

Guidance Document for GLP inspectors and GLP test facilities

Cross-contamination of control samples with test item in animal studies

General introduction

In order to ensure as far as possible a common understanding among the Member States as well as between the Member States and the competent services of the Commission, the EU GLP Working Group issues guidance dealing with specific matters related to the practical implementation and application of Directive 2004/9/EC and Directive 2004/10/EC (hereafter called the GLP Directives).

The present guidance document does not provide a legal interpretation of the Directives. It is not legally binding and it does not modify or amend the GLP Directives in any way. Where procedures are dealt with, this does not in principle exclude other procedures that may equally satisfy the Directives.

The guidance document will be primarily of interest and use to those involved in giving effect to the GLP Directives from a technical and administrative standpoint.

It may be further elaborated, amended or withdrawn by the same procedure leading to its issue.

The European Union GLP Working Group, at its meeting on 27 September 2005, approved this guidance document and recommended it to be published on the European Commission's GLP internet site.

Purpose of this document

The purpose of this document is to give guidance to GLP inspectors on how to act when cross contamination in control samples is identified. The document is also recommended to be used by test facility management and quality assurance to assess the severity of the problem when it has been identified.

Background

Miss-dosing and/or cross-contamination of test item is always a risk in animal studies. These problems are usually detected by the presence of the test item and/or its metabolites in plasma or other biological samples from control animals. It is recognised that dietary and topical studies might lead to a higher level and incidence of contamination of test item in control animals. However, contamination of biological samples from control animals has been observed also in studies using other routes of administration, e.g. gavage, intravenous, intraperitoneal, subcutaneous or inhalation. Exposure of the control animals to the test item may compromise or invalidate the study from a scientific point of view.

These kinds of problems are of course first noticed by the Study Director. In case of multi-site study Principal Investigator at the bioanalytical test site notices contamination on the basis of analytical results. The Inspector may come across such problems during study audits of finalised studies, when auditing bioanalytical data at bioanalytical Test Sites or may be requested by Receiving Authorities to perform directed inspections/study audits of particular studies.

Definitions

Miss-dosing is where the animals have received the wrong test item or the wrong concentration of the test item solution, i.e. the wrong *in vivo* exposure.

Cross contamination can be due to mix-ups of test item and reference item or mix-ups of biological samples from the animals. The latter could be caused by *in vivo* and/or *ex vivo* exposure, depending on where the cross contamination took place.

Responsibilities

a) *Study Director*

The Study Director is responsible for ensuring that the study has been performed in accordance with the study plan and the Principles of Good Laboratory Practice. If samples from control animals contain the test item or its metabolites this should be appropriately reported within the Study Report and a full assessment made on the impact of this deviation on the quality and integrity of the study.

b) *Principal Investigator*

The Principal Investigator is responsible for ensuring that the delegated phase of the study has been conducted in accordance with the applicable Principles of Good Laboratory Practice. If samples from control animals contain test item or its metabolites this should be promptly reported to the Study Director and the reason for the unexpected results should be investigated.

c) *Quality Assurance*

The QA has the responsibility to assure test facility management that the Study Report accurately reflects the raw data and that deviations from the Study Plan are appropriately documented. It is also the duty of the QA to evaluate if identified deviations are of a sporadic or systematic nature. Because of their independence, it may be useful for QA to be involved in any investigation into cross-contamination.

d) *Test Facility Management*

Test facility management is responsible for ensuring that appropriate corrective and/or preventive actions are implemented where required on a facility wide basis to prevent the recurrence of any problems that have been identified.

e) *Inspector*

The inspector has the responsibility to ascertain the extent of compliance with the Principles of Good Laboratory Practice. In particular the inspector should establish if the cross-contamination is confined to a specific study or if it is a facility based issue.

Why is the presence of test item in biological samples from control animals a GLP problem?

In toxicological studies biological samples are taken and analysed for the content of test item and/or its metabolite(s) to monitor exposure to the treatment, to generate pharmacokinetic data, and to verify that the animals have been adequately dosed as stated in the Study Plan. Toxicokinetic monitoring is usually performed in pivotal toxicological studies such as repeat dosing, reproductive toxicological studies and carcinogenicity studies. The standard procedure to survey adequate systemic exposure of animals is to collect blood/plasma samples at different time points during the study.

When test item is identified in biological samples from control animals, this is a deviation from the experimental design of the Study Plan. From a GLP viewpoint the reason for the contamination and its magnitude should be investigated and documented in order to establish the extent of the problem and to ensure that appropriate corrective/preventive actions are implemented.

How is contamination of control animals detected?

