PS3-3 - 6135

Good Laboratory Practice (GLP) - chance and impediment for the registration of new fumigants - Phosphine residues as an example

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Abstract

Phosphine is a very effective fumigant to control pest insects. Therefore the determination of phosphine residues in treated cocoa beans is chosen as an example for a study according to the principles of GLP and exemplify how the standard requirements are concretely realized at the Institute for Stored Product Protection. For the purpose of registration of a chemical compound for pest control data on the formation and decay rate of residues in treated products have to be provided Europe-wide by applicants according to GLP.

The installation of a GLP standard in a lab requires:

- defined organisation and responsibilities,
- planned and supervised processes according to written standard operation procedures (SOPs),
- comprehensible studies.

This quality ensuring system has been established in Germany in 1990, currently about 160 test facilities have been licensed. Since April 2004 the Institute for Stored Product Protection, as the only public scientific institute dealing with aspects of storage and insect pest control in Germany owns a GLP permission for “the detection of residues”.

The demand for GLP promotes high quality of residue data on one side, but impedes on the other side strongly the introduction of new fumigants or the registration of new application fields for an existing registered fumigant. Especially for small companies, it is difficult to install and keep the complex GLP infrastructure and the specific expertise. Similar constraints apply to good experimental practice (GEP), the quality ensuring system for efficacy data.

The pros and cons of working with or without GLP standards in an analytical lab are discussed.

Key words: Good Laboratory Praxis (GLP), registration, new fumigants, phosphine, food residue.

Introduction

Since 1981 the Principles of GLP – based on regulations of the US Food and Drug Administration - have been developed by an expert group of the Organisation for Economic Co-operation and Development (OECD) and they should be applied to the non-clinical safety testing of test items.

The principles of GLP have been developed to promote the

- protection of the consumer, animals and environment,
- quality and validity of non-clinical health and environmental safety studies,
- quality of residue data,
- provision of reliable data and
- international mutual acceptance of data.

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Scope of GLP

The Principles of Good Laboratory Practice (GLP) are defined as rules and criteria for 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.

Chemicals control legislation in the OECD Member states requires a preventing risk assessment by testing chemicals to determine their potential hazards. The Principles of GLP have been developed to promote the quality and validity of those test data used for determining the safety of active chemical substances and chemical products prior to their use.

The issue of data quality has an important international dimension: If regulatory authorities in countries can have confidence in the quality of test data developed by abroad test facilities, duplicat testing can be avoided and costs can be saved to government and sponsors of studies. The mutual recognition of these data by OECD and the European Member States becomes important for data related to authorisation procedures where national or European authorities have to assess the effects of chemicals relating to human health and the environment. The principles for GLP also simplify this exchange of information between the competent authorities and harmonise the procedures.

The principles of GLP have been developed in the framework of the OECD and were first published in 1981. Meanwhile a series of documents on related issues have been established. The OECD Principles of GLP (as revised in 1997) laid down the scope of GLP:

- These Principles of GLP should be applied to the non-clinical safety testing of test items contained in pharmaceutical products, pesticide products, cosmetic products, veterinary drugs as well as food additives, feed additives, and industrial chemicals. These test items are frequently synthetic chemicals, but may be of natural or biological origin and, in some circumstances, may be living organisms. The purpose of testing these test items is to obtain data on their properties and/or their safety with respect to human health and/or the environment.
- Non-clinical health and environmental safety studies covered by the Principles of GLP include work conducted in the laboratory, in greenhouses, and in the field.
- Unless specifically exempted by national legislation, these Principles of GLP apply to all non-clinical health and environmental safety studies required by regulations for the purpose of registering or licensing pharmaceuticals, pesticides, food and feed additives, cosmetic products, veterinary drug products and similar products, and for the regulation of industrial chemicals.

