FABRIQUES DE TABAC REUNIES S.A.
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S W I T Z E R L A N D

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S U B P R O T O C O L I N H A L A T I O N

P 0268/2158

SCE FREQUENCY AND CHROMOSOME ABERRATIONS AFTER EXPOSURE TO DILUTED MAINSTREAM AND SIDESTREAM WHOLE SMOKE OF TEST CIGARETTES SIRIUS-A AND -B IN CHINESE HAMSTER EMBRYO LUNG V79 CELLS

(PT)

CONTENTS

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Remarks: This subprotocol, including title page, contains 26 pages.

ABBREVIATIONS (a,b,c)
==========

CB : team Cell Biology

IH : team Inhalation

MWS: mainstream whole smoke

PT : preliminary title

SOP: standard operating procedure

SWS: sidestream whole smoke

TPM: total particulate matter, general term of determined gravimetrically

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(a) in addition to those explained immediately on the page in the text, tables and figures

(b) Units of measure are given in accordance with SI-norms (Système International d'Unités).

(c) Abbreviations of 4 REPORTING and 5 FORMS are not included.

1 SUMMARY

1.1 Objective

In accordance with the objectives of this study as specified in the SUMMARY of the INTEGRATING PROTOCOL, the team Inhalation will be responsible for

(1) generating diluted MAINSTREAM and SIDESTREAM WHOLE SMOKE (MWS and SWS) of the 2 research cigarettes SIRIUS-A and -B,

(2) recording of smoke components and sampling of smoke for analytical determinations.

1.2 Research Cigarettes

The research cigarettes coded SIRIUS-A and -B are filter cigarettes from the FTR project SIRIUS.

1.3 Method

MWS and SWS will be simultaneously generated in a quasi-continuous flow condition using an automatic 30-port INBIFO mainstream and sidestream smoking machine. SWS will be collected by a circular hood above the cigarettes. MWS will be diluted to get the same total particulate matter (TPM) concentration as in SWS. The nominal TPM concentrations will be 0.7 milligrams/liter for MWS and SWS.

For the characterization of the smoke the following smoke parameters will be determined: TPM and carbon monoxide in the whole smoke (a).

In the cell culture medium the nicotine concentration (in the highest dose only) will be determined as a measure for the deposition/absorption.

Each cigarette type will be separately assayed.

On day 1 the monolayer cultures of Chinese hamster embryo V79 cells will be seeded in cell culture flasks. For each type of cigarette 80 cultures will be randomly allocated to 1 negative control group for MWS and SWS each, 2 positive control groups, 3 dose groups for MWS and 3 dose groups for SWS. On day 2 the cultures will be exposed. The exposure will be strokewise with the help of the piston action of a Borgwaldt smoking machine (b). The stroke volume will be 35 milliliters.

Dosing will be done by varying the number of strokes administered. For each smoke exposure chamber the stroke frequency will be 1 stroke per minute. The smoke will be drawn from the quasi-continuous smoke flow and injected into the space above the medium in the culture flasks. The dose will be expressed as the cumulative amount of TPM administered, i. e., number of strokes times stroke volume times TPM concentration in smoke. The unit of the dose will be "milligram" TPM per flask (c). The number of strokes administered will vary for MWS from 15 to 35 and for SWS from 5 to 11.

(a) further smoke parameters determined in
INBIFO study P 0268/2157,
1-Day cytotoxicity in human lung PAN-L3 cells after exposure to diluted mainstream and sidestream whole smoke of research cigarettes SIRIUS-A and -B (PT),
Study director: Dr.rer.nat. D. Prühs
not yet reported

(b) Borgwaldt smoking machine RM4/CS-R04.01

(c) the "milligram" TPM dose refers to TPM in smoke, not in medium

According to a stroke volume of 35 milliliters of diluted smoke with a concentration of 0.7 milligrams TPM/liter the doses will be between 0.4 and 0.9 "milligrams" TPM per flask for MWS and between 0.1 and 0.3 "milligrams" TPM per flask for SWS.

The negative control groups for MWS and SWS of each cigarette type will be sham-exposed with unlit cigarettes of each type (a).

