JUSTIFICATION FOR DEVELOPMENT OF AN OECD GUIDANCE DOCUMENT ON THE APPLICATION OF GOOD LABORATORY PRACTICE (GLP) PRINCIPLES TO *IN VITRO* TESTING

INTRODUCTION

1. The purpose of the OECD "Principles of Good Laboratory Practice" is to ensure the generation of high quality and reliable test data related to the safety of chemicals and chemical products in the framework of harmonizing testing procedures for the mutual acceptance of data in all OECD Member countries. These Principles establish the quality system concepts covering the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported.

2. To date, in addition to the OECD "Principles of Good Laboratory Practice," Guidance, Advisory, and Consensus Documents have been developed by the OECD Working Group on Good Laboratory Practice (GLP). These Documents cover issues ranging from guidance on compliance of laboratory suppliers with GLP Principles to the application of GLPs to field studies, computerized systems, and short-term tests. A short-term study is defined as one of short duration using widely used, routine techniques; short-term biological studies include acute toxicity studies, some mutagenicity studies, and acute ecotoxicological studies. However, experts involved in the conduct of *in vitro* studies (ECVAM Workshop Report 37, "The Principles of Good Laboratory Practice: Application to *In Vitro* Toxicology Studies," 1999) have suggested that additional guidance is needed to cover the application of GLPs to specific aspects of *in vitro* studies that are considered to be not, or insufficiently, covered in existing Consensus Documents. Additional relevant considerations are provided in a Report of the ECVAM Good Cell Culture Practice Task Force (Hartung et al., 2002). The OECD Working Group on GLP has considered this issue but is not yet convinced of a need for further guidance beyond the revised "OECD Principles on Good Laboratory Practice" (OECD, 1998) and the revised "Consensus Document on the Application of the GLP Principles to Short Term Studies" (OECD, 1999). - The Interagency Coordinating Committee on the Validation of Alternative Toxicological Methods (ICCVAM) has reviewed these documents and reports, and recommends that an OECD Guidance Document on the Application of Good Laboratory Practice (GLP) Principles to *In Vitro* Testing should be developed. Justification was prepared by the ICCVAM Subcommittee on GLPs, and endorsed by the ICCVAM at its January 30, 2003 meeting. This justification will be provided to participants at the March 4, 2003 Consultation meeting between members of the OECD Working Group on GLP and ECVAM/ICCVAM on the need for further guidance on the application of the Principles of GLP to *in vitro* studies.

THE NEED FOR AN OECD CONSENSUS DOCUMENT ON THE APPLICATION OF THE GLP PRINCIPLES TO *IN VITRO* STUDIES

3. Although a number of the ECVAM Workshop Report No. 37 recommendations are addressed to some extent in the OECD "Consensus Document on the Application of the GLP Principles to Short Term Studies," there is still a critical need for a stand-alone OECD "Guidance Document on the Application of the GLP Principles to *In Vitro* Studies". Justification for such a stand-alone document includes both general and specific issues, as described below.

GENERAL JUSTIFICATIONS FOR A STAND-ALONE GLP DOCUMENT FOR *IN VITRO* STUDIES

4. The focus of OECD GLPs has been primarily on ensuring that *in vivo* toxicological studies provide high quality and reliable test data. Historically, and for the most part, the regulatory decision-making process has been based almost exclusively on data generated by *in vivo* studies. However, within the last decade, there has been considerable effort to reduce, replace and refine animal use by developing and validating alternative *in vitro* test methods. An OECD "Consensus Document on the Application of the GLP Principles to *In Vitro* Studies" would offer numerous advantages, including, but not limited to, the following:

- facilitate the conduct of high quality *in vitro* studies in accordance with GLPs;
- help increase the confidence of regulatory authorities in the outcome of *in vitro* testing;
- provide GLP guidance for the new technologies (e.g. toxicogenomics, proteomics, genetically engineered cell systems) for which currently available guidance is not generally applicable;
- provide GLP guidance for high throughput methodologies and other increasingly complex current and anticipated in vitro tests, for which practical guidance is currently lacking;
- offer guidance for ensuring regulatory compliance with GLP requirements;
- signal the importance of *in vitro* tests in contemporary modern safety evaluations and provide clarity on the application of GLPs to *in vitro* studies;
- increase clarity and consistent interpretation for persons involved in directing, conducting or assuring the quality of *in vitro* tests by providing guidance specific to these test methods;
- create a more user friendly document, especially for end users interested in conducting *in vitro* studies only, by eliminating guidance applicable to *in vivo* studies;
- create a single document that can be more easily revised as the application of GLPs to *in vitro* testing continues to evolve; and

