FABRIQUES DE TABAC REUNIES S.A.
CH-2003 Neuchatel
Switzerland

25.Mar.86
FTE/SSU

COPY NO.:

REPORT P 0268/2132

Mutagenicity of Mainstream and Sidestream Whole Smoke Condensate of Test Cigarettes SLOW-72 and SLOW-77 on Salmonella Typhimurium Strains TA98 and TA100

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2026009339

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Sitz der Gesellschaft: Köln HR B 367, 29. Oktober 1959
Institutsleiter und Geschäftsführer: Dr. med. Ulrich Hackenberg

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8.2 Tables and Figures

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Remarks: This report, including title page, contains 148 pages.

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

2-AA : 2-aminoanthracene
ABT : American Board of Toxicology
AC : team Analytical Chemistry
2-AF : 2-aminofluorene
AHM : aryl hydrocarbon monooxygenase (EC 1.14.14.2)
AT : team Animal Treatment
B(a)P: benzo(a)pyrene
BC : team Biochemistry
BSA : bovine serum albumin
BW : body weight
CFU : colony forming unit
CV : cylinder volume
DIN : Deutsches Institut für Normung (German Committee of Standards)
DMSO : dimethyl sulfoxide
DPM : dry particulate matter
EC : enzyme code according to the "International Union of Biochemistry Commission on Enzymes"
EDTA : ethylenediaminetetraacetic acid
Ex : x as exponent to the base 10, e. g. E2 = 10^2
FID : flame ionization detection
x g : centrifugal force in terms of the constant of gravitation (1 x g = 9.81 m/s2)
.GE. : greater than or equal to
G6P : glucose-6-phosphate
G6PDH: glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
.GT. : greater than
HPLC : high performance liquid chromatography
L : light
L/D : light/dark
.LT. : less than

(a) in addition to those, which are explained immediately on the same page
(b) Units are given in accordance with SI-norms (Système International d'Unités).

ABBREVIATIONS (continued)
==========

M : arithmetic mean
MB : team Microbiology
MMS : methyl methanesulfonate
MWSC-I: mainstream whole smoke condensate collected with impaction trap
N : number of individual values
NAD : nicotinamide adenine dinucleotide
NADP : nicotinamide adenine dinucleotide phosphate
O.V. : oven volatiles
PCB : polychlorinated biphenyls
PT : preliminary title
QA : Quality Assurance Unit
rpm : revolutions per minute
RSD : relative standard deviation
RT : room temperature
RTD : resistance to draw
S9 : supernatant of 9000 x g centrifugation
SE : standard error
SOP : standard operating procedure
SPF : specific pathogen free
SVT : sucrose-versene-Tris
SWSC-I: sidestream whole smoke condensate collected with impaction trap
TPM : total particulate matter (determined gravimetrically)
Tris : tris(hydroxymethyl)aminomethane
U : unit
v/v : volume/volume
WSC-I : whole smoke condensate collected with impaction trap

0 : no response
+ : response
- : not assayed

1 SUMMARY

1.1 Objective

This study was designed to investigate the mutagenicity of MAINSTREAM and SIDESTREAM WHOLE SMOKE CONDENSATE collected with impaction traps (MWSC-I and SWSC-I) of 2 test cigarettes of the FTR project SLOW in the Salmonella typhimurium reverse mutation assay with metabolic promutagen activation. Salmonella typhimurium strains TA98 and TA100 were used to detect frameshift mutagens and mutagens causing base-pair substitution respectively.

1.2 Cigarettes

The cigarettes coded SLOW-72 and SLOW-77 are filter cigarettes belonging to the FTR project SLOW. SLOW-72 is the reference test cigarette (a).

1.3 Experimental

Mainstream and sidestream whole smokes of each test cigarette were generated simultaneously from approx. 300 cigarettes for each condensate preparation with 1 automatic INBIFO smoking machine under standard conditions. The condensates collected with glass impaction traps (WSC-I) were suspended in dimethyl sulfoxide.

The mutagenicity of MWSC-I and SWSC-I of each test cigarette was assayed in the plate incorporation assay at the doses 0, 0.05, 0.10 and 0.15 milligrams dry condensate per plate following the

(a) SLOW-77 (magnesium salt impregnated cigarette paper) cigarettes appearing to be "wet" after the conditioning contained approx. 10 times more acetic acid in the cigarette paper than not conditioned SLOW-77 cigarettes.

INBIFO standard procedure. This means that with each tester strain 2 independent consecutive substudies were performed using 2 individual condensate batches, 4 doses and 4 plates per dose in each substudy. A homogenate (S9 protein) from Aroclor 1254-induced rat liver was used for metabolic promutagen activation of WSC-I.

Mutation events were detected in tester strain bacteria reverted from histidine auxotrophy to prototrophy by growth on histidine-deficient agar plates. The number of revertants was used to calculate the dose-response relationship. The specific mutagenicity was calculated from the dose-response curve as the extrapolated increase in the number of revertants per milligram dry condensate. The total mutagenicity was calculated from the specific mutagenicity and the dry condensate yield per cigarette and was expressed as the total number of revertants induced by the dry condensate yield of 1 individual cigarette.

