endpoints. Environment, Health and Safety Publications, Series on Testing and Assessment No.285, Organisation for Economic Cooperation and Development, Paris.

(3) OECD (2001), Test No. 416: Two-Generation Reproduction Toxicity, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264070868-en

(4) OECD (2016), Test No. 421: Reproduction/Developmental Toxicity Screening Test, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264380-en

(5) OECD (2016), Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264403-en

(6) OECD (2007), Test No. 426: Developmental Neurotoxicity Study, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264067394-en

(7) OECD (2012), Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264185371-en

(8) OECD (2000). Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluations, Environment, Health and Safety Publications, Series on Testing and Assessment, (No. 19), Organisation for Economic Cooperation and Development, Paris.

(9) Kavlock R.J. et al. (1996). A Simulation Study of the Influence of Study Design on the Estimation of Benchmark Doses for Developmental Toxicity. Risk Anal. 16, 399-410.

(10) Kimmel, C.A. and Francis, E.Z. (1990). Proceedings of the Workshop on the Acceptability and Interpretation of Dermal Developmental Toxicity Studies. Fundam. Appl. Toxicol. 14, 386-398.

(11) Wong, B.A., et al. (1997). Developing Specialized Inhalation Exposure Systems to Address Toxicological Problems. CIIT Activities 17, 1-8.

(12) US Environmental Protection Agency (1985). Subpart E - Specific Organ/Tissue Toxicity, 40 CFR 798.4350: Inhalation Developmental Toxicity Study.

(13) Salewski, E. (1964). Faerbermethode zum Makroskopischen Nachweis von Implantations Stellen am Uterus der Ratte. Naunyn-Schmeidebergs Arch. Pharmakol. Exp. Pathol. 247, 367.

(14) Edwards, J.A. (1968). The External Development of the Rabbit and Rat Embryo. In: Adv. Teratol. D.H.M. Woolam (ed.), Vol. 3. Academic Press, NY.

(15) Inouye, M. (1976). Differential Staining of Cartilage and Bone in Fetal Mouse Skeleton by Alcian Blue and Alizarin Red S. Congen. Anom. 16, 171-173.

(16) Igarashi, E. et al. (1992). Frequency of Spontaneous Axial Skeletal Variations Detected By the Double Staining Technique for Ossified and Cartilaginous Skeleton in Rat Foetuses. Congen. Anom. 32, 381-391.

(17) Kimmel, C.A. et al. (1993). Skeletal Development Following Heat Exposure in the Rat. Teratology 47, 229-242.

(18) Marr, M.C. et al. (1988). Comparison of Single and Double Staining for Evaluation of Skeletal Development: The Effects of Ethylene Glycol (EG) in CD Rats. Teratology 37, 476.

(19) Barrow, M.V. and Taylor, W.J. (1969). A Rapid Method For Detecting Malformations in Rat Foetuses. J. Morphol. 127, 291-306.

(20) Fritz, H. (1974). Prenatal Ossification in Rabbits as Indicative of Foetal Maturity. Teratology 11, 313-320.

(21) Gibson, J.P. et al. (1966). Use of the Rabbit in Teratogenicity Studies. Toxicol. Appl. Pharmacol. 9, 398-408.

(22) Kimmel, C.A. and Wilson, J.G. (1973). Skeletal Deviation in Rats: Malformations or Variations? Teratology 8, 309-316.

(23) Marr, M.C. et al. (1992). Developmental Stages of the CD (Sprague-Dawley) Rat Skeleton after Maternal Exposure to Ethylene Glycol. Teratology 46, 169-181.

(24) Monie, I.W. et al. (1965). Dissection Procedures for Rat Foetuses Permitting Alizarin Red Staining of Skeleton and Histological Study of Viscera. Supplement to Teratology Workshop Manual, pp. 163-173.

(25) Spark, C. and Dawson, A.B. (1928). The Order and Time of Appearance of Centers of Ossification in the Fore and Hind Limbs of the Albino Rat, With Special Reference to the Possible Influence of the Sex Factor. Am. J. Anat. 41, 411-445.

(26) Staples, R.E. and Schnell, V.L. (1964). Refinements in Rapid Clearing Technique in the KOH-Alizarin Red S Method for Fetal Bone. Stain Technol. 39, 61-63.

(27) Strong, R.M. (1928). The Order, Time and Rate of Ossification of the Albino Rat (Mus norvegicus albinus) Skeleton. Am. J. Anat. 36, 313-355.