I N B I F O
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Forschung GmbH

(a) for further handling of the cultures see SUBPROTOCOL CB1

2 RESPONSIBILITY

.....
Dr.rer.nat. D. Prühs
Biologist (Diplombiologe)

Quality Assurance:

.....
E. Römer
Biologist (Diplombiologe)

3 METHOD

3.1 Chronology of Smoke Analyses (see FIGURE A)

3.2 Generation of Mainstream Whole Smoke (a)

Principle:	open-end smoking, puffing by positive pressure

Equipment

Smoking machine

Type:	30-port INBIFO mainstream and side-stream smoking machine (see FIGURE B)
Number of machines:	1
Machine no.:	RM7
Flowmeter:	Rota, Dr. Henning KG, D-7867 Wehr 2/Baden

Cigarettes

Research cigarettes:	SIRIUS-A and -B
Internal control:	2R1

Procedure

Loading of cigarettes:	automatically or in special cases manually
Lighting of cigarettes:	automatically or manually with an iodine spot lamp
Ejection of cigarettes:	automatically or in special cases manually at defined butt length (automatic scanning with infrared photodiode)

(a) Smoke generation and exposure of cultures will take place in room R511/R512.

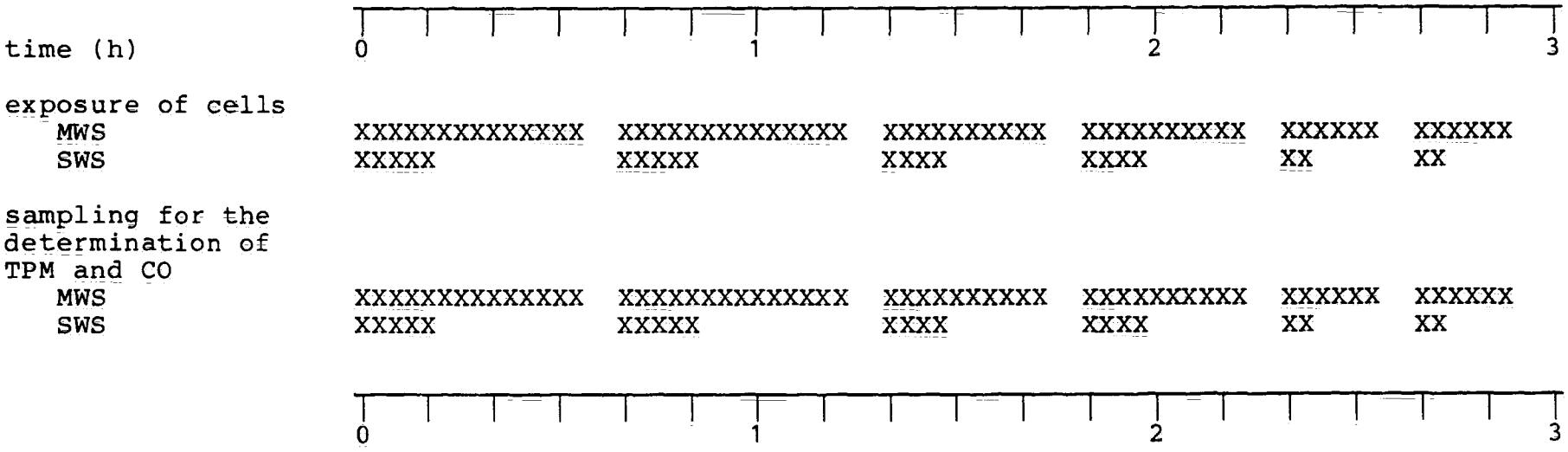

FIGURE A

CHRONOLOGY OF SMOKE ANALYSES

Remarks: sham exposure with unlit cigarettes 35 min for MWS and 11 min for SWS prior to start of smoke exposure