1.4 Results

1.4.1 Specific mutagenicity of mainstream whole smoke condensate

The mutagenic activity of MWSC-I of both test cigarettes obtained in substudy 1 was statistically not different from that in substudy 2 with the exception of cigarette SLOW-72 with respect to base-pair substitution, which showed a relative difference greater than the limit of 0.25. The mean specific mutagenicity was:

MUTAGENIC EFFECT	CIGARETTE	RELATIVE DIFFERENCE BETWEEN 2 SUBSTUDIES (ABSOLUTE)	MEAN SPECIFIC MUTAGENICITY (rev./mg dry condensate)	STATISTICAL SIGNIFICANCE (a)
frameshift mutation	SLOW-72	0.00	1752	-
	SLOW-77	0.00	2046	0
base-pair substitution	SLOW-72	0.42	(1060)	-
	SLOW-77	0.13	1250	(0)

For frameshift mutation as well as for base-pair substitution the specific mutagenicity of MWSC-I of cigarette SLOW-77 was numerically higher than that of the reference cigarette SLOW-72. In both cases the difference was statistically not significant but approached the borderline.

The mean specific mutagenicity of MWSC-I of cigarette SLOW-72 which showed a significant difference between both substudies is shown in brackets and has to be taken with reservation.

1.4.2 Specific mutagenicity of sidestream whole smoke condensate

The mutagenic activity of SWSC-I of both test cigarettes in substudy 1 was statistically not different from that in substudy 2. The mean specific mutagenicity was:

(a) relative difference to the reference cigarette SLOW-72 .GT.0.16

MUTAGENIC EFFECT	CIGARETTE	RELATIVE DIFFERENCE BETWEEN 2 SUBSTUDIES (ABSOLUTE)	MEAN SPECIFIC MUTAGENICITY (rev./mg dry condensate)	STATISTICAL SIGNIFICANCE (a)
frameshift mutation	SLOW-72	0.02	1367	-
	SLOW-77	0.10	2156	+
base-pair substitution	SLOW-72	0.06	1312	-
	SLOW-77	0.02	2043	+

For frameshift mutation as well as for base-pair substitution the specific mutagenicity of SWSC-I of cigarette SLOW-77 was higher than that of the reference cigarette SLOW-72. In both cases the difference was statistically significant.

1.4.3 Total mutagenicity of mainstream and sidestream whole smoke condensate

For MWSC-I the total mutagenicity was found to be practically the same for both test cigarettes with respect to frameshift mutation as well as to base-pair substitution. The dry condensate yield was approx. 15 percent lower for cigarette SLOW-77. The total mutagenicity of MWSC-I of cigarette SLOW-72 which showed a significant difference between both substudies with respect to base-pair substitution is shown in brackets and has to be taken with reservation.

(a) relative difference to the reference cigarette SLOW-72 .GT.0.16

For SWSC-I, the total mutagenicity of cigarette SLOW-72 was found to be higher than that of cigarette SLOW-77 which showed an approx. 45 percent lower yield of dry condensate. With respect to base-pair substitution this difference was statistically significant:

MUTAGENIC EFFECT	CONDEN-SATE	CIGARETTE	TOTAL MUTAGENICITY (1E3 rev./ cig.)	STATISTICAL SIGNIFICANCE (a)
frameshift mutation	MWSC-I	SLOW-72	28.1	-
		SLOW-77	27.6	0
	SWSC-I	SLOW-72	32.8	-
		SLOW-77	28.0	0
base-pair substitution	MWSC-I	SLOW-72	(17.0)	-
		SLOW-77	16.8	(0)
	SWSC-I	SLOW-72	31.4	-
		SLOW-77	26.5	+

1.4.4 Comment

Based upon the specific and total mutagenicity of their condensates, the cigarettes SLOW-72 and SLOW-77 are considered to be equal with regard to MWSC-I. With regard to SWSC-I the specific mutagenicity of SLOW-72 is considered to be lower, but the total mutagenicity of SLOW-72 is considered to be higher than that of SLOW-77.

(a) relative difference to the reference cigarette SLOW-72 .GT.0.16

2 RESPONSIBILITY

2.1 Project Management

Study Director:

.....
Dr.rer.nat. F. Tewes
Biologist (Diplombiologe)

2.2 Contributing Teams

Analytical Chemistry:

.....
Dr.rer.nat. B. Gerstenberg
Food Chemist (Staatl. geprüfter Lebensmittelchemiker)

Biochemistry:

.....
Dr.rer.nat. R.-A. Walk
Biologist (Diplombiologe),
Biochemist and Toxicologist (ABT)

Microbiology:

.....
Dr.rer.nat. F. Tewes
Biologist (Diplombiologe)

3 QUALITY ASSURANCE STATEMENT

The study was conducted according to the Good Laboratory Practice Regulations (a).

Inspections on this study were performed by the quality assurance unit on 9. and 24.Oct.85. All findings were immediately reported to the study director and to the general management.

The report accurately reflects the study carried out and the results obtained.

.....

Quality Assurance Manager
E. Römer
Biologist (Diplombiologe)

(a) Food and Drug Administration, USA, Federal Register, 22.Dec.78, Part 2, pp. 59986-60025

4 TEST SUBSTANCE

4.1 General Specification

Test substance: mainstream and sidestream whole smoke condensate of 2 test cigarettes (a) collected with impaction traps

Cigarette

Code: (1) SLOW-72
(2) SLOW-77 (b)

Source: FTR

Date of receipt at INBIFO: 20.Sep.85

Superscription on container: (1) PROJET SLOW
PROTOTYPE 072C1

CIG. PAPER 30-1000-

PROD. DATE 22.08.85

(2) PROJET SLOW
PROTOTYPE 077C1

CIG. PAPER 30-0061-D

PROD. DATE 22.08.85

(a) In addition WSC-I of the standard reference cigarette 2R1 was used as an internal control.

