Glossary

All terms and their descriptions should be considered as working definitions for the purpose of this Guidance Document only.

Acceptance criteria: Criteria for when results can be accepted, i.e. a set of well-defined parameters describing aspects of the *in vitro* method such as range for positive and negative controls.

Accuracy: Refers to the closeness of a measured value to a standard or known value.

Authentication: Authentication of a cell line is the sum of the process by which a line's identity is verified and shown to be free of cross-contamination by other cell lines and/or contamination caused by bacteria, yeast or fungi, mycoplasm.

Adverse Outcome Pathway (AOP): An analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse human health or environmental effect.

American National Standards Institute (ANSI): A private non-profit organisation that oversees the development of voluntary consensus standards for products, services, processes, systems, and personnel in the United States. https://www.ansi.org/

American Society for Testing and Materials International (ASTM): An international standards organisation that develops and publishes voluntary consensus technical standards for a wide range of materials, products, systems, and services. https://www.astm.org/

Amplicon: A piece of DNA or RNA that is the source and/or product of natural or artificial amplification or replication events. It can be formed using various methods including Polymerase Chain Reactions (PCR), ligase chain reactions, or natural gene duplication.

Apoptosis: Process of programmed cell death generally characterised by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptosis is considered an essential component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death.

Archive: A designated area or facility (e.g., cabinet, room, building or computerised system) for the secure storage and retention of records and materials.

Assay: A defined laboratory procedure for qualitatively or quantitatively measuring the presence or amount or the functional activity of a target or analyte. An assay can be considered as a technical operation that consists of determination of one or more characteristics of a given product, process or service according to a specified procedure.

Batch/Lot: A specific quantity of a test item, reference item or test system such as cells, tissues, assay reagent or other consumable, produced during a defined cycle in such a way that it could be expected to be of a uniform character and should be designated as such.

BenchMark Dose (BMD) or Concentration (BMC): The dose or concentration associated with a pre-specified biological response. It was developed as an alternative to the use of No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL).

Best practice: Generally accepted optimal methods or techniques that have consistently shown superior results among different labs as compared to those achieved with other means, and that is used as a benchmark. The term is also used to describe the process of developing and following a standard way of doing things that multiple organisations can use. Best practices are a snapshot and can be subject to change based on ongoing scientific dialogue and advancements.

Between-laboratory assessment: Phase of method validation, also often referred to as between (or inter) laboratory validation, in which different operators in different laboratories perform (or run) the *in vitro* method independently to establish whether or not an *in vitro* method can be successfully established in different laboratories, e.g., to assess the between laboratory reproducibility (BLR).

Biokinetics: Time-course of a chemical (substance and mixture) and its metabolites in a living organism, i.e., increase or decrease of substance concentration at the site of measurement due to transport or due to formation or breakdown.

Biological pathway: A series of actions among molecules in a cell that leads to a certain product or a change in the cell. Such a pathway can trigger the assembly of new molecules, such as a lipid or protein. Pathways can also turn genes on and off, or spur a cell to move. Some typical types of biological pathways are metabolic pathways and signalling pathways.

Carcinogenicity: The property of any agent (chemical, physical or biological agent) directly involved in causing cancer (carcinogen). Carcinogenicity results in an increased incidence of tumours, increased proportion of malignant tumours or a reduction in the time to appearance of tumours, compared with concurrent control groups. The process of carcinogenesis involves the transition of normal cells into cancer cells via a sequence of stages that may entail both genetic alterations (i.e. mutations) and non-genetic events.

Coefficient of Variation (CV): A measure of spread that describes the amount of variability relative to the mean. Because the coefficient of variation is per definition unrelated to the magnitude of the mean and also unitless, it can be used instead of the standard deviation to compare the spread of data sets that have different units or different means.

Comparative Genomic Hybridisation analysis (aCGH): A molecular cytogenetic method for analysing copy number variations relative to ploidy level in the DNA of a test sample compared to a reference sample, without the need for culturing cells. The aim of this technique is to quickly and efficiently compare two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or subchromosomal regions (a portion of a whole chromosome).

Computerised system: A computerized system is a function (process or operation) integrated with a computer system and performed by trained personnel. The function is

controlled by the computer system. The controlling computer system is comprised of hardware and software. The controlled function is comprised of equipment to be controlled and operating procedures performed by personnel.