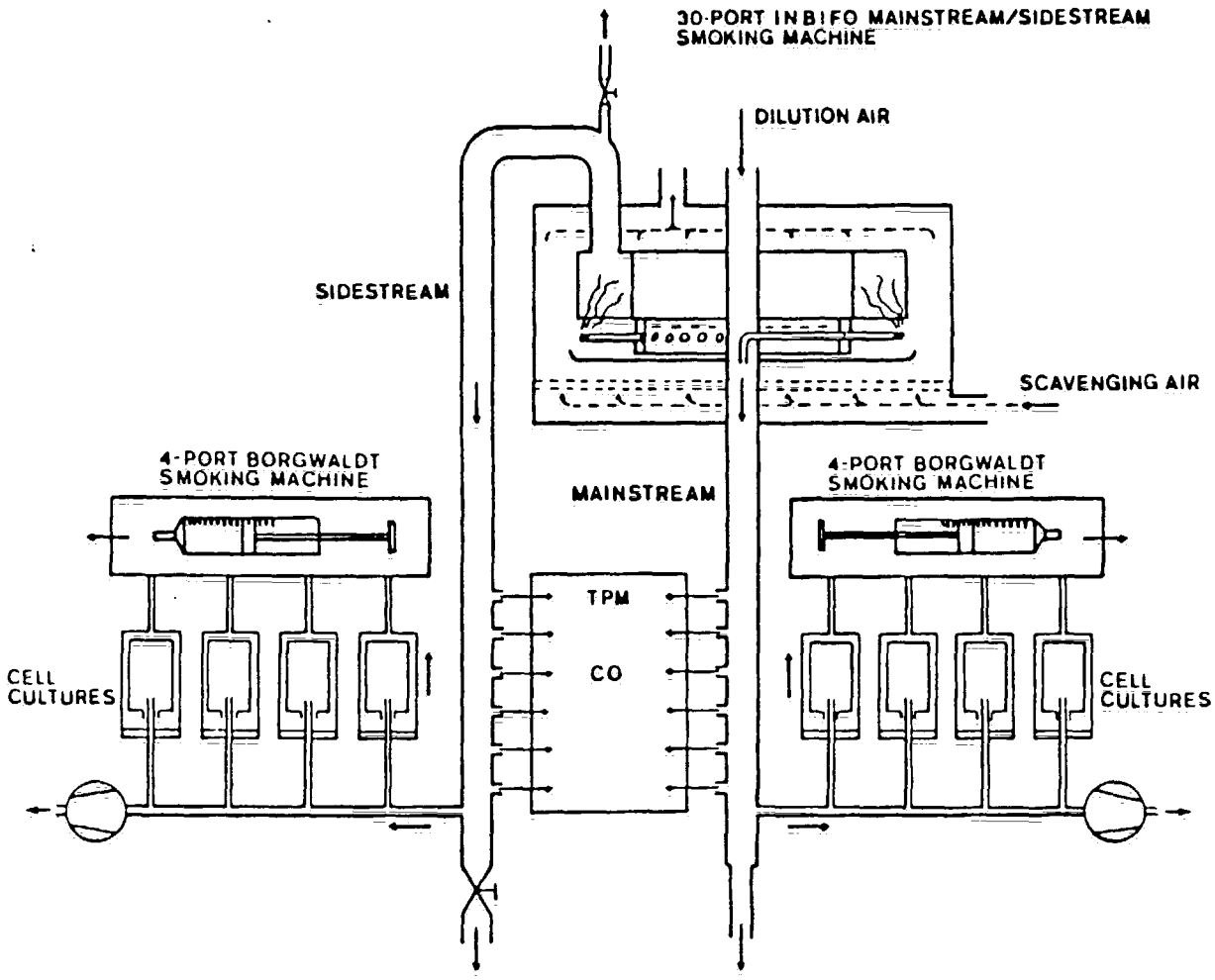

FIGURE B

SMOKE GENERATION, EXPOSURE SYSTEM, AND SAMPLING SITES

Dilution of MWS:	dilution with air, age of smoke at dilution approx. 0.3 s SIRIUS-A dilution approx. 1 : ... (a) SIRIUS-B dilution approx. 1 : ... (a) 2R1 dilution approx. 1 : ..., flow ... l/min
Positive pressure air system:	production of a positive pressure inside the smoking machine with blower, filtration of dilution air through prefilter, and mechanical filter class S
Mean puff volume (ml):	35
Pressure in smoking machine (kPa):	SIRIUS-A: 1.177 SIRIUS-B: 1.177 2R1: 1.079
Butt length (mm):	approx. 23
Puff count/cigarette (puffs):	SIRIUS-A: approx. ... (a) SIRIUS-B: approx. ... (a) 2R1: approx. ... (a)
Puff frequency/cigarette (puffs/min):	1
Puff duration (s):	approx. 2 minus time for change of position
Puff frequency of smoking machine (puffs/min):	30
Production of "steady state" conditions:	lighting according to the following sequence of cigarette positions: 1, 8, 15, 23, 2, 9, 16, 24, and so on
Consumption (b):	SIRIUS-A: ... cigarettes (a) SIRIUS-B: ... cigarettes (a) 2R1: ... cigarettes (a)
Scientific version:	SOP IH 26/1, IH 74/5
Text version:	12.Nov.87

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(a) will be given in SUBREPORT IH
(b) without cigarettes for the calibration of the setup

3.3 Generation of Sidestream Whole Smoke (a)

Principle:	cigarettes puffed by mainstream standard conditions (puffing by positive pressure), quantitative collection of sidestream with a circular hood inside the smoking machine

Equipment

Smoking machine (b)

Type:	30-port INBIFO mainstream and sidestream smoking machine (see FIGURE B)
Number of machines:	1
Machine no.:	RM7

Cigarettes

Research cigarettes:	SIRIUS-A and -B
Internal control:	2R1

Procedure

Loading of cigarettes:	automatically or in special cases manually
Lighting of cigarettes:	automatically or manually with an iodine spot lamp
Ejection of cigarettes:	automatically or in special cases manually at defined butt length (automatic scanning with infrared photodiode)