GLP regulations and requirements

In the Series on Principles of Good Laboratory Practice and Compliance Monitoring the following OECD papers are published (http://www.oecd.org):

No 1: OECD Principles on Good Laboratory Practice
No 2: Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice
No 3: Revised Guidance for the Conduct of Laboratory Inspections and Study Audit
No 4: Quality Assurance and GLP (revised 1999
No 5: Compliance of Laboratory Suppliers with GLP Principles(revised 1999)
No 6: The Application of the GLP Principles to Field Studies(revised 1999)
No 7: The Application of the GLP Principles to Short&Term Studies (revised 1999)
No 8: The Role and Responsibilities of the Study Director in GLP Studies (revised 1999)
No 9: Guidance for the Preparation of GLP Inspection Reports
No 10: The Application of the Principles of GLP to Computerised Systems(1995
No 11: The Role and Responsibility of the Sponsor in the Application of the Principles of GLP

No 12: Requesting and Carrying Out Inspections and Study Audits in Another Country
No 13: The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies
No 14: The Application of the Principles of GLP to in vitro Studies

All terms and definitions cities below are requirements test management have to establish in an test facility:

I. Terms concerning the organisation of a test facility

- Test facility means the persons, premises and operational unit(s) that are necessary for conducting the non-clinical health and environmental safety study
- Test facility management means the person(s) who has have the authority and formal responsibility for the organisation and functioning of the test facility according to these Principles of GLP.
- Study Director means the individual responsible for the overall conduct of the non-clinical health and environmental safety study.
- Quality Assurance Programme means a defined system, including personnel, which is independent of study conduct and is designed to assure test facility management of compliance with these Principles of GLP.
- Standard Operating Procedures (SOPs) means documented procedures which describe how to perform tests or activities normally not specified in detail in study plans or test guidelines. SOPs should be available for, but not be limited to, the following categories of test facility activities.

1. Test and Reference Items Receipt, identification, labelling, handling, sampling and storage.
2. Apparatus, Materials and Reagents
3. Record Keeping, Reporting, Storage, and Retrieval
4. Test System (where appropriate)
5. Quality Assurance Procedures

- Master schedule means a compilation of information to assist in the assessment of workload and for the tracking of studies at a test facility.

II. Terms concerning the Non-Clinical Health and Environmental Safety Study

- Non-clinical health and environmental safety study means an experiment or set of experiments in which a test item is examined under laboratory conditions or in the environment to obtain data on its properties and/or its safety, intended for submission to appropriate regulatory authorities.
- Study plan means a document which defines the objectives and experimental design for the conduct of the study, and includes any amendments.
- Test system means any biological, chemical or physical system or a combination thereof used in a study.
- Raw data means all original test facility records and documentation, or verified copies thereof, which are the result of the original observations and activities in a study. Raw data also may include, for example, photographs, microfilm or microfiche copies, computer readable media, dictated observations, recorded data from automated instruments, or any other data storage medium that has been recognised as capable of providing secure storage of information for a time period.
- Storage and Retention of Records and Materials

All original documents citied above and activities of the OECD concerning GLP are available from the OECD website: http://www.oecd.org/ehs/

Community legislation and legal regulations for the European Authorisation of Plant Protection products

The Member States of the European Community recognised that there was a need to protect environment and to create standards to protect consumers. For this reason, the first Community environment legislation dealt with

products and chemicals establishing registration requirements and procedures for most of the existing chemicals.

Pesticides - plant protection products (PPP) and biocidal products (BP) - belonging to these groups need to be assessed and authorised before they can be placed on the market. In the case of PPP the European Community has developed a regulatory framework, Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market – defining rules and common standards for the authorisation of PPP. The Directive requires risk assessments for effects on health and environment and maximum residues levels in food to be carried out, before an active substance (existing or new) can be listed in the positive list of Annex I by the Commission and before Member States authorise marketing and use of PPP. The legal provisions concerning GLP principles are summarized on the website of the European Union (EU) citied below: http://ec.europa.eu/enterprise/chemicals/legislation/glp/product/pesticides_en.htm.

- The introductions to annexes II and III to the Directive require that tests regarding the safety for human health and the environment for active ingredients and plant protection products must be conducted in accordance with the principles laid down in Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. Directive 2004/9/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of GLP lays down the obligation of the Member States to designate the authorities responsible for GLP inspections in their territory for mutual acceptance of data. The Directive requires that the OECD Revised Guides for Compliance Monitoring Procedures for GLP and the OECD Guidance for the Conduct of Test Facility Inspections and Study Audits must be followed during laboratory inspections and study audits.
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of GLP and the verification of their applications for tests on chemical substances requires from Member States to take all measures necessary to ensure that laboratories carrying out safety studies on chemical products comply with the OECD Principles of GLP.
- Article 13 (1) of Directive 91/414/EEC provides that applicants for the authorisation of a plant protection product have to submit a dossier satisfying the requirements of Annexes II and III which detail the data to be submitted.
- reference to test guidelines which intends to ensure that the data submitted are scientifically acceptable
- the circumstances in which studies have or have not to be submitted
- the application of GLP
- The Commission adopted temporary derogations for the application of the principles of GLP for residue trials started at the latest on 31.12.1997 and for honeybee and beneficial arthropod testing started at the latest on 31.12.1999 (Directives 93/71/EEC and 95/35/EC both amending Directive 91/414/EEC; available in EUR-Lex http://ec.europa.eu).
- Since the Directive provides in general terms for the application of GLP to studies on the properties and/or safety with respect to human health or the environment, the Standing Committee on Plant Health agreed that guidance document 7109/VI/94 rev. 6 contains the correct interpretation of which individual studies of Annexes II and III, part A are related to human health or the environment and therefore should be subject to the GLP requirements.