Cytotoxicity: General cytotoxicity (or basal cytotoxicity) is the result of toxic effects on structures and functions common to all cells of the body, such as DNA, chromosomes, mitochondria, the cytoskeleton and various membranes.

Data (derived data): Derived data depend on raw data and can be reconstructed from raw data (e.g., final concentrations as calculated by a spreadsheet relying on raw data, result tables as summarised by a LIMS, etc.).

Data (raw data): Data (raw data) may be defined as measurable or descriptive attribute of a physical entity, process or event. The GLP Principles define raw data as all laboratory records and documentation, including data directly entered into a computer through an automatic instrument interface, which are the results of primary observations and activities in a study and which are necessary for the reconstruction and evaluation of the report of that study.

Data integrity: The extent to which all data are complete, consistent and accurate throughout the data lifecycle.

Data lifecycle: All phases in the life of the data (including raw data) from initial generation and recording through processing (including transformation or migration), use, data retention, archive / retrieval and destruction (if applicable).

Defined Approach to Testing and Assessment: A defined approach consists of a fixed data interpretation procedure (DIP) (e.g. statistical, mathematical models) applied to data (e.g., in silico predictions, in chemico, in vitro data) generated with a defined set of information sources to derive a prediction. In contrast to the assessment process within Integrated Approaches to Testing and Assessment (IATA), that necessarily involves some degree of expert judgment, predictions generated with defined approaches are rule-based and can either be used on their own if they are deemed to fit-for-purpose or considered together with other sources of information in the context of IATA.

Design Qualification (DQ): Documented verification that the proposed design of the facilities, equipment, or systems is suitable for the intended purpose. This definition is applicable for complex instrumentation (computerised systems).

Effective Concentration 50 (EC_{50}) and Inhibition Concentration 50 (IC_{50}): In *in vitro* cell and tissue culture, EC_{50} is the concentration causing a half-maximal response for any measured biological effect of interest, and is equivalent to median effective dose (ED_{50}) and median lethal dose (LD_{50}) used in animal experiments. EC_{50} is used for read-outs that increase with concentration, whereas IC_{50} is used in case of an *in vitro* method where there is a decline in read-out. IC_{50} is therefore the test item concentration causing a reduction of response/binding etc. by half.

Emulsion: A stable dispersion of liquid droplets in another liquid, where the two are immiscible.

Engelbreth-Holm-Swarm (EHS) gel: Gelatinous protein mixture secreted by an EHS mouse sarcoma cells which are rich source of basement membrane components often used in cell and tissue culture work.

EURL ECVAM Database service on Alternative Methods (DB-ALM): A database providing comprehensive descriptions of non-animal methods together with related

information. Method descriptions are provided at two levels of detail, such as Summary Descriptions in an OECD compliant format (OECD, 2014) and/or detailed Standard Operating Procedures. DB-ALM originates from a requirement for EURL ECVAM to establish and manage public databases on alternative approaches as described in Annex VII of Directive 2010/63/EU on the protection of animals used for scientific purposes. https://ecvam-dbalm.jrc.ec.europa.eu

EURL ECVAM Scientific Advisory Committee (ESAC): Advises the European Union Reference Laboratory (EURL) ECVAM on scientific issues. ESAC's main role is to conduct independent peer reviews of validation studies of alternative test methods, assessing their scientific validity for a given purpose.

European Union Reference Laboratory on Alternatives to Animal Testing (EURL ECVAM): ECVAM was established in 1991 pursuant to a requirement in Directive 86/609/EEC that the European Commission (EC) and its member states actively support the development, validation, and acceptance of methods to replace, reduce, or refine the use of animals in laboratories. The activities of ECVAM were assumed by the European Union Reference Laboratory on Alternatives to Animal Testing (EURL ECVAM), which was formally established in 2011 as the Union Reference Laboratory specified in section 47, Article 48, and Annex VII of the European Commission's Directive 2010/63/EU. https://eurl-ecvam.jrc.ec.europa.eu/

European Chemicals Agency (ECHA): Agency of the European Union (EU) that manages technical, scientific and administrative aspects of EU chemicals legislation, notably the regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). https://echa.europa.eu/

European Directorate for the Quality of Medicines & HealthCare (EDQM): Organisation that is responsible for the European Pharmacopoeia and the European Biological Standardisation Programme. https://www.edqm.eu/

