ANNEX 1
PROJECT PLAN

CONTENTS

Project Plan prepared by

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1 Initial Note

The intended use of the 1st Draft Project Plan (16 April 1997) was to inform on the status of preparation of this specific part of the ECVAM Project ***"Evaluation of the Prevalidation Process",*** *thus aiding putative laboratories to decide about their definite participation in this part of the study. Since these decisions are made now, the present final Project Plan is on one hand a status update and on the other hand it contains corrections of the study timing. Compared to the Draft the working programme for Phases I, II and III has not changed.*

2 Objective of the ECVAM Project "Evaluation of the Prevalidation Process"

Since experience has shown that the outcome of large and expensive validation studies can be compromised if their managers do not insist that optimised test protocols and proof of their performance are submitted before the start of the formal validation study, ECVAM is implementing a prevalidation scheme. This has been outlined in the first report of the ECVAM Task Force on Prevalidation[1] and includes three main phases: (**I**) protocol refinement; (**II**) protocol transfer; and (**III**) protocol performance. The objective of the prevalidation process is to ensure that any method included in a formal validation study adequately fulfils the criteria defined for inclusion in such a study, so that financial and human resources are used more efficiently, and so that there is a greater likelihood that the expectations of those in the scientific, regulatory and animal welfare communities, who seek the replacement of current animal tests by relevant and reliable alternative methods, will be met.

3 Background

The Project "Evaluation of the Prevalidation Process" was announced by ECVAM in June 1995 in the Official Journal of the EU (95/S91-46800). Microbiological Associates Ltd., Stirling, UK (in the following referred as MA Ltd.) was selected by ECVAM as the main contractor and principle investigator, who is responsible for the whole project. Depending on the tests selected for "Evaluation of the Prevalidation Process" MA Ltd. is fulfilling this task through subcontracts with laboratories familiar in routinely conducting *in vitro* tests according to GLP and by subcontracting special tasks, like chemical coding and distribution. In 1996 out of several candidate tests, five tests were selected to be suitable for the project:

(a) Bovine Cornea Opacity and Permeability (BCOP) test *(Eye irritation)*
(b) Fluorescein Leakage (FL) test *(Eye irritation)*
(c) Embryonic stem cell test (EST) *(Embroytoxicity)*
(d) EpiDerm™ Phototoxicity Test *(Phototoxicity)*
(e) EpiDerm™ Skin Corrosivity Test *(Skin corrosivity)*

Reasons for selection of (a) and (b) were experiences made in the EC/Home Office Study. Test (c) was selected because the test is promising, but there was no interlab experience. Tests (d) and (e) were selected since due to the discontinuation of the production of Skin² (ATS) two promising test protocols were existing, both worth to be adapted to an available model (EpiDerm™).

Whereas for tests (a), (b) and (c) laboratories are subcontracted and acting already, due to the late decision in November 1996 to include tests (d) and (e), so far only ZEBET is subcontracted for adoption of the existing Skin² test protocols to the EpiDerm™ model. Presently, ZEBET has conducted Phase I to nearly 100% for the EpiDerm™ Phototoxicity test and Phase I to about 30% for

[1] Curren, R.D., Southee, J.A., Spielmann, H., Liebsch, M., Fentem, J.H. & Balls, M. (1995). The role of prevalidation in the development, validation and acceptance of alternative methods. *ATLA* **23**, 211-217

the EpiDerm™ Skin Corrosivity test. ZEBET has now the task to acquire definite partner labs for conducting Phases II and III for each of the two sub-projects.

4 Study Design *(for details see 10: work programme)*

Phase I, Protocol Refinement,

The existing Skin² ZK 1351 methodology is refined by ZEBET (Laboratory 1) and adapted to the EpiDerm™ Epi-200 model. A Draft SOP covering the complete methodology including test acceptance criteria and a prediction model is elaborated and **about ten chemicals are tested several times** according to the refined SOP.