Determination of phosphine in cocoa beans according to the GLP-guidelines

The phosphine residues were detected according to a written study plan and based on a

large number of SOPs dealing with descriptions of organization, apparatures and methods.

Examples of used organisational SOPs are:

- Documentation of any working step in the lab,
- Drawing up of study plans and final reports,
- Management of data retrievals and unchanged data,
- Archiving and storage of documents and papers

Examples of used methodical SOPs are:

- Handling with materials and reagents,
- Handling with test and reference items,
- Sample taking, sample taking systems and sample transport,
- Sample extraction and PH_3 detection,
- Checking, Excel program with example figures,
- Validation of the system "gas-chromatograph with mass selective detector"

Examples of used apparatus SOPs are:

- Refrigerator, freezer, balance, gas-chromatograph with mass selective detector

Aim

The use of GLP shall be demonstrated with the determination of residue for registration of a phosphine releasing product. The Detia Gas Ex B forte sachets should be tested for their suitability to be used for cocoa bean disinfestations.

In order to comply with the fixed maximum residue limits (MRLs) for cocoa beans (0,01 mg/kg), applicants have to provide research results of the agent's residues in treated raw cocoa according to GLP. It is then in the admission process for plant protecting substances, e.g. when determining waiting times and ceiling values of the substance to be applied, that these data are used for a thorough risk assessment. Before the actual test in the fumigation station of the BBA in Berlin, Germany, a gas-proof steel chamber filled with raw cocoa was fumigated according to the data of a potential applicant. Before fumigation and at fixed times after the process, samples were taken and investigated for their residues of phosphine.

Method development, method validation and storage stability studies

A large number of methodical SOPs were developed to validate the PH_3 residue determination method in cocoa beans. The aims were:

- the detection of recovery rates,
- the determination of the quantitative detection limit,
- the determination of the qualitative detection limit,
- the investigation of the linearity, accuracy and precision of the phosphine detection and
- the investigation of the storage stability of phosphine residues in fumigated cocoa beans and stored at –18 °C.

Often the testing facilities do not develop a method for the analytical work since the method is always very specified. Prior to the analysis of samples a method had to be developed a method to detect the PH_3 residues in cocoa beans. A test procedure by Nowicki, recorded by Noack et.al. (1983) was applied, where PH_3 residues are extracted from the samples by diluted sulphuric acid in a vacuum and their quantities determined by a mass spectrometer.

Prior to analysis, the storage of treated cocoa beans was performed at –18 °C. Investigation of the storage stability of the phosphine residues in fumigated cocoa beans revealed (Figure 1) that no relevant decay occurred for 56 days of storage at this temperature. The average of the recovery rate ranged within the required limits in Germany of 70 % and 110 %.

Fumigation

Two packages of Detia Gas Ex B forte (weight per package: 9 g of substance, content: 66 % Mg_3P_2, equals 3 g PH_3) were used for four bags of raw cocoa in the fumigation process, as shown in Figure 2. Based on a volume of the fumigation chamber of 0.5 m^3, the dose was 12.0 g/m^3 of the applied PH_3. The dose was 18 g Mg_3P_2/ 0.5 m^3 and 6 g PH_3/ 0.5 m^3, respectively. With a weight of the treated cocoa of 100 kg, this

corresponds to an application of 180.0 g of Mg_3P_2 per ton or 60.0 g of PH_3 per ton of cocoa. The fumigation time was 60 hours, whereas subsequent ventilation lasted 24 hours.