European Food Safety Authority (EFSA): Agency of the European Union that provides independent scientific advice in the fields of food and feed safety, animal health and welfare, plant protection and plant health and communicates on existing and emerging risks associated with the food chain. http://www.efsa.europa.eu/

European Medicines Agency (EMA): Agency of the European Union that is responsible for the protection of public and animal health through the scientific evaluation and supervision of medicines. http://www.ema.europa.eu/

European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL): A network of highly qualified laboratories to (1) respond to some of the provisions of Directive 2010/63/EU, (2) generate *in vitro* method information that is reliable, relevant and based on current best quality and scientific practices, (3) increase the European Commission's validation capacity of *in vitro* methods and (4) provide a laboratory network knowledgeable on the routine implementation of good *in vitro* method practices for regulatory use in human safety assessment. https://eurl-ecvam.jrc.ec.europa.eu/eu-netval

Foetal Bovine/Calf Serum (FBS/FCS): Foetal bovine serum or often referred to as foetal calf serum is the liquid fraction of clotted blood (depleted of cells, fibrin and clotting factors, but containing a large number of nutritional (e.g., amino acids, sugars, lipids) and macromolecular factors (e.g., growth factors and hormones) considered by a large community to be essential for cultured cell growth. FBS/FCS is derived from the

blood drawn from a bovine foetus via a closed system of collection at the slaughterhouse. It is the most widely used growth supplement for cell and tissue culture media because of its high content of embryonic growth promoting factors and its low level of antibodies. When used at appropriate concentrations it may supply many defined but also undefined components that have been shown to satisfy specific metabolic requirements for the culture of cells and tissues.

Genetically Modified Organisms (GMOs): An organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Good Cell Culture Practice (GCCP): Guidelines developed in 2005 to define minimum standards in cell and tissue culture work. This GCCP guidance lists a set of six principles intended to support best practice in all aspects of the use of cells and tissues *in vitro*, and to complement, but not to replace, any existing guidance, guidelines or regulations.

Good Laboratory Practice (GLP): A quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported. GLP in this document refers to the OECD Principles of GLP (OECD, 1998a) unless otherwise stated.

Hazard: An intrinsic feature of a stressor (e.g., chemical or physical in nature) to cause harm or adverse effects to human health and to the environment. It is a qualitative (for example in the case of classifications) or quantitative expression of the adverse effects elicited by a test item under defined conditions of exposure.

High Performance Liquid Chromatography (HPLC): High performance liquid chromatography (or high-pressure liquid chromatography) is a chromatographic technique that can separate a mixture of compounds when in solution and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.

High-Efficiency Particulate Air (HEPA): A type of air filter, also sometimes called high-efficiency particulate arrestance, with a minimum efficiency rating of 99.97% for the removal of 0.3 μm diameter or larger particulate matter[1].

High-Throughput Screening (HTS): A scientific approach relevant to chemistry and biology in which a very large number (e.g., tens of thousands per day) of experimental samples are subjected to testing under given conditions in a prescribed procedure.

***In silico*:** The technique of performing experiments via computer simulations. Examples are Structure-Activity Relationships (SAR) and Quantitative Structure-Activity Relationships (QSAR).

***In vitro*:** The technique of performing a given experiment in a test tube, or, more generally, in a controlled environment outside of a living organism.

***In Vitro* to *In Vivo* Extrapolation (IVIVE):** The qualitative or quantitative transposition of experimental results or observations made *in vitro* to predict phenomena *in vivo*, i.e. in whole organisms.

***In vivo* :** Experimentation using a whole, living organism as opposed to a partial or dead organism, or an *in vitro* controlled environment. Animal testing and clinical trials are two forms of *in vivo* research.

Inhibitor or spiked up control: Mix of test item and positive control to assess any effect of inhibition of the test item on the test system endpoint measurements.

Installation Qualification (IQ): The documented verification that the facilities, systems and equipment, as installed or modified, comply with the approved design and the manufacturer's recommendations. This definition is applicable for complex instrumentation (computerised systems).

Integrated Approaches to Testing and Assessment (IATA): IATA are pragmatic, science-based approaches for chemical hazard characterisation that rely on an integrated analysis of existing information coupled with the generation of new information using testing strategies.