(b) Cigarette SLOW-77 appeared to be "wet" in substudy 1 after conditioning. This appearance was found only in the top layer (1 out of 8 cigarettes layers) and only on the upper side of these cigarettes (approx. 30 percent of the cigarette paper area). It was found that wet cigarettes contained approx. 10 times more acetic acid in the cigarette paper (6 mg acetic acid/cigarette paper, see INBIFO study P 5086) than not conditioned SLOW-77 cigarettes. All cigarettes were used for condensate preparation without selection. In substudy 2, the wetness was prevented by a sheet of paper placed on top of the cigarette layers. Nevertheless, no influence of the wetness of test cigarette SLOW-77 was observed either on condensate yield and composition (water, nicotine and catechol) in both substudies.

Amount:	(1) approx. 5000 loose cigarettes in 11 plastic boxes (2) approx. 5000 loose cigarettes in 22 plastic boxes
Storage:	in walk-in cold room (R907), approx. 4 degrees centigrade, relative humidity uncontrolled prior to conditioning stored for approx. 12 h at -20 degrees centigrade
Conditioning:	in conditioning room (R326) for 6 to 8 d prior to use at approx. 23 degrees centigrade, 60 0/0 relative humidity cigarettes taken out of their packaging and were placed in 8 horizontal layers
Selection:	no selection

Condensate

Preparation:	see 5.2 Condensate Preparation, Suspension, Storage and Analyses
Number of condensate batches:	4 condensate batches/condensate type, 2 condensate batches/substudy
Number of single cigarettes per smoking process:	300
Solvent:	DMSO
Specification:	amount of dry condensate, water, nicotine and catechol and puff count determined for each condensate batch
Storage:	in the dark at 4 degrees centigrade, 7 or 8 days prior to mutagenicity assay

Scientific version:	SOP MB 84/1
Text version:	5.Jan.86

4.2 Supplier's Specification

Cigarette and filter:	see PAGE 4-3
Smoke and filler:	see PAGE 4-4

Supplier's specification:

LIST NO. : _____ CODE : 3LC-11

CHARACTERISTICS OF EXPERIMENTAL CIGARETTES (I)

DATE : 4/9/85 REMARKS : _____

CIGARETTE	Units	72	77		
Cigarette Length, Total	mm	84	84		
Butt Length	mm	28	28		
Filling Power CV	ml/20g				
Cig. Weight, Total	mg	965	990		
Filter + Paper Weight	mg	210	226		
Tobacco Weight	mg				
Cigarette Diameter	mm	7.92	7.89		
Cigarette RTD	mm/H20	88	90		
Ventilation Cigarette	%	26	26		
Firmness at 12.5% O.V.	mm	3.21	2.96		
FILTER					
Filter Length, Total	mm	20	20		
Tipping Length on Cig.	mm	24	24		
Tipping Paper Type		1C	1C		
Tipping Perfor. Type		MLPS	MLPS		
Tipping, Perfor. Lines	nbr	4	4		
Filter Weight	mg	161	162		
SN Filter	mg				
Filter Efficiency	%				
Filter RTD	mm/H20	68	68		
Filter Type		S	S		
Filter Material		CA	CA		

Observations :

2026009358

LIST NO. : _____ CODE : [illegible]

CHARACTERISTICS OF EXPERIMENTAL CIGARETTES (II)

DATE : 9/9/85 · REMARKS : _____

SMOKE	Units	72	77		
CO Carbon Monoxide	mg/cig	13.9	13.9		
NO Nitrogen Monoxide	mg/cig	0.16	0.16		
T P M	mg/cig	18.3	16.0		
H20 in TPM	mg/cig	2.1	1.8		
D P M	mg/cig	16.2	14.2		
Tar	mg/cig	15.0	13.2		
SN Smoke Nicotine	mg/cig	1.20	0.97		
Ratio SN/Tar	%	8.0	7.4		
Puff Count		10.1	8.6		
FILLER					
Alkaloids, Total	%	2.03	2.04		
Reducing Sugars	%	10.3	9.7		
Nitrate Nitrogen	%	0.17	0.16		
Ammonia Nitrogen	%	0.19	0.17		
Tobacco Oven Volatiles	%	12.6	12.8		
Weight at 12.5% O.V.	mg/cig	754	761		
Density	mg/ml	239	243		

Observations :

2026009359

5 METHOD

5.1 Chronology (see FIGURE A)

5.2 Condensate Preparation, Suspension, Storage and Analyses

5.2.1 Mainstream whole smoke condensate preparation

Principle:	mechanical open-end smoking to a defined butt length in automatic negative pressure (vacuum pump) smoking machine, condensate collection in impaction trap
Time:	simultaneously to the sidestream whole smoke condensate from the identical cigarettes (see 5.1 Chronology)
Sample material and quantity:	cigarettes, approx. 300/batch (see 4 TEST SUBSTANCE)

Equipment

Smoking machine

Type:	30-port automatic INBIFO smoking machine
Number of machines:	2
Machine no.:	0035, 0036
Loading of cigarettes:	automatically
Lighting of cigarettes:	automatically or manually with an iodine spot lamp iodine spot lamp: Halogen-Bellaphot, 15 V, 150 W, gold-plated reflector, Osram, no. 64635, R. Schahl, D-8000 München 71
Ejection of cigarettes:	automatically at butt length of at least 23 mm, but not less than the length of the filter + 8 mm or not less than the length of the tipping paper + 3 mm, in accordance with DIN 10240