Surveillance of the fumigation parameter

The analytical detection of the fumigant during the fumigation with Detia Gas Ex B forte was carried out online with an IR-spectrometer Miran 1B (Wilks scientific corporation, Norwalk, USA), see Figures 3 and 4. Figure 5 shows the temperature curve during the fumigation period. The average temperature was 19.2 °C.

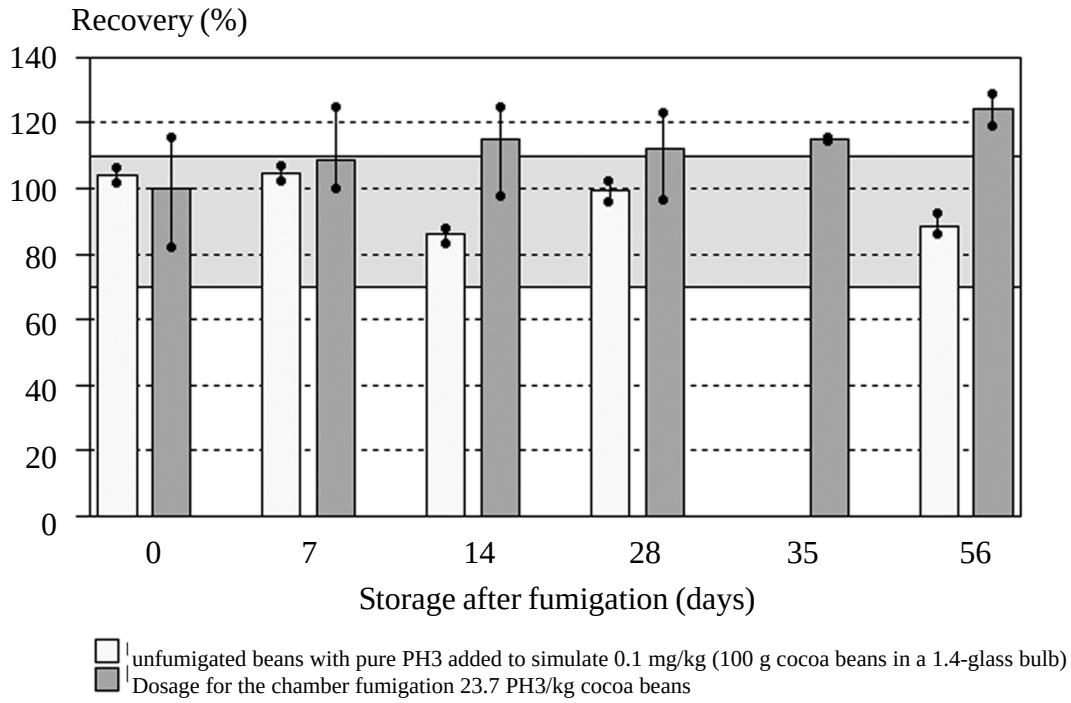


Figure 1. Stability of phosphine residues in cocoa beans during storage after treatment.

Figure 2. Cocoa beans in the fumigation chamber before fumigation with Detia Gas Ex B forte.

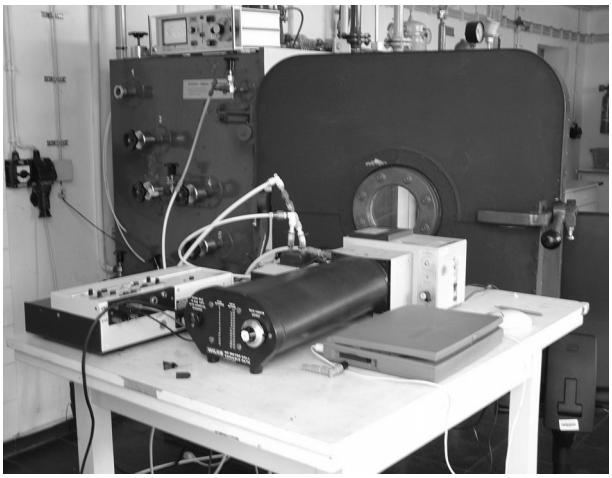


Figure 3. Miran 1B (Wilks scientific corporation, Norwalk) in front of the fumigation chamber.