The principal way of identifying that miss-dosing has occurred is to examine the bioanalytical results of biological samples taken from the animals. The easiest or perhaps the only way to notice this is if biological samples from control animals contain test item or its metabolite(s). The Study Director and the study personnel notice the problem when they receive the bioanalytical results and should of course take necessary corrective measures.

Investigation strategy of the inspector.

There are two possibilities for the Inspector to identify cross-contamination problems. In most cases the Inspector gets the information from the Receiving Authority or from another Monitoring Authority. The second possibility is that the Inspector identifies the problem during a surveillance inspection.

Step 1: Investigation of a particular study

There may be many reasons behind the presence of test item in biological samples from control animals. First of all the Inspector should expect that the Study Director has highlighted it as a deviation and that both the Study Director and Test Facility Management have performed a thorough investigation as to why there is test item in biological samples from control animals. This investigation should be documented. QA should also identify the problem when the final draft report is reviewed. Contamination of control samples in toxicological studies shall be reported appropriately. If no such investigation has been performed and/or the problem has not been accurately documented and reported the Study Director has not fulfilled his responsibility, which is a serious GLP deviation. Test Facility Management also needs to find out whether the contamination is specific to a particular study or whether other studies may have been affected. QA may assist in these investigations. It is the responsibility of the Receiving Authority to decide if the study is scientifically valid or not. Therefore, a thorough and careful investigation of all the aspects causing a possible cross-contamination should be carried out. The role of the Monitoring Authority is to support the Receiving Authority by performing a thorough inspection/study audit to establish the extent of compliance with the Principles of GLP.

There are two types of problems that should be investigated by the Inspector. From a scientific point of view, which is of interest for the Receiving Authority, it is vital to be able to separate between:

1. Are detected levels of test item in biological samples from control animals due to *in vivo* exposure? If yes, special attention should be paid to the administration procedure where miss-dosing could be the main cause of contamination.
2. Are detected levels of test item in biological samples from control animals due to *ex vivo* contamination during or after sampling?

If the outcome of the investigation unequivocally shows that the control animals have not been exposed and there is another cause for the anomalous results, this is still a serious deviation and the study may not be GLP compliant. In this situation it is up to the Receiving Authority to decide on the scientific validity of the study concerned.

In order to differentiate the stage where the test item was introduced, all steps in the study process should be reviewed.

Organisation:	Does the organisation have a documented procedure to investigate cross-contamination?
Quality Assurance:	Where cross-contamination occurs, has an investigation been carried out and documented? Has an impact assessment on the integrity and quality of the data been made and documented?
Characterisation:	Is an appropriate level of characterisation of the test item available to ensure that the correct receipt, storage and handling conditions have been followed?
Formulation /dispensing:	Analysis of dose samples; materials accountability; measures to separate test item from control item etc; labelling/coding
Animal dosing:	Are documented procedures in place for handling; separation etc.,
Animal care:	Are documented procedures in place for reporting and handling accidents such as feed spill, loose animals, animal group separation
Sampling of biological samples	Are documented procedures in place to address hygiene, measures to avoid contamination, logistic of sampling, labelling
Sample preparation:	Are documented procedures in place for handling, labelling; storage; shipping. Equipment and procedures adapted to physical-chemical properties of test item, i.e. hydrophobic/sticky compounds etc
Analysis:	In the case where the test item is metabolised <i>in vivo</i> and no metabolites are detected in biological samples from control

animals this is a good indication that the animals have not been exposed.

Good housekeeping/
Concurrent studies: Is there appropriate separation between concurrently run studies in terms of facilities, equipment and personnel used, are appropriate environmental conditions like ventilation and animal cage cleaning in place and being monitored?
Has appropriate clean-up between studies been undertaken? For topical studies has the study plan specified procedures to prevent cross-contamination?

Administrative issues: Does the Master Schedule indicate excessive work load for some staff; have there been similar problems in other studies?

An *in vivo* exposure has most probably been caused by mis-dosing. If such an exposure cannot be excluded the Inspector should continue his/her investigation.

Step 2: Test facility investigation procedures

When contamination problems have been verified in a study the Inspector is expected to expand his/her inspection to see if other studies have similar problems. If that is the case a thorough facility inspection is necessary to establish the GLP compliance status of the test facility.

Inspection report

The Inspector's findings and his/her conclusion about the impact of them on the GLP status of the audited study and/or the test facility should be documented in the Inspection Report.

- For study audits/non-routine inspections - the Inspector should describe in detail his/her investigation procedure and its results;
- For routine inspections - the report should give information about the extent of the problem, corrective actions recommended and plans for follow-up inspection.

If systematic problems have been identified the relevant Receiving Authority(ies) and EU GLP Working Group should be informed.