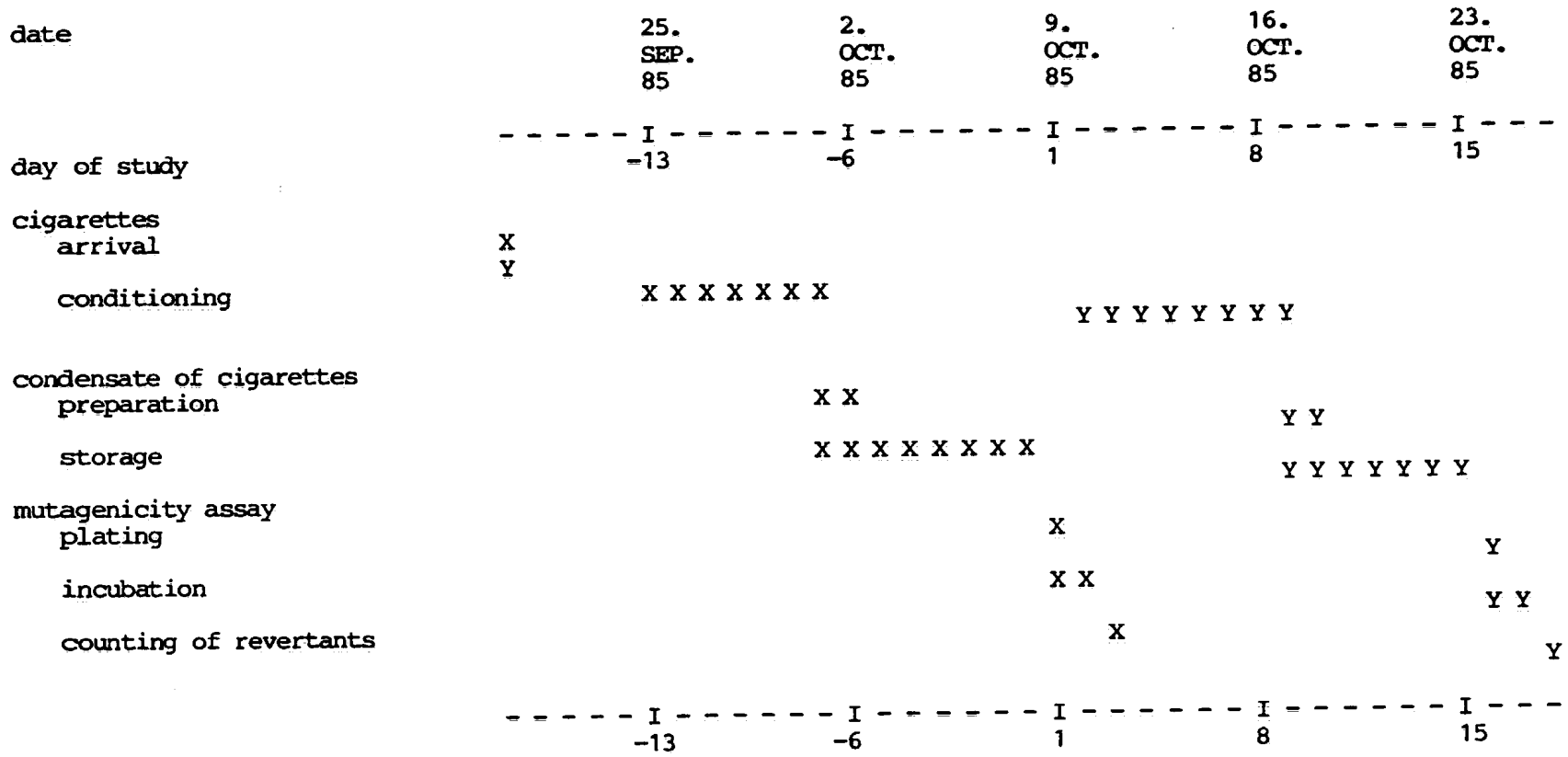


FIGURE A

CHRONOLOGY

Remarks: X: substudy 1
Y: substudy 2

Vacuum pump:	membrane vacuum pump N 0135/AVE, K. Neuberger KG, D-7800 Freiburg-Munzingen
Flowmeter:	rotameter, L 4/160, Rota, Dr. Henning KG, D-7867 Wehr/Baden soapfilm flowmeter, Faust GmbH, D-5000 Köln 90

Impaction trap

Type:	glass "impaction trap for cigarette smoke condensate collection" according to Philip Morris (see FIGURE B), Faust GmbH, D-5000 Köln 90
Capillary:	length: 5 mm bore: 0.4 mm
Mode of installation of the impaction trap insert:	distance of 0.5 mm between capillary tip and wall of flask calibrated with 0.5 mm thick teflon sheet spacer
Connection of impaction trap to smoking machine:	impaction trap lies horizontally below smoking machine connected via glass tubes dimension of connecting glass tubes (between impaction trap and smoking machine): length: 51 cm outer diameter: 13 mm inner diameter: 8 mm

Procedure

Puffs/cigarette:	see TABLE 1.3
Puff frequency/cigarette:	1 puff/min
Puff duration:	approx. 2 s minus time for change of position