Dilution of SWS:	by positive pressure air device, flow through the vertical sidestream tube: SIRIUS-A dilution approx. ... l/min SIRIUS-B dilution approx. ... l/min 2R1 dilution approx. ... l/min

(a) smoke generation and exposure of cultures will take place in room R511/R512

(b) same smoking machine for MWS and SWS generation

Positive pressure air system:	production of a positive pressure inside the smoking machine with blower, filtration of scavenging air through prefilter and mechanical filter class S
Mean puff volume (MWS) (ml):	35
Pressure in smoking machine (kPa):	SIRIUS-A: 1.177 SIRIUS-B: 1.177 2R1: 1.079
Butt length (mm):	approx. 23
Puff count/cigarette (puffs):	SIRIUS-A: approx. ... (a) SIRIUS-B: approx. ... (a) 2R1: approx. ... (a)
Puff frequency/cigarette (puffs/min):	1
Puff duration (s):	approx. 2 minus time for change of position
Production of "steady state" conditions:	lighting according to the following sequence of cigarette positions: 1, 8, 15, 23, 2, 9, 16, 24, and so on
Scientific version:	SOP IH 26/1, IH 74/5
Text version:	12.Nov.87

3.4 Adjustment of Smoke Concentration

Principle:	adjustment of positive pressure in smoking machine for the achievement of a nominal average puff volume (35 ml/puff) determined behind the burning cigarette, adjustment of the calculated dilution air flow for the nominal TPM

(a) will be given in SUBREPORT IH

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Equipment:	U-tube manometer: Trox, D-4133 Neukirchen Vluyn flowmeter: Rota, Dr. Henning KG, D-7867 Wehr 2/Baden

Procedure

Determination of positive pressure inside smoking machine:	by U-tube manometer
Determination of flow:	determination of dilution air with flowmeter
Adjustment and regulation of air flow:	positive pressure air for smoking machine regulated by pneumatic actuated throttle
Scientific version:	SOP IH 26/1, IH 74/5
Text version:	27.Apr.87

3.5 Determination of CO Concentration

Principle:	determination of pressure in a chamber filled with defined amount of infrared light absorbing gas (CO) placed at the distal end of an infrared light beam passing through the measuring cuvette