Phase II, Protocol Transfer

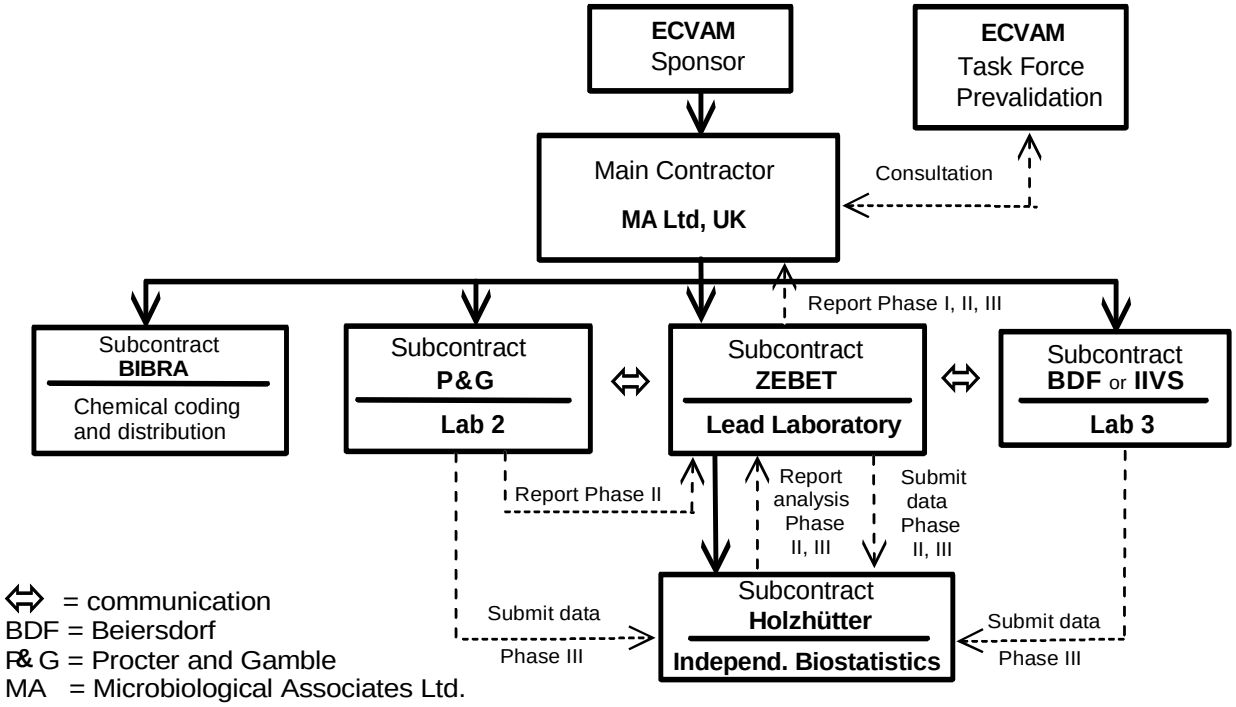
The test is transferred to Laboratory 2 and established by repeated testing of a positive and a negative chemical. If and where necessary, the SOP is amended according to experiences made during the process of transfer. Then, for assessment of intralaboratory repeatability and interlaboratory comparability, Laboratory 1 and Laboratory 2 are testing **5 selected chemicals several times**. Finally, the SOP is refined in cooperation between Laboratory 1 and Laboratory 2 and submitted to *INVITTOX* by Laboratory 1.

Phase III, Protocol Performance

The test is transferred to Laboratory 3 and established by repeated testing of a positive and a negative chemical. Ten chemicals, taken from a list of 20 recommended by ZEBET are selected by BIBRA, coded and distributed to Laboratories 1, 2 and 3, which will test each of the **10 chemicals twice** on separate occasions. A biostatistical analysis is performed at the end.

5 Management

Dr. Jacqueline A Southee (MA Ltd, Stirling, UK) will be the Principal Investigator, who oversees the prevalidation process and activities of the participating laboratories. ZEBET at the BgVV (Berlin, Germany) will be the Lead-Laboratory, which is involved in all three project phases and coordinates activities through the Study Manager, Dr. Manfred Liebsch. The Study Manager has to acquire Laboratories 2 and 3 and to ensure that each Laboratory focuses on the Workscope assigned. He also has to ensure communications between all laboratories and a biostatistical analysis of the data. The Principle Investigator reports progress to the ECVAM Task Force Prevalidation, which will assist the Project in case critical decisions become necessary.