Integrated Testing Strategies (ITS): ITS provide guidance on how various types of available data (including those obtained from *in vitro* testing methods or assays) should be evaluated, and addresses additional aspects on some elements such as the use of other toxicity data or weight of evidence analysis of existing and relevant data.

Intellectual Property Rights (IPR): The rights given to persons over the creations of their minds. They usually give the creator an exclusive right over the use of his/her creation for a certain period of time (WTO)[2].

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM): ICCVAM is a permanent committee of the NIEHS under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). ICCVAM is composed of representatives from 16 U.S. Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information.https://ntp.niehs.nih.gov/pubhealth/evalatm/iccvam/index.html

International Cell Line Authentication Committee (ICLAC): ICLAC is a voluntary, independent scientific committee that aims to make cell line misidentification more visible and promote authentication testing to combat this problem. http://iclac.org/

International Council for Harmonisation (ICH): ICH's mission is to achieve greater harmonisation worldwide to ensure that safe, effective, and high quality medicines are developed and registered in the most resource-efficient manner. The ICH brings together the receiving authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration. Harmonisation is achieved through the development of ICH Guidelines via a process of scientific consensus with regulatory and industry experts working side-by-side. Key to the success of this process is the commitment of the ICH regulators to implement the final Guidelines. http://www.ich.org/home.html

International Organization for Standardization (ISO): ISO is an independent, non-governmental international organisation that brings together experts to share knowledge and develop voluntary, consensus-based, market relevant International Standards that support innovation and provide solutions to global challenges. https://www.iso.org/

International Uniform ChemicaL Information Database (IUCLID): A software application designed to capture, store, maintain and exchange data on intrinsic and hazard properties of chemicals (substances and mixtures). The freely downloadable tool assists chemical companies globally to fulfil their obligation to submit data to the EU Chemicals Agency (ECHA) under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (Regulation (EC) No 1907/2006).

***In vitro* method developer**: The person or entity who develops an *in vitro* method destined for regulatory use in human or environmental safety assessment.

ISO 9000: The ISO 9000 family of quality management standards is designed to help organisations ensure that they meet the needs of customers and other stakeholders while meeting statutory and regulatory requirements to a product or program. ISO 9000 deals with the fundamentals of quality management systems.

Japanese Center for the Validation of Alternative Methods (JaCVAM): Promotes the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establishes guidelines for new alternative experimental methods through international collaboration. http://www.jacvam.jp/en/

Korean Centre for the Validation of Alternative Methods (KoCVAM): KoCVAM was established in 2009 as part of the National Institute of Food and Drug Safety Evaluation (NIFDS) under the Korean Food & Drug Administration (KFDA). In March 2013, the KFDA was restructured and renamed as the Ministry of Food and Drug Safety (MFDS). http://www.nifds.go.kr/en/inter/kocvam.jsp

Lactate DeHydrogenase (LDH): An enzyme that helps the process of turning sugar into energy for cells to use. LDH is present in many kinds of organs and tissues throughout the body, including the liver, heart, pancreas, kidneys, skeletal muscles, brain, and blood cells. An LDH assay is a means of measuring either the number of cells via total cytoplasmic LDH or membrane integrity as a function of the amount of cytoplasmic LDH released into the medium.

Limit Of Detection (LOD): The LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 1%).

Limits Of Quantification (LOQ): The Lower Limit Of Quantification (LLOQ) and Upper Limit Of Quantification (ULOQ) are the lowest and highest quantity of a substance that can be quantitatively determined with a stated acceptable precision and accuracy, under stated experimental conditions.

Lipophilicity: The ability of a chemical (substance and mixture) to dissolve in non-polar environments such as oils, lipid membranes, and non-polar solvents such as hexane or toluene.

Mass Spectrometry (MS): An analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules such as peptides and other chemical compounds.

Medical device: An article, instrument, apparatus or machine that is used in the prevention, diagnosis or treatment of illness or disease, or for detecting, measuring, restoring, correcting or modifying the structure or function of the body for some health purpose. Typically, the purpose of a medical device is not achieved by pharmacological, immunological or metabolic means (WHO[3]).

Metadata: Metadata are data that describe the attributes of other data, and provide context and meaning. Typically, these are data that describe the structure, data elements, inter-relationships and other characteristics of data. They also permit data to be attributable to an individual.