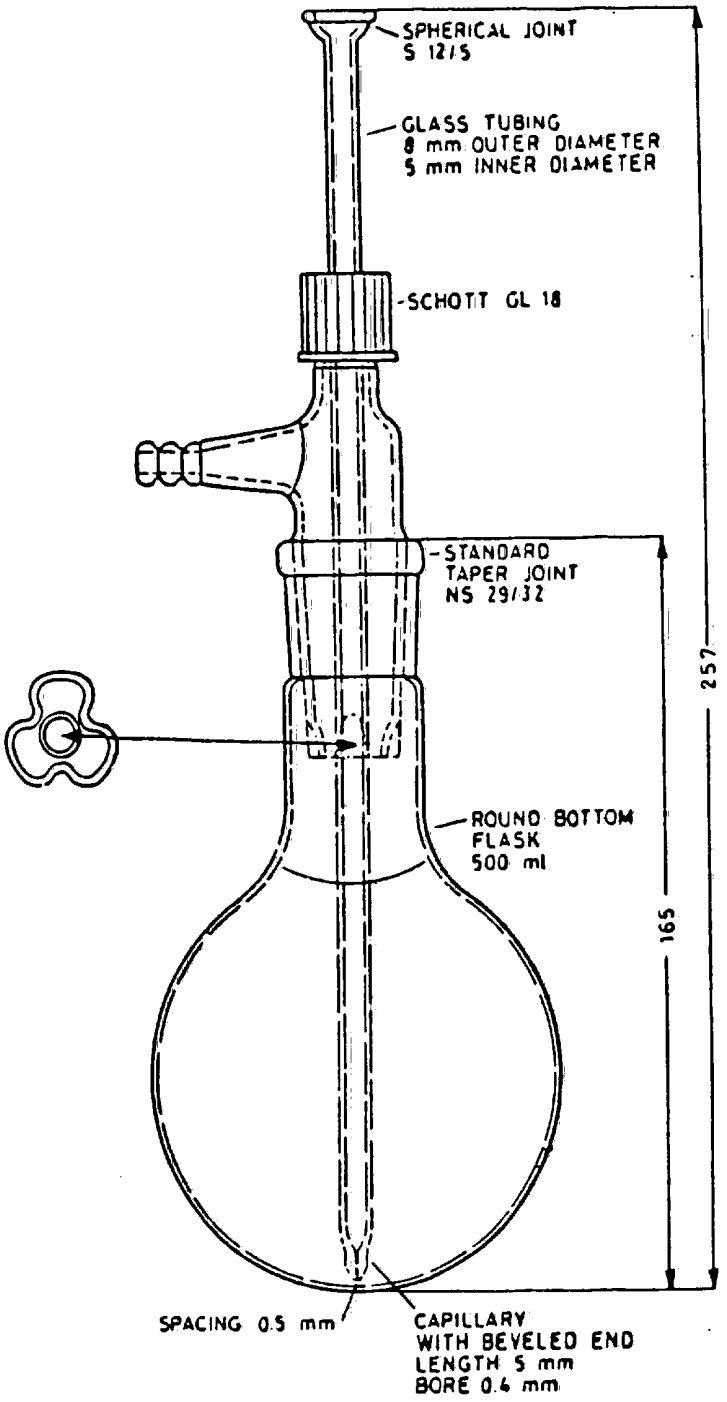


FIGURE B

GLASS IMPACTION TRAP FOR MAINSTREAM WSC-I COLLECTION

Puff volume:	35 ml parameter checked and regulated during condensation with a rotameter or soap-film flowmeter
Suction volume:	1.05 l/min
Pressure in impaction trap:	approx. 4E4 Pa (0.4 bar)
Scientific version:	SOP AC 41/1
Text version:	30.Jan.86

5.2.2 Sidestream whole smoke condensate preparation

Principle:	mechanical open-end smoking to a defined butt length in automatic negative pressure (vacuum pump) smoking machine, sidestream smoke collection by means of a circular hood and condensate collection with a special impaction trap
Time:	simultaneously to the mainstream whole smoke condensate from the identical cigarettes (see 5.1 Chronology)
Sample material and quantity:	cigarettes, approx. 300/batch (see 4 TEST SUBSTANCE)

Equipment

Smoking machine

Type:	30-port automatic INBIFO smoking machine with circular hood for sidestream smoke collection
Number of machines:	2
Machine no.:	0035, 0036
Loading of cigarettes:	automatically

Lighting of cigarettes:	automatically or manually with an iodine spot lamp iodine spot lamp: Halogen-Bellaphot, 15 V, 150 W, gold-plated reflector, Osram, no. 64635, R. Schahl, D-8000 München 71
Ejection of cigarettes:	automatically at butt length of at least 23 mm, but not less than the length of the filter + 8 mm or not less than the length of the tipping paper + 3 mm, in accordance with DIN 10240
Vacuum pump:	water ringpump LRKA 10603, SIHI Halberg, via Hartmann und Essen GmbH und Co., D-5000 Köln 91

Impaction trap

Type:	glass impaction trap for sidestream smoke condensate collection (see FIGURE C), Faust GmbH, D-5000 Köln 90
Outlet nozzle:	annular fissure of 88 mm length and 0.1 mm width
Distance of the impaction plate from the outlet nozzel:	approx. 0.1 mm
Installation of impaction trap:	in vertical position in an ice/water bath below smoking machine connected via copper tube with sidestream smoke collection hood dimension of copper tube: length: 70 cm outer diameter: 35 mm inner diameter: 30 mm

