DRAFT UPDATED TEST GUIDELINE 407

Repeated Dose 28-Day Oral Toxicity Study in Rodents; Updated with Parameters for Endocrine Effects

INTRODUCTION

u1a The OECD initiated a high-priority activity in 1998 to revise existing guidelines and to develop new guidelines for the screening and testing of potential endocrine disruptors (8). One element of the activity was to update the existing OECD guideline for "repeated dose 28-day oral toxicity study in rodents" (TG 407) by parameters suitable to detect endocrine activity of test substances. This procedure underwent an extensive international program to test for the relevance and practicability of the additional parameters, the performance of these parameters for chemicals with (anti)oestrogenic, (anti)androgenic, and (anti)thyroid activity, the intra- and interlaboratory reproducibility, and the interference of the new parameters with those required by the prior TG 407. The chemicals used in this international program were ethinylestradiol, genistein, nonylphenol, tamoxifen, CGS 18320 B, methyl testosterone, flutamide, p,p'-DDE, propylthiouracil, and l-thyroxine. The large amount of data thereby obtained has been compiled and evaluated in detail in a comprehensive OECD report (9).This updated Test Guideline 407 is the outcome of the experience and results gained during the international test program. This TG 407 now allows to put endocrine mediated effects into context to other toxicological effects.

u1b The rational for enhancing existing test guidelines to detect endocrine mediated effects is contained in the OECD monograph 21 (10). A list of potential updates is given in this monograph and the suitability of most of them was tested in phase I of this international program with flutamide and propylthiouracile. Further compounds were investigated in phase-I as well, but these studies did not include all endpoints required by the prior TG 407 such as FOB or motor activity assessment, some of the studies were not reported adequately, and some used too low dose levels in comparison to the requirements of the prior TG 407. The experience thereby gained with regard to practicability, reproducibility and sensitivity of the updated parameters led to the selection of those to be added to the prior TG 407 for the international phase II testing. The results obtained by this phase II testing are the basis of the current TG 407.

u1c This assay serves as an in vivo method providing data about multiple endocrine mechanisms and effects and should be seen in the context of the "OECD Conceptual Framework of the Testing and Assessment of Endocrine Disrupting Chemicals" (Annex 2). In this Conceptual Framework this TG 407 is contained in level 4. It provides data on and gives an indication of the dose response relationship of multiple endocrine mechanisms and effects in addition to the information provided by the prior TG 407 (11).

u1d In the course of the international evaluation of this TG 407 test procedure, strongly acting endocrine active chemicals with (anti) oestrogenic, (anti) androgenic or (anti) thyroidal activity were clearly identified with appropriate sensitivity. There also was a good indication by the data obtained with methyl testosterone and p,p'-DDE that this TG 407 will detect chemicals exerting a weak activity on the thyroid. Regarding genistein (phytoestrogen), nonylphenol (weak estrogenic potential), and p,p´-DDE (weak anti-androgenic potential) the hormonal activity was too low compared to other effects to give a clear response in hormone related parameters up to the highest dose tested or results were equivocal (genistein) across the laboratories.

u1e A detailed analyses of the results obtained by the international testing program led to the conclusion that some further updates to the prior TG 407 might increase the sensitivity especially for weak oestrogens and (anti) androgens (9). It is recommended to perform histopathology of the female as well as of the male mammary gland and to evaluate the histopathology of the female reproductive tract not only for clear pathological changes but especially also for synchronisation with the oestrous cycle as determined by vaginal smears. These investigations were not contained in the protocol provided to the laboratories participating in the international program.

u1f The results obtained by the international program could not give clear guidance whether the determination of thyroid hormones should be included in the TG 407(9). There was a good indication that T4, T3 and TSH should be added as updated parameters.

u1g The international program showed that the quality of data obtained by this TG 407 will depend much on the experience of the test laboratory. This relates specifically to the histopathological determination of cyclic changes in the female reproductive organs and to the weight determination of the small hormone dependent organs which are difficult to dissect. Thus, appropriate quality controls are recommended for laboratories carrying out TG 407 tests.