Method endpoint: Quantitative or quantitative measurable characteristics that serve as indicators of a putatively pathologic process or related biochemical or molecular events, e.g., measured absorbance in a cytotoxicity assay or a skin irritation *in vitro* method.

Me-too test method: A colloquial expression for a test method that is structurally and functionally similar to a validated and accepted reference test method. Such a test method would be a candidate for catch-up validation (OECD, 2005).

Microorganism: Any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture.

Minimal Essential Medium (MEM): A synthetic cell culture media for *in vitro* cell and tissue culture work, developed by Harry Eagle, one of the most widely used synthetic cell culture media.

Minimum Significant Ratio (MSR): Parameter that can be used to quantify assay reproducibility and resolution (the smallest ratio between two compound potencies which can be detected in the *in vitro* method).

Ministry of Agriculture, Forestry and Fisheries (MAFF): Japanese Ministry related to agricultural, forestry and fisheries products, covering from production to consumption and also to rural development and promotion of the welfare of rural inhabitants with a view to achieving a stable supply of food, sound development of the agriculture, forestry and fisheries industries and upgrading of the welfare of rural inhabitants. http://www.maff.go.jp/e/

Ministry of Health, Labour and Welfare (MHLW): Japanese Ministry responsible for the approval and administration of drugs, medical devices and cosmetics in Japan. http://www.mhlw.go.jp/english/

Mixture: A combination of two or more chemicals (liquid or solid) that do not react with each other.

Molecular Initiating Event (MIE): The initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway.

Multi-constituent substance: A substance, defined by its quantitative composition, in which more than one main constituent is present in a concentration ≥ 10% (w/w) and < 80% (w/w). A multi-constituent substance is the result of a manufacturing process. The difference between mixture and multi-constituent substance is that a mixture is obtained by blending of two or more substances without chemical reaction. A multi-constituent substance is the result of a chemical reaction.

Mutual Acceptance of Data (MAD): The OECD MAD is a multilateral agreement which states that test data generated in any member country in accordance with OECD Test Guidelines and GLP shall be accepted in other member countries for assessment purposes and other uses relating to the protection of human health and the environment. The application of MAD avoids unnecessary and costly duplication of testing as well as non-tariff barriers to trade. In addition, it reduces the number of laboratory animals used for *in vivo* testing.

Nanomaterial: A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm[4].

Negative control: Separate part of a test system treated with an item for which it is known that the test system should not respond; the negative control provides evidence that the test system is not responsive under the actual conditions of the assay.

Nephelometry: A technique for determining the amount of turbidity in a solution based upon the measurement of scattering of light.

OECD Harmonised Templates (OHTs): Standard data formats for reporting information used for the risk assessment of chemicals, mainly studies done on chemicals to determine their properties or effects on human health and the environment, but also for storing data on use and exposure.

OECD Test Guidelines (TG): OECD Test Guidelines are harmonised test methods included in the OECD Council Decision on Mutual Acceptance of Data. This means that "data generated in the testing of chemicals in an OECD Member country (or some non-member economies) in accordance with OECD Test Guidelines and OECD principles of Good Laboratory Practice shall be accepted in other Member countries (or non-member economies) for purposes of assessment and other uses relating to the protection of man and the environment".

Official Medicines Control Laboratories (OMCLs): European laboratory network supporting receiving authorities (including the issuing of guidelines) in the area of quality control of marketed medicinal products for human and veterinary use. https://www.edqm.eu/en/news/omcl-network

Omics: A general term for a broad discipline of science and engineering for analysing the interactions of biological information objects in various omes (these include genome, transcriptome, proteome, metabolome, expressome, and interactome).

Some examples of 'Omics' technologies:

- genomics
- proteomics
- metabolomics
- transcriptomics

Operational Qualification (OQ): Documented verification that all aspects of the facility, systems and equipment which can affect product quality; performs as intended throughout all anticipated operating ranges. This definition is applicable for complex instrumentation (computerised systems).

Particulates/Particulate Matter (PM): Tiny subdivisions of solid matter suspended in a gas or liquid (also known as particulate matter, fine particles and soot).

Performance Based Test Guideline (PBTG): A test guideline that contains one or more *in vitro* methods that are mechanistically and functionally similar. A PBTG defines the important components of the *in vitro* method and describes in detail characteristics and performance standards that a new *in vitro* method should meet in order to be considered as an additional method.