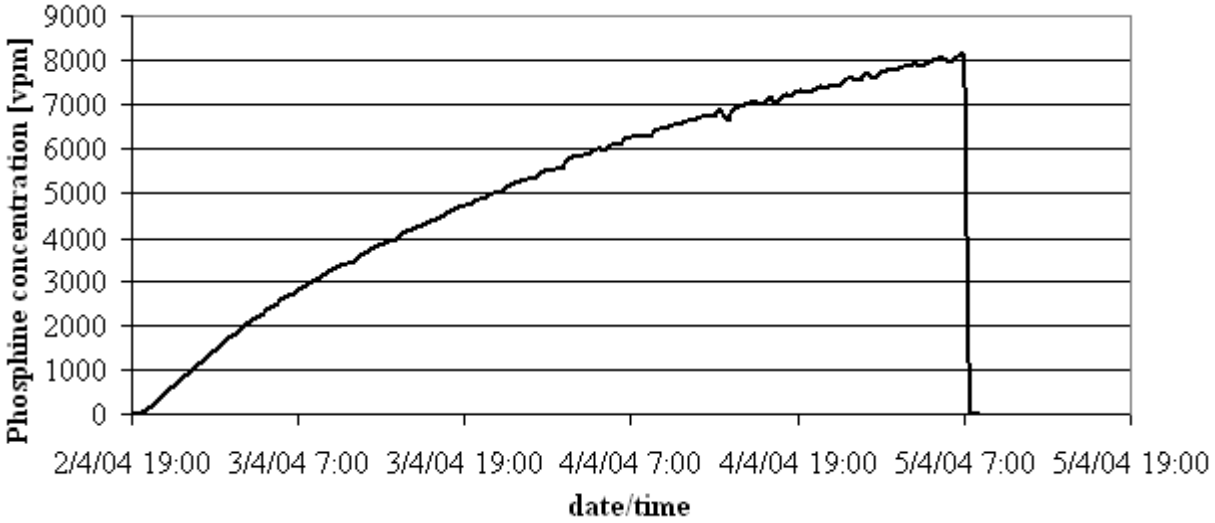


Figure 4. Phosphine concentration during the fumigation period with Detia Gas Ex B forte.

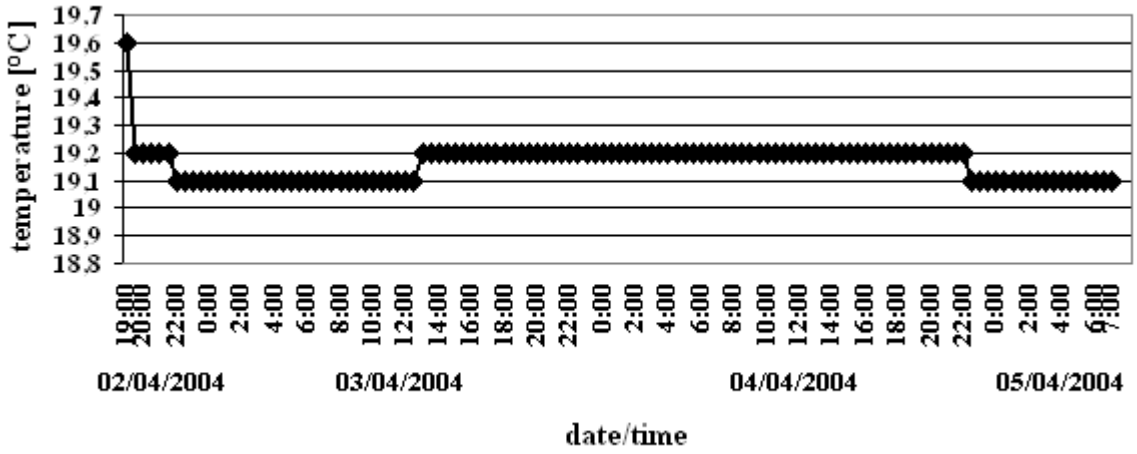


Figure 5. Temperature curve in the fumigation chamber filled with cocoa beans during the fumigation with Detia Gas Ex B forte.

Detection of phosphine residues

A head space technique was used to determine the phosphine contents in the cocoa beans. The analytical detection of PH_3 residues is performed later on by gas-chromatography with mass selective detector. As shown in Table 1 and in Figure 6, respectively the PH_3 contents in cocoa beans were detected after 1, 3, 7, 10 and 14 days after a fumigation with Detia Gas Ex B forte. The phosphine residues were in the range of 0.051 mg/kg on day "1" after the fumigation end and as soon as 10 days after storage, the residues had dropped down to about 0.01 mg/kg. After 14 days, the phosphine values were below the detection limit of 0.01 mg/kg, the European maximum residue limit for these products.