Time

Sampling:	during exposure
Determination:	1., 2., and 8.Dec.87
Sample material and quantity:	diluted MWS and SWS, taken from the vertical manifold (see FIGURE B)
Results expressed in:	CO concentration (ul/l)

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Equipment:	1-beam infrared gas analyzer: UNOR 2, Maihak AG, D-2000 Hamburg 60 recorder: Servogor 210, Metrawatt GmbH, D-8500 Nürnberg integrator: BC1, Kipp und Zonen Vertriebs GmbH, D-6242 Schönberg
Chemicals:	test gas: 1454 ul CO/l synthetic air for SWS, 425 ul CO/l synthetic air for MWS, Messer Griesheim GmbH, D-4100 Duisburg
Procedure:	continuous sampling of diluted whole smoke from special outlets on the vertical manifold, separation of the particle phase with 2 glass wadding filters
Computation:	registration of CO concentration on recorder mean CO concentration (ul/l) calculated on the basis of electronically integrated CO concentration during the exposure periods range of determination: 0 to 3000 ul/l for MWS and SWS
Scientific version: Text version:	SOP IH 16/1 11.Nov.87

3.6 Determination of TPM Concentration

Principle:	determination of the weight difference of a Cambridge filter prior to and after passage of defined volume of diluted smoke

Time	
Sampling:	during the exposure time, successive samples (10 min max.)
Determination:	1., 2., and 8.Dec.87
Sample material and quantity:	diluted MWS and SWS on Cambridge filter, 15 l smoke/filter, flow 1.5 l/min, 2 to 8 determinations/group sample material taken from the vertical manifold (see FIGURE B)
Results expressed in:	TPM concentration (mg/l)
Equipment:	scales: no. 404/13, A. Sauter KG, D-7470 Ebingen 1 Cambridge filter no. 6004300 (filter unit with 2 tips), Gelman Sciences, Inc., Medical Device Division, Ann Arbor, Michigan, USA pump: type MW63, KNF Neuberger, D-7800 Freiburg
Procedure:	weighing of a Cambridge filter unit before and after TPM sampling, sample taken at the vertical manifold, smoke flow through Cambridge filter 1.5 l/min (see FIGURE A) continuous sampling (10 min max.) of TPM on Cambridge filter
Scientific version:	SOP IH 15/1
Text version:	11.Nov.87

3.7 Dosing (a)

Principle:	strokewise exposure of cell cultures in flasks with the help of the piston action of a pump (Borgwaldt smoking machine). Dosing by variation of the number of strokes administered, expression of the dose as the cumulative amount of TPM administered
Time	
Sampling:	during the exposure time, successive samples
Determination:	1., 2., and 8.Dec.87
Sample material and quantity:	diluted MWS and SWS on Cambridge filter, 2 to 8 determinations/group, sample material taken from the vertical manifold (see FIGURE B)
Results expressed in:	"mg" TPM/flask
Equipment:	4-port smoking machine type RR04.01, control unit type RS04.01, stroke volume: 35 ml, H. Borgwaldt, D-2000 Hamburg
Procedure:	determination of TPM concentration and number of strokes, calculation: number of strokes times stroke smoke volume (b) times TPM concentration
Scientific version:	SOP IH 82/1
Text version:	11.Nov.87

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(a) the dose refers to TPM in smoke, not in medium
(b) 35 ml/stroke

4 REPORTING

Subreport

Language: English

Concept (chapters):

1 SUMMARY
2 RESPONSIBILITY
3 METHOD
4 RESULTS AND DISCUSSION

Tables:

(1) CO CONCENTRATION, SIRIUS-A
(2) " SIRIUS-B
(3) " 2R1
(4) TPM CONCENTRATION, SIRIUS-A
(5) " SIRIUS-B
(6) " 2R1
(7) GROUPS AND DOSES, SIRIUS-A
(8) " SIRIUS-B
(9) " 2R1

5 FORMS

TITLE	TEXT VERSION	IDENTIFICATION (a)
ERRECHNUNG DES ZUGVOLUMENS	15. KW 86	A
EINSTELLUNG DER RAUCHMASCHINE	10. KW 86	B
ZIGARETTENVERBRAUCH	13. KW 85	C
TPM-KONZENTRATION IM VERDÜNNTEN RAUCH	14. KW 85	D
CO-KONZENTRATION IM VERDÜNNTEN RAUCH	14. KW 84	E
BESTIMMUNG VON ...	30. KW 85	F
BESONDERE VORKOMMNISSE	10. KW 86	G

TABLE A

TITLE, TEXT VERSION AND IDENTIFICATION OF FORMS

(a) arbitrary identification for this subprotocol

ERRECHNUNG DES ZUGVOLUMENS

P

FÜR ZUG-NR.