5. While the current OECD "Consensus Document on the Application of the GLP Principles to Short Term Studies" is applicable to both *in vivo* and *in vitro* methods, there is a greater emphasis on *in vivo* test methods. The inclusion of principles and guidance that principally apply to *in vivo* test methods (e.g., animal husbandry) may be open to subjective interpretation and confusion as to if and how they should be applied to *in vitro* test methods. A stand-alone document focusing exclusively on the application of GLPs to *in vitro* test methods would not include discussions applicable only to *in vivo* methods, and would clarify and emphasize the principles specifically relevant to *in vitro* test systems. A more specific document should result in increased compliance and improved quality assurance oversight of *in vitro* studies. Furthermore, such a document would provide the regulatory community with specific information and guidance to better assess the quality and integrity of *in vitro* studies submitted to them for regulatory purposes. This stand-alone document would contain a glossary of terms, as they relate specifically to the application of GLPs to *in vitro* test methods. This glossary of new *in vitro* terminology and definitions could also include relevant existing terminology and definitions available in other OECD principles, guidance, advisory, and consensus documents devoted to GLPs.

6. The most dramatic changes in regulatory test methods used for regulatory purposes are manifest in the *in vitro* arena, as evidenced by the acceptance in 2002 of four new OECD test guidelines for *in vitro* methods by the OECD WNT National Coordinators for the Test Guidelines Program). In the last few years, there has been an enormous increase of *in vitro* assays and proprietary non-animal test systems, and this can be expected to accelerate as new technologies, high throughput methods, and other alternative methodologies are incorporated into testing approaches that lend themselves to research and regulatory purposes (e.g., toxicogenomics, proteomics, transgenic models, etc.). As with recently adopted *in vitro* test systems, these new systems will likely have unique quality issues and concerns, and will raise new questions regarding compliance. With the evolutionary changes occurring in toxicology and related fields and their ever-increasing technological complexity, it is necessary to provide relevant, specific, detailed GLP guidance that is directly applicable to test methods that will ultimately arise from such revolutionary technologies. Some of these issues are as follows:

- requirements for consistency and calibration of *in vitro* systems obtained from various sources, including proprietary methods;
- specific responsibilities of the study director for handling and calibrating *in vitro* test systems that incorporate these new technologies;
- application of GLP requirements related to *in vitro* study protocols, including those that use proprietary methods or kits; and
- GLP requirements related to proprietary test methods and *in vitro* study reports.

A stand-alone OECD "Guidance Document on the Application of the GLP

Principles to *In Vitro* Studies" would also simplify its revision, which is likely to be needed to meet the GLP needs of this evolving and rapidly expanding field of *in vitro* methods.

7. Many new *in vitro* test systems involve the use of proprietary materials or test kits. Guidance is necessary to ensure that study directors are cognizant of the need to verify that the test kit or *in vitro* test system is functioning properly when it is used. Test systems must also be evaluated to ensure their structural and functional integrity, according to specifications or other performance standards set forth for the initial test system that was validated and approved. Appropriate reference chemicals, including negative and positive controls, should be used for this purpose.

8. The OECD "Consensus Document on the Application of the GLP Principles to Short Term Studies" is directed at short-term studies (i.e., studies of short duration using widely used, routine techniques) for both *in vivo* and *in vitro* test methods. However, *in vitro* studies are not necessarily limited to studies of short duration or to those that would be based on widely used, routine techniques. For example, some *in vitro* neoplastic transformation assays take between one and two months to conduct. It is also conceivable that *in vitro* complex organ systems will be developed using co-cultured human cells derived from different tissues that that can be used as test systems for evaluations occurring over extended periods of time. Such test systems might eventually allow, for example, an *in vitro* assessment of systemic toxicity over an extended timeframe, assessment of repeat dose effects, and assessment of recovery of *in vitro* test systems following an acute toxicity.

SPECIFIC JUSTIFICATIONS FOR A STAND-ALONE GLP DOCUMENT FOR *IN VITRO* STUDIES

9. Examples are provided below for some of the ten subsections of the current GLP Principles where additional guidance relevant to *in vitro* test methods could be provided. The title of each subsection addressed is provided in bold type. An OECD "Guidance Document on the Application of the GLP Principles to *In Vitro* Studies" would contain information in the following areas, as they apply specifically to *in vitro* studies. These include, but would not be limited to:

(1.) Test Facility Organization and Personnel

10. Study director responsibilities as they relate specifically to *in vitro* test methods could be expanded upon. For example, study director issues related to a review of the quality of proprietary test kits and materials, such as evaluating quality control documents for completeness and acceptability, could be included

(2.) Facilities

11. The OECD GLPs largely focus on facilities (i.e., separate rooms for separate studies, environmental controls for rooms), as they apply to animal studies. In *in vitro* studies,

- *in vitro* studies do not require dedicated facilities that are exclusionary of other *in vitro* studies, and often share in the use of equipment and supplies;
- the focus should be on conducting multiple studies in the same physical environment and on ensuring the appropriate separation of co-existing studies; and
- equipment (e.g., incubator, laminar flow safety hoods) rather environmental (i.e., room) controls become more important and often, equipment (as opposed to environmental) monitoring measurements need to be continuous rather than sporadic.