u2a This TG 407 is the outcome of a meeting of the Validation Management Group on mammalian tests (VMG-mammalian) of the Endocrine Disruptor Testing and Assessment Task Force (EDTA), held in Washington in April 2006 (12) and of the EDTA, held in Stockholm in April 2006 (13). It is based on the data obtained by the international program during development of this method.

u2b Taking account of the large number of animals, the high costs, and the long time required for performing an investigation according to the TG 407 it was and it is not appropriate to carry out a full validation program as required for example for in vitro tests. Neither animal welfare considerations nor economic aspects would justify such a complete validation effort. It is recognized that some of the parameters to be investigated within this TG 407 need further validation and possibly refinements. Such additional experience will be gained in the course of routine applications of this new test procedure.

u2c It is proposed that this TG 407 should be used in the years to come to test for effects arising from repeated exposure including those related to the endocrine system. After further experience has been gained with this test procedure worldwide a group of experts should be called together in order to modify, refine or endorse the TG 407. For future modifications, if necessary, specific emphasis should be placed on the value of some of the endpoints that at present cannot be judged definitively, e.g. histopathology of the mammary gland, synchronisation of cyclicity of the female reproductive organs, and thyroid related hormones.

INITIAL CONSIDERATIONS AND LIMITATIONS

u3 In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute toxicity testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time including effects on the endocrine system. The method comprises the basic repeated dose toxicity study (11) that maybe used for chemicals on which a 90-day study is not warranted (e.g. when the production volume does not exceed certain limits) or as a preliminary to a long-term study. The duration of exposure should be 28 days.

u4a The TG 407 has been modified to include endpoints to identify chemicals that interfere with thyroid physiology and affect the male and/or female reproductive organs in young adult animals, while still investigating all other toxicological parameters required under the prior TG 407. On the basis of data

generated in the validation process, it must be emphasized that the sensitivity of this assay is not sufficient to identify all substances with (anti)androgenic or (anti)estrogenic modes of action. The TG is not performed in a life-stage that is most sensitive to endocrine disruption. The TG nevertheless identifies strong or moderate endocrine active substances but in most cases will fail to identify weak endocrine active substances. Thus it can't be described as a screening assay for endocrine activity.

4a' Consequently, the lack of effects related to these modes of action can not be taken as evidence for the lack of effects on the endocrine system. Regarding endocrine mediated effects, compound characterization should not therefore be based on the results of this TG alone but should use a weight of evidence approach using all existing data on a chemical to characterise potential endocrine activity. Regulatory decision making on endocrine activity (compound characterisation) should be based on this weight of evidence and not be solely on results from application of this TG.

u4b The TG 407 is designed to identify possible chemical hazards from the toxic effects observed in the study, including effects related to the endocrine system. That is, the results from the TG 407 should be used for hazard identification and risk assessment. The results obtained by the endocrine related parameters should be seen in the context of the "OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals" (Annex 2).

u4c It is acknowledged that all animal-based procedures will conform to local standards of animal care; the descriptions of care and treatment set forth below are minimal performance standards, and will be superseded by local regulations. Further guidance of the humane treatment of animals is given by the OECD (14)**.**

5. Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

6. The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals which die or are killed during the test are necropsied and at the conclusion of the test surviving animals are killed and necropsied.

DESCRIPTION OF THE METHOD

Selection of animal species

u7. This guideline specifically relates to the rat, since in the international testing program the rat was the only species used. If the parameters specified within this TG 407 are investigated in another rodent species a detailed justification should be given and it should be demonstrated that the species selected will respond to the updated parameters with a sensitivity comparable to the rat. Commonly used laboratory strains of young healthy adult animals should be employed. Females should be nulliparous and non pregnant. Dosing should begin as soon as possible after weaning when the animals are 7 weeks old. In any case, at the start of the study the animals must not be older than 9 weeks. At the commencement of the study the weight variation of animals used should be minimal and not exceed ± 20% of the mean weight of each sex. When a repeated oral dose is conducted as a preliminary to a longer-term study, preferably animals from the same strain and source should be used in both studies.