Performance Qualification (PQ): The documented verification that the facilities, systems and equipment, as connected together, can perform effectively and reproducibly, based on the approved process method and product specification. This definition is applicable for complex instrumentation (computerised systems).

Performance Standards (PS): The purpose of performance standards is to provide the basis by which new or modified *in vitro* methods, both proprietary (i.e. copyright, trademarked, registered) and non-proprietary, can be deemed to be structurally and mechanistically similar to a validated reference method and demonstrate to have sufficient reliability and relevance for specific purposes (i.e. in accordance with the principles to OECD GD 34).

Physiologically-Based ToxicoKinetic (PBTK[5]) models: Physiologically based toxicokinetic, or alternatively referred to as physiologically based pharmacokinetic or biokinetic models, are quantitative descriptions of absorption, distribution, metabolism, and excretion (ADME, possibly including toxicity as ADMET) of synthetic or natural chemical substances in humans and other animal species. PBTK models are increasingly being used as an effective tool for designing toxicology experiments and for conducting extrapolations essential for risk assessments (e.g., in pharmaceutical research and drug development, and in health risk assessment for cosmetics or general chemicals).

Polymerase Chain Reaction (PCR): A molecular biology technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules[6]. It is based on the natural process of DNA replication.

Population Doubling Level (PDL): Refers to the total number of times the cells in the population have doubled since their primary isolation *in vitro*, and are usually an estimate rounded off to the nearest whole number.

Positive control: Separate part of the test system treated with an item for which it is known that the test system should respond. The positive control provides evidence that the test system is responsive under the actual conditions of the assay. The positive control is endpoint specific to the test system.

Prediction model: The method by which the *in vitro* endpoint value(s) is used to predict the *in vivo* equivalent activity (i.e., degree of toxicity).

Proficiency chemicals (substances): A set of chemicals recommended within OECD Test Guidelines to be used by laboratories to demonstrate their technical proficiency prior to the routine use of a test method falling within the adopted OECD test guideline. These chemicals represent either a subset of the reference chemicals included in the Performance Standards relating to the OECD TG, or chemicals used in the validation studies of the test method falling within the OECD TG. Selection criteria for these test chemicals include, to the extent possible, chemicals that: i) represent the range of responses to be predicted, ii) have high quality reference data available; iii) cover the method's dynamic range of responses; iv) were correctly predicted by the test method during its validation study; v) cover a wide and representative range of relevant physical states, chemical classes, organic functional groups and structures falling within the applicability domain of the in vitro method; vi) are commercially available, and vii) are not associated with prohibitive acquisition and/or disposal costs.

Provenance: Describes the origin and culture history of a cell line, including its transfers among laboratories and repositories, its manipulation (physicochemical or genetic), tests for and the detection and elimination of contamination by other cell lines and/or contamination caused by bacteria, yeast or fungi, mycoplasma, genotypic and phenotypic characteristics, and verification of its identity.

Quality Assurance (QA)*:* All the planned and systematic actions by which adherence to laboratory testing standards, requirements, and record keeping procedures, and the accuracy of data transfer, are assessed by individuals independent of those performing the testing.

Quality assurance programme: A defined system, including personnel, which is independent of study conduct and is designed to assure test facility management of compliance with the Principles of Good Laboratory Practice. (OECD, 1998a).

Quality Control (QC): Operational techniques and activities that are used to fulfil given requirements for quality.

Quality Management System (QMS): Can be expressed as the organisational structure, procedures, processes and resources needed to implement quality management. GLP specifically refers to a quality system of management controls for test facilities and organisations to try to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of test item non-clinical safety tests. Of all QMS regimes, the ISO 9000 family of standards is probably the most widely implemented worldwide.

Reagent: A substance or mixture for use in cell culture media, chemical analysis or other reactions.

Reference item: An article used to provide a basis for comparison with the test item.

Relevance: The term "Relevance" describes whether a procedure is meaningful and useful for a particular purpose.

Reliability: The term "Reliability" describes whether a procedure can be performed reproducibly within and between laboratories and over time.

Replace, Reduce, Refine (3Rs): A term describing current internationally accepted strategies for minimising use and suffering of laboratory animals used in experimental research. The optimal solution is to Replace the test method requiring animal experiments with one or several *in vitro* methods; if this is not possible at least it might be possible to modify the methods in order to Reduce the number of animals being used in each study without compromising data quality; if this is also not possible it might at least be possible to Refine the test method so that experiments are conducted in a way minimising stress and other impact on the animals.