6 Present Status

The Skin² ZK 1351 test protocol developed by ATS and ZEBET in 1993 and modified by ZEBET for the use in the EU/COLIPA "In Vitro Photoirritation" project phase II, has been adapted by ZEBET to the EpiDerm™ model EPI-200 during Dec. 96 - Jan. 97. Five UV-sensitivity experiments and 30 chemical tests on 10 chemicals were performed to form a data base. A positive control, assay quality criteria and a prediction model have been defined. A 1st Draft SOP (dated 13 January 1997), was circulated to members of the ECVAM TF Prevalidation, to MatTek (Ashland) as well as to putative partner labs Procter & Gamble (Ohio), Novartis (Basle) and Beiersdorf AG (Hamburg) for comments. In addition, ZEBET circulated the data base obtained until 13. January 1997 to the above labs.

ZEBET received comments on data and Draft SOP from MatTek, Novartis, MA Ltd. and P&G. Most of them were considered in the refined SOP (30. May 1997). According to suggestions from Dr. Frank Gerberick (P&G), during April/May ZEBET has performed time course experiments (3, 6, and 21 hours preincubation) with the chemicals *Anthracene*, *Bergamot oil* and *Musk ambrette*. Experiments revealed in a reproducible manner that there is no significant difference in the sensitivity of the assay between 6 hrs and 21 hrs (the period originally recommended in the 1st Draft SOP. The sensitivity of the EpiDerm™ Epi-200 tissues was confirmed in four additional experiments performed during April/May 1997. UV sensitivity criteria were included in the final refined Draft SOP. Thus, the work programme of Phase 1 is successfully fulfilled. The refined Draft SOP, the full data base, a MS Excel spreadsheet for data collection and a comments on the data will be circulated by ZEBET early June 1997.

7 Participants

- **Procter & Gamble** (Dr. Frank Gerberick) has indicated definite interest in acting as Laboratory 2 and is in the Process of being contracted.
- **Beiersdorf AG** (Dr. W. Diembeck, U. Pfannenbecker) has indicated definite interest in acting as Laboratory 3 (which means participation in Phase III only), provided Phase III will not start before July 1997. Beiersdorf is presently in the process of being contracted.

- In addition, **Institute for In Vitro Science, IIVS** (Dr. Rodger Curren) had indicated interest in acting as Laboratory 3 (which means participation in Phase III only). It is planned to consider the additional participation of IIVS as a fourth lab if there is enough budget to cover the costs.
- **Novartis** (Dr. Anne de Fraissinette) indicated interest to participate in the study and commented on the 1st Draft SOP. Later, we were informed that Novartis had decided to switch from Skin² to SkinEthic and did not want to set up a second epidermal model for in house testing, since each model needs to be fine tuned.

The expected advantage of four participants in the blind trial (phase III) is that the produced data base might be sufficient to avoid a formal validation as a follow up of the PV study. With regard to the fact, that the intended use of the assay is safety testing, which is in contrast to hazard /risk identification, not regulated. Industry might decide to use this assay as a necessary adjunct on the basis of the unbiased data base produced in the PV study.

8 Contracts

In March 1997, ZEBET was subcontracted by MA Ltd. with a contract covering the total work programme of Laboratory 1 for Phase I, Phase II and Phase III including biostatistical analysis.

Based on the 1st Draft Project Plan, MA Ltd. has meanwhile sent subcontract Drafts for Phase II and Phase III to P&G and Beiersdorf, which are both in the process of being contracted.