Housing and feeding

8. The temperature in the experimental animal room should be 22°C (± 3°C). Although the relative humidity should be at least 30% and preferably not to 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. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance when administered by this method. Animals may be housed individually, or be caged in small groups of the same sex; for group caging, no more than five animals should be housed per cage.

u8a The feed should be regularly analysed for contaminants. A sample of the diet should be retained until finalisation of the report. In case of unexpected results an analysis of the diet for oestrogenic compounds may be considered.

Preparation of animals

9. Healthy young adult animals are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimized. The animals are identified uniquely and kept in their cages for at least five days prior to the start of the study to allow for acclimatisation to the laboratory conditions.

Preparation of doses

10. The test compound is administered by gavage or via the diet or drinking water. The method of oral administration is dependent on the purpose of the study, and the physical/chemical properties of the test material.

11. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

PROCEDURE

Number and sex of animals

12. At least 10 animals (five female and five male) should be used at each dose level. If interim kills are planned, the number should be increased by the number of animals scheduled to be killed before the completion of the study. Consideration should be given to an additional satellite group of ten animals (five per sex) in the control and in the top dose group for observation of reversibility, persistence, or delayed occurrence of toxic effects, for at least 14 days post treatment.

Dosage

13. Generally, at least three test groups and a control group should be used, but if from assessment of other data, no effects would be expected at a dose of 1000mg/kg bw/d, a limit test may be performed. If there are no suitable data available, a range finding study may be performed to aid the determination of the doses to be used. Except for treatment with the test substance, animals in the control group should be handled in an

identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

14. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

u14a Endocrine-mediated effects may be a secondary consequence of toxicity. In order to correctly identify such interference or confounding there should be at least 2 doses below the overtly toxic dose.

Limit test

15. If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

Administration of doses

u16. The animals are dosed with test substance daily 7 days each week for a period of 28 days for males; females are treated for a period of 28 to 32 days depending on the day they are found in dioestrus. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. 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/100g body weight except in the case of aqueous solutions where 2 ml/100 g body weight may be used. Except for irritating or corrosive substances, which will normally reveal exacerbated effects with higher concentrations, variability in test volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels.

17. For substances administered via the diet or drinking water it is important to ensure that the quantities of the test substance involved do not interfere with normal nutrition or water balance. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used must be specified. For a substance administered by gavage, the dose should be given at similar times each day, and adjusted as necessary to maintain a constant dose level in terms of animal body weight. Where a repeated dose study is used as a preliminary to a long term study, a similar diet should be used in both studies.

Observations

u18. The observation period should be 28 days for males and for individual females until completion of an oestrus cycle, maximally 32 days (see paragraph 16). Animals in a satellite group scheduled for follow-up observations should be kept for at least 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.

19. General clinical observations should be made at least once a day, preferably at the same time(s) each day and considering the peak period of anticipated effects after dosing. The health condition of the animals should be recorded. At least twice daily, all animals are observed for morbidity and mortality.

20. Once before the first exposure (to allow for within-subject comparisons), and at least once a week thereafter, detailed clinical observations should be made in all animals. These observations should be made outside the home cage in a standard arena and preferably at the same time, each time. They should be carefully recorded, preferably using scoring systems, explicitly defined by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observations are preferably conducted by observers unaware of the treatment. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) should also be recorded (2).

21. In the fourth exposure week sensory reactivity to stimuli of different types (2) (e.g. auditory, visual and proprioceptive stimuli) (3)(4)(5), assessment of grip strength (6) and motor activity assessment (7) should be conducted. Further details of the procedures that could be followed are given in the respective references. However, alternative procedures than those referenced could also be used.