(3.) Apparatus, Materials, and Reagents

12. Current OECD GLP Guidance provides only brief information on the types of apparatus, materials, and reagents required for *in vitro* studies. An OECD "Guidance Document on the Application of the GLP Principles to *In Vitro* Studies" would indicate the need to ensure that the quality of equipment and cell culture materials are sufficient to assure reproducible and accurate results. In particular, a stand-alone document would indicate that:

- slight variations of these materials can impact study results, and therefore should be monitored and documented;
- equipment and instruments should be maintained and calibrated properly (e.g., control of temperature and CO_2 levels of incubators);
- all materials employed should be stored under appropriate conditions to protect them from damage, infestation, or contamination;
- composition of culture media for routine cultures (maintenance media) and/or experimental cultures, and supplements/additives (e.g. serum, heat-inactivation or irradiation of serum, growth factors, hormones, antibiotics) should be defined, to the extent possible;
- non-defined preparations (e.g., serum-replacements, growth factor mixtures) should not be considered acceptable;
- cell/tissue/organ culture medium volumes used and feeding cycles should be defined;
- changes of batches of materials should be monitored with regard to their influence

on *in vitro* growth conditions, inhibitory factors, and principal endpoints of the study;
- culture vessels (flasks, Petri dishes, bottles, roller cultures, etc.) should be defined and sterility methods should be documented and verified;
- culture substrata and/or coating materials/procedures (e.g., collagen, fibronectin, laminin) should be defined;
- names and addresses of manufacturers and suppliers of culture media, media supplements, culture substrata and culture vessels should be reported; and
- lot-to-lot consistency of materials and supplies should be documented and verified.

(4.) Test Systems

13. Instead of providing guidance on the type of information needed on animals when conducting a GLP-compliant study, an OECD "Guidance Document on the Application of the GLP Principles to *In Vitro* Studies" would be able to focus exclusively on information needed on the types of cells used in *in vitro* biological test and culture systems, and their specific properties that make them appropriate for the method for which they are intended. Examples include:
- origin or source of the cell type or cell line (e.g., primary tissue explant; established cell line from a cell bank such as ATCC, ECACC, DMSZ, Riken Gene bank; depositor; laboratory of origin; original publication/patent);
- nomenclature of the cell type or line in use (e.g., ATCC designation);
- method by which cells were obtained (i.e., from a tissue biopsy or explant, shipped frozen or in liquid medium);
- chronology of custody (historical tracking) of cell/tissue culture;
- mode of culture initiation (species, organ, tissue, lineage, mode of transformation, genetic modification, sublines/hybrid cells; in case of humans: donor characteristics such as race, sex, age, disease, health status and medication, biopsy, tumour, if such information is available);
- basic morphological description of cultured cells, including stability of the phenotype and expected population doubling times;
- culture conditions and subcultivation intervals (cell density, confluent/subconfluent cultures, cell harvest, split ratio, initial passage number, number of passages in culture, effect of passage number on principal endpoint of test);
- conditions for freezing/thawing, including cryoprotectant, storage conditions, viability, plating/cloning efficiency (if available);
- indicators of the state of differentiation and expressing activities, where available;
- precise definition of measures undertaken to maintain or induce differentiation; and
- test methods and test interval for screening for mycoplasma and other adventitious agents and other appropriate tests to ensure the use of non-contaminated cells.

(5.) Test and Reference Items

14. The types of information needed in the test protocol regarding the concurrent negative, solvent, reference, and positive controls generally included in *in vitro* test methods or in characterizing *in vitro* systems, or in identifying acceptable reagents (e.g., serum supplements) compatible with the *in vitro* system would be indicated here.

15. The extent and manner to which GLP procedures apply to proprietary materials and test kits used in some *in vitro* studies would be discussed. For example, guidance would be provided on what factors should be evaluated to determine that such materials and kits are of sufficient quality (e.g., review of quality control data for relevant test kit batches) for them to be considered acceptable.

(6.) Performance of the Study

16. *In vitro* test method specific procedures relevant to acceptance criteria (e.g., the use of negative, solvent, reference, and/or positive control substances and historical control data) would be discussed.

(7.) Reporting of Study Results

17. Additional information relevant to *in vitro* testing that should be included in the study report would be discussed, including many of the details above. Further examples are provided in recent recommendations for minimum information that should be included in the study report for *in vitro* endocrine disrupter test methods (ICCVAM, 2003).

OTHER CONSIDERATIONS

18. There are increasing numbers of validation studies of *in vitro* testing methods, and this can be expected to continue to increase even more dramatically as new science and technologies are incorporated into *in vitro* test methods and such innovative techniques enter the regulatory arena. According to ICCVAM, ECVAM, and OECD guidances, validation studies should ideally be conducted in accordance with GLPs. A user-friendly, clear and concise document devoted to the application of GLPs to *in vitro* methods could help encourage the use of GLPs for validation studies, thereby helping to facilitate and increase confidence in the validation of *in vitro* test methods.

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