Procedure

Puff frequency/cigarette:	1 puff/min

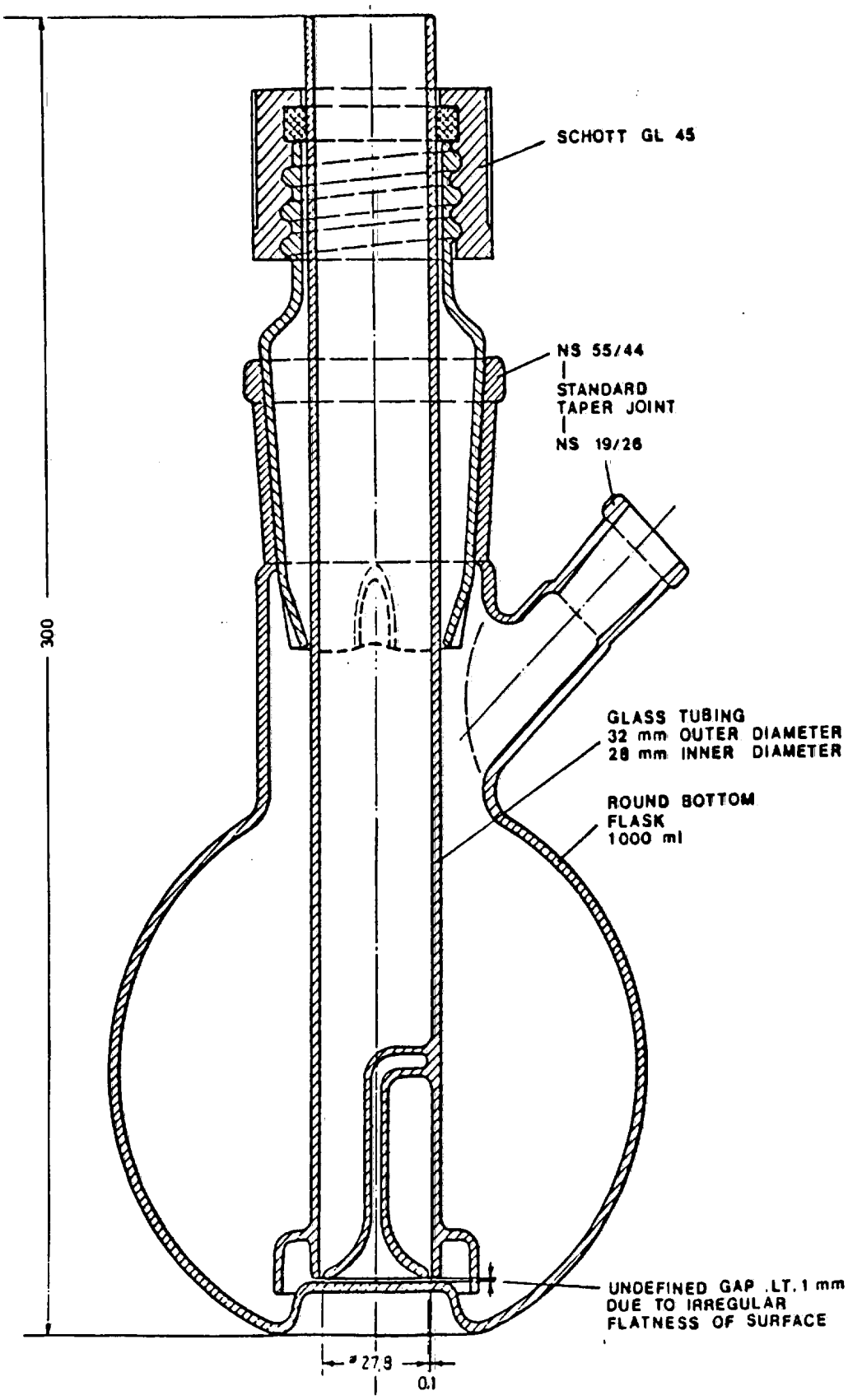


FIGURE C

GLASS IMPACTION TRAP FOR SIDESTREAM WSC-I COLLECTION

Puff duration:	approx. 2 s minus time for change of position
Puff volume:	35 ml
Suction volume for sidestream collection:	approx. 120 l/min
Pressure in impaction trap:	approx. 6E4 Pa (0.6 bar) regulated with pressure gauge
Scientific version:	SOP AC 42/2
Text version:	30.Jan.86

5.2.3 Suspension and storage of condensate

Principle:	suspension of WSC-I in DMSO by sonication
Time:	immediately after WSC-I preparation
Sample material and quantity:	total WSC-I of each condensate batch
Results expressed in:	g/l
Equipment:	sonication water bath: Sonorex RK 100, Bandelin KG, D-1000 Berlin
	brown glass bottles, 250 ml, no. 9072144, with teflon-lined screw caps, Faust GmbH, D-5000 Köln 90
	brown glass vials, 8 ml, no. 224814, screw caps, no. 240409, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main

Chemicals:	DMSO, no. 2950, E. Merck, D-6100 Darmstadt 1
Procedure:	WSC-I washed out of trap approx. 4 times with approx. 50 ml portions of DMSO repeatedly after sonication (water bath) for approx. 5 min, washings transferred to a 250-ml volumetric flask and filled up to volume with DMSO amount of WSC-I calculated from weight of impaction trap before and immediately after condensate preparation concentration of dry condensate calculated from WSC-I and water concentration of suspension (determination of water concentration: see 5.2.4 Analyses of condensate suspension)
Storage:	in sterile brown glass bottles at 4 degrees centigrade for 7 days, 5-ml aliquots at -75 degrees centigrade labeling of the bottles: study no., batch no., date of condensate preparation
Scientific version: Text version:	SOP AC 52/1, AC 74/2 30.Jan.86