9 Timing

With regard to the 1st Draft Project Plan the timing given below had to be slightly corrected due to the fact that both, P&G (Lab 2) and Beiersdorf (Lab 3) will be available for experimental work not before July. Nevertheless, with some efforts, the deadline originally envisaged for completion this specific PV sub-project has to be shifted for 2 weeks only:

End of **05 / 1997**	Completion of Phase I (ZEBET). Subcontracting of Laboratory 2.
07 and 08 / 1997	Conduction of Phase II (ZEBET and Laboratory 2). Subcontracting of Laboratory 3
09 and 10 / 1997	Conduction of Phase III (ZEBET, Laboratory 2 and Laboratory 3).
mid **11 / 1997**	Biostatistical Analysis and final Report (responsible: ZEBET)

10 Work Programme

PHASE I PROTOCOL REFINEMENT (Laboratory 1)

ZEBET's tasks for Phase I are almost fulfilled. Tasks and the degree of completion are listed in the following table:

Task	Description	Degree of completion
SOP	elaboration of a GLP compliant test protocol, including assay acceptance criteria and prediction model	**100%** 1st Draft (13 January 97) Refined SOP: 30 May 1997
method documentation sheet	GLP compliant documentation of experimental steps	**100%**
data recording software	MS Excel spreadsheet to import reader files and calculate results	**100%** to be distributed to labs 2 and 3

Positive control	Identification of a positive control chemical and data base for this chemical	**100%**
Testing	8-10 chemicals tested at least twice	**100%** **35** tests on **10** chemicals performed including time course experiments and verification experiments for UV sensitivity of Epi-200 tissues.
Project Plan	Project Plan covering the actual status of Phase I, as well as the work programme for Phase II and Phase III. Project Plan + SOP + test data shall allow potential participants a definite decision on their participation in Phases II and III	**100%** • 1st Draft circulated 13 January 97 • Final Project Plan (30 May 1997)
Report Phase I	Report actions and data and refined SOP to Principle Investigator	**~ 90%** all data and SOP sent to Principle Investigator. Comments on data still necessary. Finalise 1st week of June

PHASE II PROTOCOL TRANSFER (Laboratory 1 & Laboratory 2)

Tasks of Laboratory 1 (ZEBET):

(1) **Test transfer**:
Provide Laboratory 2 with refined SOP + data recording software + data of Phase I. Supervise transfer of method to Laboratory 2. Give advice whenever necessary.

(2) **Chemical selection**
Select, in cooperation with Laboratory 2, five test chemicals for testing intra- and interlaboratory reproducibility.

(3) **Testing of reproducibility**:
Test one chemical five times independently (= 5 tests).
Test four chemicals three times independently (= 12 tests).
(*Data obtained in Phase I may be used, provided they were obtained by applying definite SOP).*

(4) **Submit SOP to *INVITTOX***
Refine, in cooperation with Laboratory 2, the SOP and submit the definite SOP to be used in Phase III to *INVITTOX* methods data bank (Nottingham, UK).

(5) **Report**:
Collect and submit data of Phase II to Dr. Holzhütter. Include biostatistical analysis of reproducibility in report to the Principle Investigator.

Tasks of Laboratory 2:

(1) **Establish EpiDerm™ Phototoxicity assay**
Test Chlorpromazine (positive control) and a negative chemical 3 times on different batches of EpiDerm™ EPI-200 at separate occasions (= 6 tests).
Test UV-sensitivity of EpiDerm™ EPI-200 twice on different batches of EpiDerm™ at separate occasions (= 2 tests).

(2) **Testing of reproducibility**:
Test one chemical five times independently (= 5 tests).
Test four chemicals three times independently (= 12 tests).

(3) **Data:**
Submit data obtained during (1) and (2) to ZEBET.

(4) **2nd Refinement of SOP:**
After data of Phase II have been analysed, communicate with Laboratory 1 for data discussion and final refinement of SOP.
(*To achieve this, a meeting of Laboratory 1, Laboratory 2 and the biostatistician may be necessary. In addition attendance of Laboratory 3 would help to quickly establish the test for Phase III in this lab.)*

PHASE III PROTOCOL PERFORMANCE (Lab 1 & Lab 2 & Lab 3)

Tasks of Laboratory 1:

(1) **Chemicals:**
Provide a list of at least 20 test materials (if achievable, including formulations) to *BIBRA*. (*BIBRA to select 10 chemicals out of this list, code and distribute them to Laboratory 1, Laboratory 2, and Laboratory 3.*

(2) **Testing:**
Test 10 coded test materials twice at separate occasions (= 20 tests).