22. Functional observations conducted in the fourth exposure week may be omitted when the study is conducted as a preliminary study to a subsequent subchronic (90-day) study. In that case, the functional observations should be included in this follow-up study. On the other hand, the availability of data on functional observations from the repeated dose study may enhance the ability to select dose levels for a subsequent subchronic study.

23. Exceptionally, functional observations may also be omitted for groups that otherwise reveal signs of toxicity to an extent that would significantly interfere with the functional test performance.

u23a At the end of the 4th week, the oestrus cycle of all females shall be determined daily by taking vaginal smears for at least 5 consecutive days starting on day 24 at the latest. Vaginal smears should be evaluated blind to the treatment groups, although with some knowledge of the previous days'identity.

u23b. It should be understood that the limited number of days that the oestrus cycle is evaluated will not permit a reliable determination of cycle irregularities, but will allow females to be sacrificed on a comparable day. This will be during dioestrus following 28-32 days chemical administration. Female not found in dioestrus during this time period are necropsied on day 32.

Body weight and food/water consumption

24. All animals should be weighed at least once a week. Measurements of food consumption should be made at least weekly. If the test substance is administered via the drinking water, water consumption should also be measured at least weekly.

Haematology

25. The following haematological examinations should be made at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and a measure of blood clotting time/potential.

u26. Blood samples should be taken from a named site just prior to or as part of the procedure for killing the animals, and stored under appropriate conditions. Animals will not be fasted prior to killing.

Clinical biochemistry

u27. Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, should be performed on blood samples obtained of all animals just prior to or as part of the procedure for killing of the animals (apart from those found moribund and/or intercurrently killed). Blood shall be collected from females at the same stage of the oestrus cycle. Investigations of plasma and serum shall include sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl trans-peptidase and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids may provide useful information under certain circumstances.

28. Optionally, the following urinalysis determinations could be performed during the last week of the study using timed urine volume collection; appearance, volume, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

u29. In addition, studies to investigate serum markers of general tissue damage should be considered. Other determinations that should be carried out, if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include calcium, phosphate, triglycerides, specific hormones, methaemoglobin, and cholinesterase. These need to be identified for chemicals in certain classes or on a case-by-case basis.

u29a Although in the international evaluation of the endocrine related endpoints a clear advantage for the determination of thyroid hormones (T3, T4) and TSH could not be demonstrated, it may be helpful to retain blood samples to measure T3, T4 and TSH if there is an indication for an effect on the pituitary-thyroid axis. The following factors may influence the variability and the absolute concentrations of the hormone determinations:

- time of sacrifice because of diurnal variation of hormone concentrations
- method of sacrifice to avoid undue stress to the animals that may affect hormone concentrations
- test kits for hormone determinations that may differ by their standard curves.

The benefit of these determinations should be re-evaluated after more experience has been gained with the application of this guideline. However, widespread and routine analyses appear to be redundant to the histopathology for identification of thyroid-active chemicals.

u29b. Plasma samples specifically intended for hormone determination should be obtained at a comparable time of the day, preferably during a period between late morning and early afternoon, when TSH is highest. It is recommended that consideration should be given to T_3, T_4 and TSH determinations triggered based upon alterations of thyroid histopathology. The numerical values obtained when analysing hormone concentrations differ with various commercial assay kits. Consequently, it may not be possible to provide performance criteria based upon uniform historical data. Alternatively, laboratories should strive to keep control coefficients of variation below 25 for T_3 and T_4 and below 35 for TSH. All concentrations are to be recorded in ng/ml.

30. Overall, there is a need for a flexible approach, depending on the species and the observed and/or expected effect with a given compound.

31. If historical baseline data are inadequate, consideration should be given to determination of haematological and clinical biochemistry variables before dosing commences.