5.2.4 Analyses of condensate suspension

5.2.4.1 Determination of water concentration

Principle:	titration according to Karl Fischer modified by E. Scholz (1984)
Time:	within 48 h after preparation of WSC-I suspension

Sample material and quantity:	WSC-I/DMSO suspension, 0.5 ml, 2 determinations/suspension
Results expressed in:	g/l and mg/cigarette
Equipment:	Karl Fischer-Titrator E452, Deutsche Metrohm GmbH, D-7024 Filderstadt
Chemicals:	Hydranal-Composite 5 K solution, no. 34816, Hydranal-Arbeitsmedium K, no. 34817, Riedel-de Haen, D-3016 Seelze 1 DMSO, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure

Titration:	4 ml "Arbeitsmedium K" titrated with Composite 5 K solution to dryness, stop time 20 s for titration of the sample the method "fliegender Start" used e. g. start of the titration and within 20 s addition of 0.5 ml of the sample solution
Computation:	0.5 ml DMSO titrated before preparation of WSC-I suspension 0.5 ml DMSO with 10 g H2O/l titrated before preparation of WSC-I suspension 0.5 ml DMSO titrated after preparation of WSC-I suspension calculation of water concentration according to the formula

$$\text{water conc. (g/l)} = 10 \frac{M3 - MX}{M2 - M1}$$

$$MX = \frac{ME - M1}{t2 - t1} (t3 - t1) + M1$$

M1: reagent consumption for DMSO before preparation of WSC-I suspension
M2: reagent consumption for DMSO + 10 g water/l before preparation of WSC-I suspension
M3: reagent consumption for sample
MX: time depending reagent consumption for DMSO
ME: reagent consumption for DMSO after preparation of WSC-I suspension
t1: start time of preparation of WSC-I suspension (a)
t2: stop time of preparation of WSC-I suspension (a)
t3: actual time of preparation of WSC-I suspension (a)

Detection limit:	0.5 g/l
Reproducibility (relative standard deviation):	4 0/0 (determined at 5 g/l, N: 8)
Scientific version:	SOP AC 16/3
Text version:	30.Jan.86

5.2.4.2 Determination of nicotine concentration

Principle:	gas chromatography after extraction with dichloromethane computer integration of peaks
Time:	within 48 h after preparation of WSC-I suspension
Sample material and quantity:	WSC-I/DMSO suspension, 1 ml, 2 determinations/suspension
Results expressed in:	g/l, g/kg dry condensate and mg/cigarette

(a) times expressed in decimal values rounded to quarters of hours

Equipment:

gas chromatograph: HP 5710 A,
detector: FID,
automatic sampler: HP 7671 A,
laboratory data system: HP 3351 A,
Hewlett-Packard GmbH,
D-6000 Frankfurt/Main

recorder: Servogor 210,
Metrawatt GmbH,
D-8500 Nürnberg

centrifuge: model J6-B,
rotor: JS-4.2,
Beckman Instruments GmbH,
D-8000 München 40

Chemicals:

quinoline, no. 802407,
dichloromethane, no. 822271,
dimethyl sulfoxide (DMSO), no. 2950,
sodium hydroxide, no. 5594,
sulfuric acid, no. 9074,
E. Merck,
D-6100 Darmstadt 1

nitrogen,
hydrogen,
air (synthetic),
Linde AG,
D-5000 Köln 50

nicotine, no. 77635,
Serva Feinbiochemica GmbH und Co. KG,
D-6900 Heidelberg 1

Procedure

Extraction:

addition of 1 ml of the internal standard solution (0.5 mg quinoline/ml (0.1 mol/l) sulfuric acid), 1 ml sodium hydroxide (200 g/l) and 10 ml dichloromethane to 1 ml WSC-I/DMSO suspension, after agitation (5 min) and centrifugation (approx. 7.8E3 m/s2 (= 800 x g), 5 min, approx. 10 degrees centigrade), injection of 1 ul of the lower phase into the gas chromatograph

Gas chromatography

Column:

2 m x 2 mm inner diameter, glass

Column packing:	100 g Apiezon L and 100 g potassium hydroxide per kg Chromosorb W-AW DMCS (a), 80 to 100 mesh
Carrier gas and flow rate:	nitrogen, 30 ml/min
Oven temperature:	175 degrees centigrade
Injection port temperature:	200 degrees centigrade
Detector temperature:	200 degrees centigrade
Computation:	1 ml of a standard solution (1 mg nicotine and 0.5 mg quinoline/ml (0.1 mol/l) sulfuric acid) diluted with 1 ml DMSO and 1 ml sodium hydroxide (200 g/l) and extracted as described above determination of calibration factor
Detection limit:	0.02 g/l
Recovery:	98.5 0/0
Reproducibility (relative standard deviation):	0.5 0/0 (1 g/l nicotine, N: 10)
Scientific version:	SOP AC 9/4
Text version:	30.Jan.86