The criteria for monitor the methods (requirements to recovery rates, relative standard deviations and initial dose) described in the guideline "Methods of Residue Analysis for Monitoring" by Hänel and Siebers (1998) were complied with in the investigation of the samples.

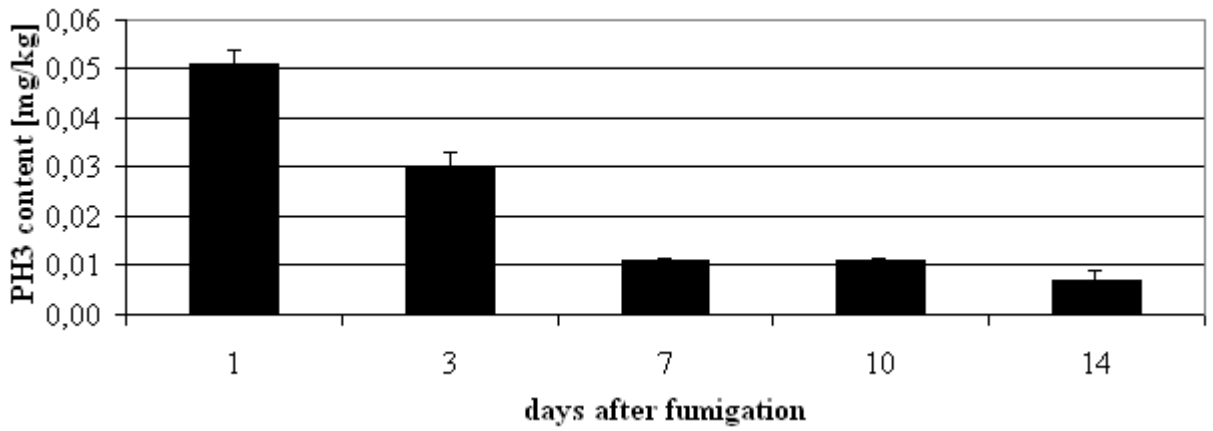
Table 1. PH_3 contents in cocoa beans.

Days after the end of fumigation	Sample number	PH_3 content (mg/kg)	Mean value PH_3 content (mg/kg)	Absolute standard deviation	variation coefficient
Before fumigation	UK1	nd	nd	not calculated	not calculated
	UK2	nd			
	UK3	nd			
1	P1_1	0.047	0.051	0.003	6.5
	P1_2	0.050			
	P1_3	0.055			
3	P2_1	0.028	0.030	0.002	6.8
	P2_2	0.033			
	P2_3	0.030			
7	P3_1	0.011	0.011	0.0	4.4
	P3_2	0.010			
	P3_3	0.011			
10	P4_1	0.011	0.011	0.0	4.4
	P4_2	0.011			
	P4_3	0.010			
14	P5_1	0.007	0.007	0.002	20.2
	P5_2	0.010			
	P5_3	0.006			
	P5_4	0.006			
	P5_5	0.008			
stability tests	L1a	0.076	0.081	0.006	7.0
	L1b	0.089			
	L1c	0.078			

nd = not detectable.

P1_1 = sample 1_1.

L1a = stability sample added with 0.01 mg phosphine /kg cocoa beans.



MRL = maximal residue limit = 0.01 mg/kg

Figure 6. Phosphine content in cocoa beans after application of Detia Gas Ex B fort.

The storage stability of the phosphine residues in fumigated cocoa beans was investigated simultaneously to the detection of these phosphine residues after the fumigation in the fumigation chamber. The storage stability results presented in Table 1, reveal a recovery rate of 81 % ± 6 %. This recovery rate range within the required limits in Germany of 70 % and 110 %.

Conclusion

This demand for GLP protects on one side but impedes on the other strongly the introduction of new fumigants or the registration of new application fields for an existing registered fumigant. This is why a sufficient number of these labs in the individual member countries is missing. This shortage is coursed by the limited number of GLP investigations, which require a high level of specific expertise and very expensive specific apparatus. The limited number depends strongly on the amount of money, which can be earned by this business.

The biggest advantages of working under the GLP guidance are:

- better comprehensibility,
- increasing protection of the consumer, animals and environment,
- increasing quality and validity of studies,
- increasing quality of residue data,
- standardisation of methods and procedures,
- introduction of a high level of specific expertise and
- international mutual acceptance of data.

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