Robustness: The insensitivity of test results to departures from the specified test conditions when conducted in different laboratories or over a range of conditions under which the test method might normally be used. If a test is not robust, it will be difficult to use in a reproducible manner within and between laboratories.

Saturation concentration: The maximum dissolved concentration of a test chemical that can be achieved under the test conditions.

Sensitivity: A measure of *in vitro* method performance that describes the proportion of all evaluated test items that are classified as positive for a particular toxicological endpoint, which are predicted as positive by the actual *in vitro* method. The terms "sensitivity" may also refer to *in vivo* tests when e.g., compared to human data.

Service Level Agreement (SLA): A contract between a service provider (either internal or external) and the end user that defines the level of service expected from the service provider.

Short Tandem Repeat (STR): Short sequences of DNA, usually of length 2-6 base pairs and directly adjacent to each other that are repeated numerous times along a given loci. They are also known as microsatellites. STRs are used to compare specific loci on DNA from two or more samples.

Signal Windows (SW): A measure of separation between maximum and minimum controls in an assay that accounts for the amount of variability in the assay (Sittampalam *et al*., 2017).

Single Nucleotide Polymorphism analysis (aSNP): Single nucleotide polymorphism or SNP (pronounced snip) analysis is a technique to detect a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide.

Solid Phase MicroExtraction (SPME): A technique for separating mixtures of compounds without the use of solvents. SPME uses a fibre coated with a polymer or sorbent extracting phase that extracts chemical compounds from liquid or gas phases.

Solubility limit in water: The maximum attainable concentration in water at thermodynamic equilibrium between the aqueous pure phase and the solid (or liquid or gaseous) pure phase.

Specificity: A measure of *in vitro* method performance that describes the proportion of all evaluated test items that are classified as negative for a particular toxicological endpoint, which are predicted as negative by the actual *in vitro* method. The terms "specificity" may also refer to *in vivo* tests when e.g., compared to human data.

Sponsor: Means an entity which commissions, supports and/or submits a non-clinical health or environmental safety study.

Standard Deviation (SD): The expected squared deviation from the mean.

Standard Operating Procedure (SOP): A documented procedure which describes how to perform testing methods or assays or activities normally not specified in detail in study plans or test guidelines.

Standard Project Submission Form (SPSF): OECD standard form which specifies the information generally required to submit a proposal for new or updated Test Guidelines or related documents to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT)[7], including the project description and the actions planned toward the development of the Test Guideline, the project milestones, and deliverables.

Structure-Activity Relationships and Quantitative Structure-Activity Relationships (SAR/QSAR): Structure-activity relationships and quantitative structure-activity relationships based on the chemical structure of a compound, collectively referred to as (Q)SARs, are simplified mathematical representations of complex chemical-biological interactions that can be used to predict the physicochemical and biological properties of molecules.

Study plan: A document which defines the objectives and experimental design for the conduct of the study, including amendments (i.e., an intended change to the study plan after the study initiation date).

Suspension: A stable dispersion of solid particles in a liquid.

Test item: An article that is the subject of a study (e.g., chemical, substance, nanomaterial, medical device, biologicals etc.). Test chemical may be used interchangeably for chemical based test items.

Test system: Any biological, chemical or physical system or a combination thereof used in a study. *In vitro* test systems are mainly biological systems (e.g., cells or tissues), although some of the more recent developments in alternatives to conventional *in vivo* testing (e.g., gene arrays for toxicogenomics) may also exhibit some attributes of physical-chemical test systems. Test kits, including proprietary test kits, should also be considered as test systems.

Testing method: A testing method, also known as assay, is a process or procedure used to obtain information on the characteristic of a substance or agent. Specific testing methods generate information regarding the ability of a substance or agent to produce a specific biological effect under specified conditions.

Toxicological endpoint: A direct marker of progression to an adverse outcome - e.g., morphological or physiological changes, functional impairments, disease symptoms or death - used to describe an adverse health effect (or a probability of that adverse effect) resulting from exposure to a test item. The test system response to an exposure of a test item may be measured by a series of endpoints. The most sensitive endpoint (critical endpoint) is the one that occurs at the lowest exposure level and associated with an adverse response (committed step).