(28) Stuckhardt, J.L. and Poppe, S.M. (1984). Fresh Visceral Examination of Rat and Rabbit Foetuses Used in Teratogenicity Testing. Teratogen. Carcinogen. Mutagen. 4, 181-188.

(29) Walker, D.G. and Wirtschafter, Z.T. (1957). The Genesis of the Rat Skeleton. Thomas, Springfield, IL.

(30) Wilson, J.G. (1965) Embryological Considerations in Teratology. In: Teratology: Principles and Techniques, J.G. Wilson and J. Warkany (eds.), University of Chicago, Chicago, IL, pp 251-277.

(31) Wilson, J.G. and Fraser, F.C. (eds.) (1977). Handbook of Teratology, Vol. 4. Plenum, NY.

(32) Varnagy, L. (1980). Use of Recent Fetal Bone Staining Techniques in the Evaluation of Pesticide Teratogenicity. Acta Vet. Acad. Sci. Hung. 28, 233-239.

(33) Staples, R.E. (1974). Detection of Visceral Alterations in Mammalian Foetuses. Teratology 9, 37-38.

(34) Van Julsingha, E.B. and C.G. Bennett (1977). A Dissecting Procedure For the Detection of Anomalies in the Rabbit Foetal Head. In: Methods in Prenatal Toxicology (eds. D. Neubert, H.J. Merker and T.E. Kwasigroch). University of Chicago, Chicago, IL, pp. 126-144.

(35) US Environmental Protection Agency (1991). Guidelines for Developmental Toxicity Risk Assessment. Fed. Register 56, 63798-63826.

(36) OECD (2008). Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. Environment, Health and Safety Publications, Series on Testing and Assessment (No. 43.), Organisation for Economic Cooperation and Development, Paris.

(37) OECD. (2009).Guidance Document for Histologic Evaluation of Endocrine and Reproductive Tests in Rodents. Environment, Health and Safety Publications, Series on Testing and Assessment (No. 106) Organisation for Economic Cooperation and Development, Paris.

(38) Wise, D.L. et al. (1997). Terminology of Developmental Abnormalities in Common Laboratory Mammals (Version 1). Teratology 55, 249-292.

Annex A. DEFINITIONS

Developmental toxicology: the study of adverse effects on the developing organism that may result from exposure prior to conception, during prenatal development, or postnatally to the time of sexual maturation. The major manifestations of developmental toxicity include 1) death of the organism, 2) structural abnormality, 3) altered growth, and 4) functional deficiency. Developmental toxicology was formerly often referred to as teratology.

Adverse effect: any treatment-related alteration from baseline that diminishes an organism's ability to survive, reproduce or adapt to the environment. Concerning developmental toxicology, taken in its widest sense it includes any effect which interferes with normal development of the conceptus, both before and after birth.

Altered growth: an alteration in offspring organ or body weight or size.

Alterations (anomalies): structural alterations in development that include both malformations and variations (38):

> **Malformation**/Major Abnormality: Structural change considered detrimental to the animal (may also be lethal) and is usually rare.
>
> **Variation**/Minor Abnormality Structural change considered to have little or no detrimental effect on the animal; may be transient and may occur relatively frequently in the control population.

Conceptus: the sum of derivatives of a fertilised ovum at any stage of development from fertilisation until birth including the extra-embryonic membranes as well as the embryo or fetus.

Implantation (nidation): attachment of the blastocyst to the epithelial lining of the uterus, including its penetration through the uterine epithelium, and its embedding in the endometrium.

Embryo: the early or developing stage of any organism, especially the developing product of fertilisation of an egg after the long axis appears and until all major structures are present.

Embryotoxicity: detrimental to the normal structure, development, growth, and/or viability of an embryo.

Fetus: the unborn offspring in the post-embryonic period.

Fetotoxicity: detrimental to the normal structure, development, growth, and/or viability of a fetus.

Abortion: the premature expulsion from the uterus of the products of conception: of the embryo or of a nonviable fetus.

Resorption: a conceptus which, having implanted in the uterus, subsequently died and is being, or has been resorbed:

> **Early resorption:** evidence of implantation without recognisable embryo/fetus.
> **Late resorption:** dead embryo or fetus with external degenerative changes.

NOAEL: abbreviation for no-observed-adverse-effect level and is the highest dose level where no adverse treatment-related findings are observed.

Thyroid activity: is the capability of a chemical to interfere with the production, transportation, and metabolism of thyroid hormones by a variety of mechanisms.