(3) **Data:**
Submit data to independent biostatistician, Dr. Holzhütter.

(4) **Data analysis and reporting**:
Submit final report, including an overall evaluation of the test and the improvements achieved by applying the prevalidation process to the test. Report has to include an evaluation of whether the test is ready for a formal validation. Report has to include a critical assessment of the practicability of the prevalidation process itself. Report Draft has to be approved by Laboratory 2 and Laboratory 3 before submission to the Principle Investigator, MA Ltd..

Tasks of Laboratory 2:

(1) **Testing:**
Test 10 coded test materials twice at separate occasions (= 20 tests).

(2) **Data:**
Submit data to independent biostatistician, Dr. Holzhütter.

(3) **Communication:**
After decoded data analysis has been presented communicate with Laboratory 1 and Laboratory 3 as necessary for preparation of final report to Principle Investigator.

Tasks of Laboratory 3:

(1) **Establish EpiDerm™ Phototoxicity assay**
Test Chlorpromazine (positive control) and a negative chemical 3 times on different batches of EpiDerm™ EPI-200 at separate occasions (= 6 tests).
Test UV-sensitivity of EpiDerm™ EPI-200 twice on different batches of EpiDerm™ at separate occasions (= 2 tests).

(2) **Testing:**
Test 10 coded test materials twice at separate occasions (= 20 tests).

(3) **Data:**
Submit data to independent biostatistician, Dr. Holzhütter.

(4) **Communication:**
After decoded data analysis has been presented communicate with Laboratory 1 and Laboratory 2 as necessary for preparation of final report to Principle Investigator.

11 Cost estimates

For the subcontract with the Principle Investigator, MA Ltd., each of the three labs shall provide it's individual cost estimates. As ZEBET is contracted already, information is given below on ZEBET's estimate, which may help Laboratory 2 and Laboratory 3 to estimate their costs.

Each test mentioned in the above work programme (paragraph **10**) will need 1 kit (24 tissues) of EpiDerm™ EPI-200. If, in the future, the positive control will be included into each single test, only one relevant concentration of Chlorpromazine will be included, and 4 instead of 5 concentrations of the test material will be tested. Thus, the kit needs will be kept at 1 kit per test material.

Laboratory 1:

Phase I:	35 kits		
Phase II:	10 kits *(use of data from Phase I!)*		
Phase III:	20 kits	**65 kits total.....**	**36.000 ECU**
Technical laboratory work for all 3 Phases (total: 3 man-months).....			**10.000 ECU**
SOP development, management and reporting for all 3 Phases.....			**2.000 ECU**
Data software and biostatistical analysis (subcontracted) for all 3 phases.....			**5.000 ECU**
			53.000 ECU

Laboratory 2:

Phase II:	25 kits	
Phase III:	20 kits	**45 kits total.....**
Technical laboratory work for Phase 2 and 3 (total: ~1.5 man-months).....		
Reporting and communication.....		

Laboratory 3:

Phase III:	28 kits	**28 kits total.....**
Technical laboratory work for Phase 3 (total: ~0.8 man-months).....		
Reporting and communication.....		

12 Total kit needs per time period

This information is necessary for **MatTek** to plan the EpiDerm™ production. It is based on the assumption that we keep within the time frame given in paragraph **9** is kept.

until end **05 / 1997**	12 kits
07 and **08 / 1997**	35 kits
09 and **10 / 1997**	68 kits

13 Actions necessary to proceed to Phases II and III

- **MA Ltd**. to subcontract P&G and Beiersdorf AG ***in process***
- **ZEBET** to circulate data and refined SOP and report Phase I ***in process***
- **MatTek** to confirm production capacity needed for the 3 Phases of this study ***open***
- **Principle Investigator** to decide about participation of **IIVS** ***open***

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