PATHOLOGY

Gross necropsy

32. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart of all animals (apart from those found moribund and/or intercurrently killed) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying.

u32a In addition, the wet weight should be determined for the following organs as soon as possible after dissection to avoid drying: paired ovaries, uterus, seminal vesicles (including coagulating glands), and prostate (dorsolateral and ventral part combined). Alternatively, seminal vesicles and prostate may be trimmed and weighed after fixation. Clamp or ligature should be present during fixation as leakage of fluid provokes damage to fine structures in seminal vesicles.

u32b The following organ weights should be determined after fixation:
Thyroid (trimming should also be done after fixation to avoid tissue damage) and dorsolateral and ventral part of the prostate separately after separation.

33. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs (preserved by inflation with fixative and then immersion), gonads, accessory sex organs (e.g. uterus, prostate), urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a fresh mounted bone marrow aspirate). The clinical and other findings may suggest the need to examine additional tissues. Also any organs considered likely to be target organs based on the known properties of the test substance should be preserved.

u33a The following tissues may give valuable indication for endocrine-related effects: Gonads (ovaries and testes), accessory sex organs (uterus, cervix, vagina, epididymides, seminal vesicles with coagulation gland, dorsolateral and ventral prostate), pituitary, male and female mammary gland and thyroid.

u33b In the international test program some evidence was obtained that subtle endocrine effects by chemicals with a low potency for affecting sex hormone homeostasis may be identified by disturbance of the synchronisation of the oestrus cycle in different tissues and not so much by frank histopathological alterations in female sex organs. Although no definitive proof was obtained for such effects, it is recommended that specific emphasis should be given by histopathology on synchronisation of cyclic alterations in ovaries (follicular, thecal, and granulosa cells), uterus, cervix, vagina, pituitary, and mammary gland in comparison to the stage of cycle as determined by vaginal smears. The validity of such detailed examinations should be evaluated at a later stage when more experience has been gained with the TG 407 .

Histopathology

34. Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

35. All gross lesions shall be examined.

36. When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

DATA AND REPORTING

Data

37. Individual 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 and the time of any death or humane kill, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity of any toxic effects, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

38. When possible, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical methods should be selected during the design of the study.

u38a For quality control it is proposed that historical control data are collected and that for numerical data coefficients of variation are calculated, especially for the updated parameters. These data can be used for comparison purposes when actual studies are evaluated.

Test report

39. The test report must include the following information:

Test substance:

- physical nature, purity and physicochemical properties;
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- number, age and sex 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 substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation;
- details of the administration of the test substance;
-conversion from diet/drinking water test substance concentration (ppm) to the actual dose (mg/kg body weight/day), if applicable;
- details of food and water quality.

Results:

- body weight/body weight changes;
- food consumption, and water consumption, if applicable;
- toxic response data by sex and dose level, including signs of toxicity;
- nature, severity and duration of clinical observations (whether reversible or not);
- sensory activity, grip strength and motor activity assessments;
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
-body weight at killing and organ weight data;
- necropsy findings;
- a detailed description of all histopathological findings;
- absorption data if available;
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

ANNEX 1

DEFINITIONS

Dose is the amount of test substance administered. The dose is expressed as weight of test substance per unit body weight of test animal per day (e.g. mg/kg body weight/day), or as a constant dietary concentration.

Dosage is a general term comprising of dose, its frequency and the duration of dosing.

Evident toxicity is a general term describing clear signs of toxicity following administration of test substance. These should be sufficient for hazard assessment and should be such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.

NOEL is the abbreviation for no-observed-effect level. This is the highest dose level where no treatment related-findings in any parameter are observed due to treatment.

NOAEL is the abbreviation for no-observed-adverse-effect level. This is the highest dose level where no adverse treatment-related findings are observed due to treatment.

Oestrogenicity is the capability of a chemical to act like a natural oestrogenic hormone (e.g. oestradiol 17ß) in a mammalian organism.

Androgenicity is the capability of a chemical to act like a natural androgenic hormone (e.g. testosterone) in a mammalian organism.