ZIGARETTENTYP

KAMMERDRUCK (mmWS)	STEIGHÖHE D. LAMELLE (mm)	ANZAHL ZIGARETTEN (N)	GESAMT-ZUGVOLUMEN (ml)	ZUGVOLUMEN (ml/Zug)	MITTELWERT (ml/Zug)	BEMERKUNGEN

N (total)	
M (total)	
RSD (0/0)	
SE	

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DATUM	
ZN	

pril 1986 QA

FORM 10. KW 86 DPR/BGR BGR3DIVB6 6063

EINSTELLUNG DER RAUCHMASCHINE

P /

RAUCHMASCHINE-NR.	
ZIGARETTENTYP	
RAUM	R

DATUM VON BIS	KAMMERDRUCK (mm WS)	VERDÜNNUNGS-LUFT (l/min)	ANZAHL DER ZIG./KRANZ	ART DER MUNDFILTER	RAUCHART	DAUER DER BERAUCHUNG (h)

BEMERKUNG:

DATUM	
ZN	

QA 0 4. März 1986

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FORM 13. KW 85 SL/UUN/HGZ UN1 (DIV) A13

ZIGARETTENVERBRAUCH

P

ZIG. TYP	DATUM	TAG DER INHAL. PERIODE	RAUCH-MASCHINE NR.	ZÄHLERSTAND VOR BE-RAUCHUNG	ZÄHLERSTAND NACH BE-BERAUCHUNG	ZIGARETTEN-VERBRAUCH (ANZAHL)	ZN

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28. März 1985 QA

BEMERKUNGEN:

FORM 14. KW. 85 DPR/UNN UN8 (DIV) B3 (HGZ)

METHODE: 302.5 TPM-KONZENTRATION IM VERDÜNNTEN RAUCH

PROJEKTNUMMER:
:

DATUM:
:

PARAMETER:
:

EXPOSITIONSTAG:

GRUPPE	SAMMEL-DAUER (min)	GEWICHT (g) (a) VORHER	GEWICHT (g) (a) NACHHER	TPM (g)	TPM-KONZEN-TRATION (mg/l)	BEMERKUNGEN

MESSUNG: _____
DATUM/ZN

LOCHUNG: _____
DATUM/ZN

(a) Gewicht von Cambridge-Filtergehäuse und Cambridgefilter

QA
-3. April 1985

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FORM 14. KW. 84 DRPR/UN UN8 (DIV) A23 (HGZ)

METHODE: 316.10 CO-KONZENTRATION IM VERDÜNNTEN RAUCH

PROJEKTNUMMER:
:

DATUM: PARAMETER:
: :

MESSBEREICH: VOL. 0/0 EXPOSITIONSTAG:

KONSTANTE	GRUPPE	SAMMEL-DAUER (min)	CO-KONZENTRATION, INTEGR.EINH.		MITTLERE CO-KONZ. (ul/l)	BEM.

MESSUNG: LOCHUNG:

DATUM/ZN DATUM/ZN

QA

-3. April 1985

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BESTIMMUNG VON .....

P /

DATUM:
INHALATIONSTAG:
TESTSUBSTANZ:

PROBE NR.	GRUPPE	DAUER (min)	FLUSS (l/min)	BEMERKUNG

DATUM	ZN

2. Juli 1985 QA

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BESONDERE VORKOMMNISSE	P /

DATUM:

VORKOMMNIS

DATUM	
ZN	

END OF SUBPROTOCOL IH

07. März 1986 QA

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