Training set: A set of test items used to develop the prediction model for an assay. The training set items should have strong reference data (i.e., values from a recognised regulatory assay and derived from multiple runs of the reference tests) against which the *in vitro* assay endpoint values can be compared.

Untreated control: Test system that receives no treatment (e.g., no test chemical or solvent) but is processed concurrently and in the same way as the test system receiving the test item.

US Department of Agriculture (USDA): USDA is the U.S. federal executive department responsible for developing and executing federal laws related to farming, agriculture, forestry, and food. It aims to meet the needs of farmers and ranchers, promote agricultural trade and production, work to assure food safety, protect natural resources, foster rural communities and end hunger in the United States and internationally. https://www.usda.gov/

US Environmental Protection Agency (EPA): The EPA is an agency of the Federal government of the United States which was created for the purpose of protecting human health and the environment. https://www.epa.gov/

US Food and Drug Administration (FDA): The FDA is a regulatory and research agency within the US Department of Health and Human Services that is responsible for "protecting the public health by assuring the safety, efficacy and security of human and veterinary drugs, biological products [for humans], medical devices, the nation's food supply [including dietary supplements], cosmetics, tobacco products and products that emit radiation". https://www.fda.gov

Validation: The process by which the reliability and relevance of a procedure are established for a specific purpose (OECD, 2005).

Validation set: A set of selected chemicals used to assess the predictive capacity of an *in vitro* method based on the performance of the endpoint values by the reference *in vitro* method results. Testing of the validation set is a principal part of *in vitro* method validation.

Vehicle/solvent control: Separate part of a test system to which the vehicle/solvent for the test item is added without the test item; the vehicle/solvent control provides evidence for a lack of influence of the chosen vehicle/solvent on the test system under the actual conditions of the *in vitro* method.

Veterinary International Conference on Harmonization (VICH): VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products. VICH was officially launched in April 1996. http://www.vichsec.org/

Within-laboratory assessment: Phase (of method validation), also often referred to as within laboratory (or intra) laboratory validation, in which one or more operators from the same laboratory perform (or run) the *in vitro* method independently and at different times to establish whether or not an *in vitro* method meets established criteria e.g., to assess within-laboratory reproducibility (WLR).

World Health Organisation (WHO): The WHO was established in 1948 as a specialised agency of the United Nations. WHO is made up of 193 Member States, most of which are also UN Member States. WHO's mission is "the attainment by all peoples of the highest possible level of health". http://www.who.int/

Xenobiotic: A chemical foreign to the biological system, structurally distinct from endogenous compounds present within the biological system. A xenobiotic may also be directly pharmacologically, endocrinologically, or toxicologically active, or undergo metabolism in target organisms such as to become biologically active or inactive.

Z-factor: A measure of the separation between solvent control and test item signal which takes into account the dynamic range of the *in vitro* method and the data variation associated with the signal and control measurements. It is suitable for *in vitro* method quality assessment.

Notes

1. See: https://www3.epa.gov/ttncatc1/dir1/ff-hepa.pdf
2. See: https://www.wto.org/english/tratop_e/trips_e/intel1_e.htm
3. See: http://www.who.int/medical_devices/definitions/en/
4. See: http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm
5. PBTK models are regarded as being synonymous to PBPK (physiologically-based pharmacokinetic), PBBK (physiologically-based biokinetic) and PBK (physiologically-based kinetic) models (Bessems *et al.*, 2014).
6. See: https://www.nature.com/scitable/definition/polymerase-chain-reaction-pcr-110
7. See: http://www.oecd.org/env/ehs/testing/testguidelinesprogrammefaqs.htm

References

Bessems, J. et al. (2014), "PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment", *Regulatory Toxicology and Pharmacology*, Vol. 68/1, pp. 119-139, http://dx.doi.org/10.1016/j.yrtph.2013.11.008. [2]

OECD (2017), *Guidance Document for Describing Non-Guideline In Vitro Test Methods*, OECD Series on Testing and Assessment, No. 211, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264274730-en. [5]

OECD (2005), *OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*, OECD Publishing. Paris. [4]

OECD (1998), *OECD Principles on Good Laboratory Practice*, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264078536-en. [3]

Sittampalam, G. et al. (2004), *Assay Guidance Manual*, Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational. [1]