Thyroid activity is the capability of a chemical to act like a natural thyroid hormone (e.g. T_3) in a mammalian organism.

Antioestrogenicity is the capability of a chemical to suppress the action of a natural oestrogenic hormone (e.g. oestradiol 17ß) in a mammalian organism.

Antiandrogenicity is the capability of a chemical to suppress the action of a natural androgenic hormone (e.g. testosterone) in a mammalian organism.

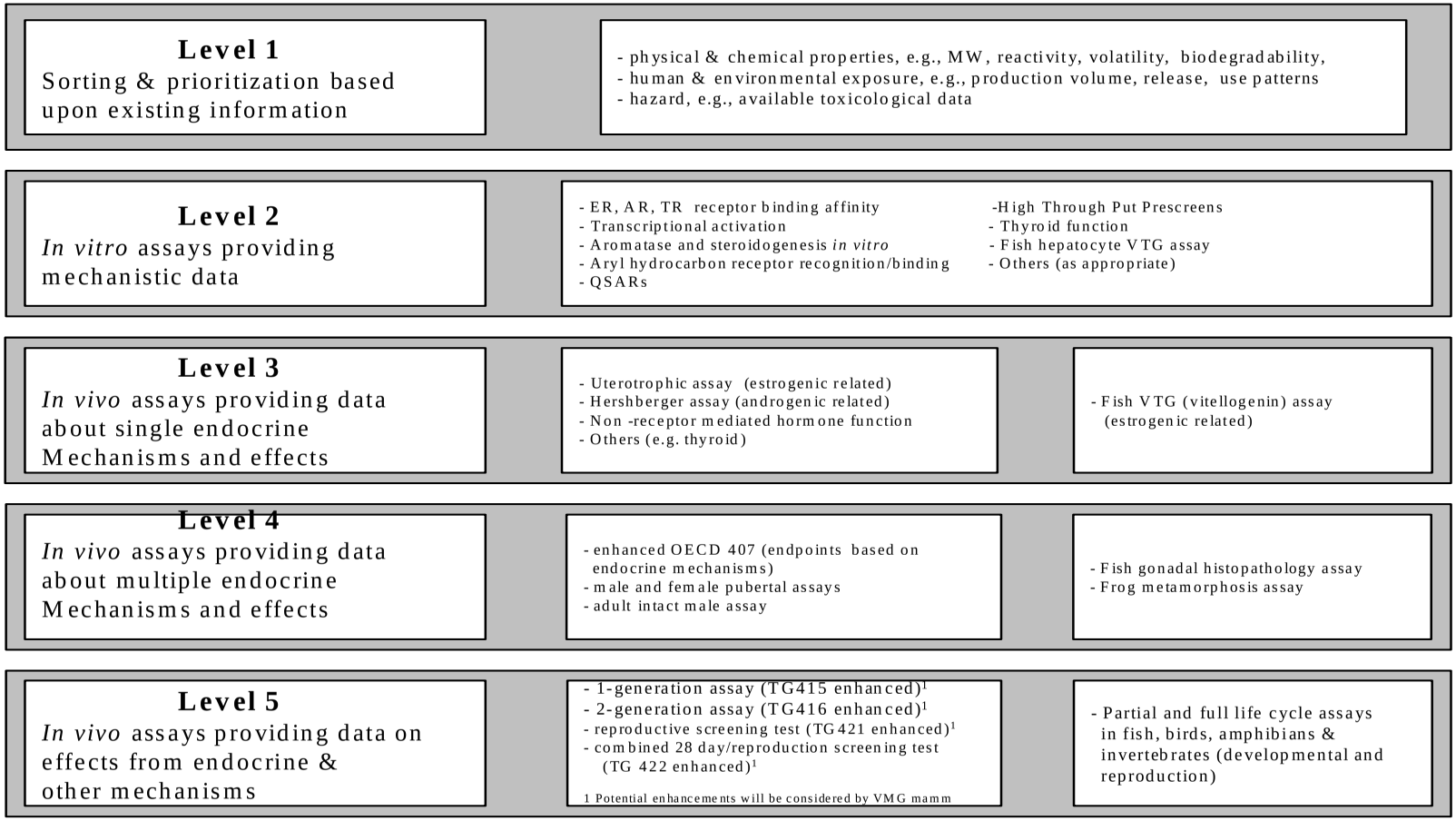
Antithyroid activity is the capability of a chemical to suppress the action of a natural thyroid hormone (e.g. T_3) in a mammalian organism.

Validation is a scientific process designed to characterise the operational requirements and limitations of a test method and to demonstrate its reliability and relevance for a particular purpose.

ANNEX 2

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals



VMG mamm: Validation Management Group on Mammalian Testing and Assessment

Notes to the Framework

Note 1: Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information needs for hazard and risk assessment purposes

Note 2: In level 5,ecotoxicology should include endpoints that indicate mechanisms of adverse effects, and potential population damage

Note 3: When a multimodal model covers several of the single endpoint assays, that model would replace the use of those single endpoint assays

Note 4: The assessment of each chemical should be based on a case by case basis, taking into account all available information, bearing in mind the function of the framework levels.

Note 5: The framework should not be considered as all inclusive at the present time. At levels 3,4 and 5 it includes assays that are either available or for which validation is under way. With respect to the latter, these are provisionally included. Once developed and validated, they will be formally added to the framework.

Note 6: Level 5 should not be considered as including definitive tests only. Tests included at that level are considered to contribute to general hazard and risk assessment.

LITERATURE

(1)OECD (Paris, 1992). Chairman's Report of the Meeting of the ad hoc Working Group of Experts on Systemic Short-term and (Delayed) Neurotoxicity.

(2)IPCS (1986). Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria Document No. 60

(3)Tupper, D.E., Wallace, R.B. (1980). Utility of the Neurologic Examination in Rats. Acta Neurobiol. Exp., 40, 999-1003.

(4)Gad, S.C. (1982). A Neuromuscular Screen for Use in Industrial Toxicology. J. Toxicol Environ. Health, 9, 691-704.

(5)Moser, V.C., McDaniel, K.M., Phillips, P.M. (1991). Rat Strain and Stock Comparisons Using a Functional Observational Battery: Baseline Values and Effects of Amitraz. Toxicol. Appl. Pharmacol., 108, 267-283.

(6)Meyer O.A., Tilson H.A., Byrd W.C., Riley M.T. (1979). A Method for the Routine Assessment of Fore- and Hindlimb Grip Strength of Rats and Mice. Neurobehav. Toxicol., 1, 233-236.

(7)Crofton K.M., Howard J.L., Moser V.C., Gill M.W., Reiter L.W., Tilson H.A., MacPhail R.C. (1991). Interlaboratory Comparison of Motor Activity Experiments: Implication for Neurotoxicological Assessments. Neurotoxicol. Teratol. 13, 599-609.

(8) OECD. (1998). Report of the First Meeting of the OECD Endocrine Disrupter Testing and Assessment (EDTA) Task Force, 10th-11th March 1998, ENV/MC/CHEM/RA(98)5.

(9) OECD. (2006). Draft Final Report of the Validation of the Updated Test Guideline 407 Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats.

(10) OECD. (1998). Detailed Review Paper on the Appraisal of Test Methods for Sex Hormone Disrupting Chemicals. OECD Monograph No 21.

(11) OECD. (1995). Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guideline for the Testing of Chemicals. Adopted 27.07.1995.

(12) Include: Report on VMG Meeting in Washington, April 2006.

(13) Include: Report on EDTA Meeting in Stockholm, April 2006.

(14) OECD (2000) Guidance document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. Environmental Health and Safety Monograph Series on Testing and Assessment No 19. ENV/JM/MONO(2000)7.