5.2.4.3 Determination of catechol concentration

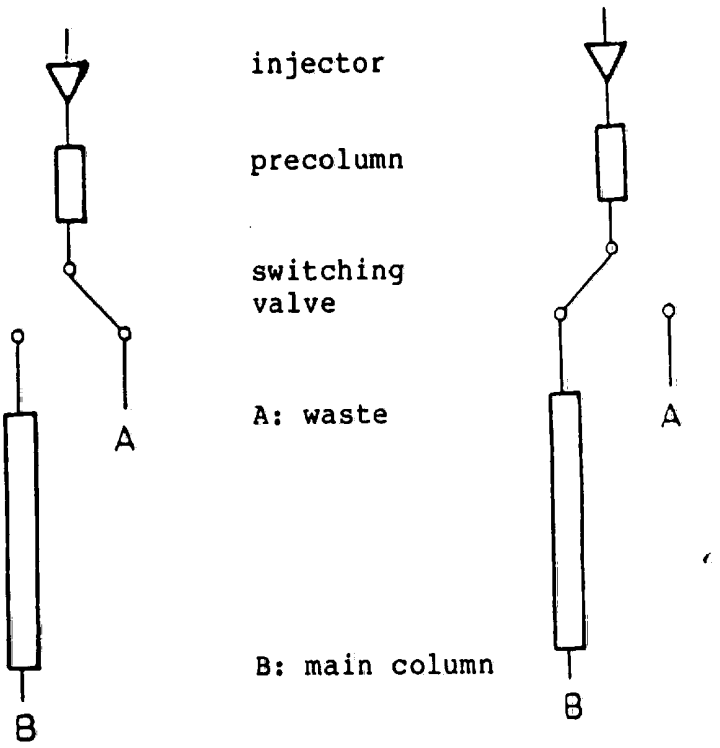
Principle:	direct HPLC determination of catechol in a diluted WSC-I/DMSO suspension by combination of 2 different columns and column switching
Time:	within 1 week after preparation of WSC-I/DMSO suspension
Sample material and quantity:	WSC-I/DMSO suspension, 200 ul, 2 determinations/suspension

(a) AW: acid-washed, DMCS: treated with dimethyldichlorosilane

Results expressed in:	g/l, g/kg dry condensate and mg/cigarette
Equipment:	high performance liquid chromatograph with automatic sampler, variable wavelength detector and integrator, HP 1084 B column: RP18, 10 um, 20 mm x 4.6 mm, no. 79916 B, Hewlett-Packard GmbH, D-6000 Frankfurt/Main precolumn: 40 mm x 4.6 mm, filled with dihydroxyboryl, silica gel, 30 um, no. -, Serva, D-6900 Heidelberg 1
Chemicals:	acetone, no. 14, methanol, no. 6007, phosphoric acid, no. 573, sodium dihydrogenphosphate, no. 6346, E. Merck, D-6100 Darmstadt 1 catechol, no. 15880, pyrogallol, no. 83130, Fluka, D-7910 Neu-Ulm standard solution: 100 g/l catechol in DMSO internal standard solution: 250 mg/l pyrogallol in acetone

Procedure

Sample preparation:	dilution of the WSC-I/DMSO suspension with the internal standard solution in a ratio of 1 : 5
HPLC determination:	see FIGURE D
Precolumn:	dihydroxyboryl silica gel, 40 mm x 4.6 mm
Column:	RP-18, 200 mm x 4.6 mm



(1) sample injection
(2) washing (methanol)

switching

(3) elution from the precolumn (buffer)
(4) separation on the main column (buffer-methanol gradient)

switching

(5) conditioning of the precolumn for the next analysis (methanol)

FIGURE D

SCHEME OF THE HPLC DETERMINATION OF CATECHOL

Elution solvents:	solvent 1: 10 mmol/l sodium dihydrogen-phosphate in water, adjusted to pH 2.8 with phosphoric acid solvent 2: methanol
Elution:	see TABLE A
Flow rate:	1.5 ml/min
Column switching:	see TABLE A
Oven temperature:	ambient
Wavelength:	280 nm
Injection volume:	100 ul
Time between the analyses:	3 min
Computation:	calibration by internal standard method, mixing 1 ml standard solution with 4 ml internal standard solution and 3-fold analysis of the mixture, evaluation of the peak area ratio by the integrator The 1st analysis in a series has to be cancelled.
Scientific version:	SOP AC 59/3
Text version:	30.Jan.86

5.2.4.4 Bacteriological examination of WSC-I/DMSO suspension

Principle:	determination of bacterial contamination of test substance assayed for mutagenicity in the plate incorporation assay detection limited to aerobic bacteria growing on minimal-glucose agar plates
Time:	at the day of mutagenicity assay
Sample material and quantity:	WSC-I/DMSO suspension, highest dose/plate, 1 WSC-I batch of each test cigarette, condensate type and substudy

TIME (min)	EVENT
0	elution: 100 0/0 methanol
from 1.0 to 2.0	elution: from 100 0/0 to 5 0/0 methanol
2.1	column switching: precolumn on-line to the main column
from 3.5 to 13.5	elution: from 5 0/0 to 70 0/0 methanol
from 13.5 to 15.0	elution: from 70 0/0 to 100 0/0 methanol
14.9	column switching: precolumn off-line to the main column
17.0	end of the analysis

TABLE A

CONDITIONS OF THE HPLC DETERMINATION OF CATECHOL

Results expressed in:	CFU/plate
Equipment:	incubator: no. 3916, Forma Scientific, via Labotect, D-3400 Göttingen petri dishes: no. 1029, 100 mm x 15 mm, polystyrene, sterilized, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1 colony counter: Colony Star 2, Funke-Gerber, D-1000 Berlin
Chemicals:	top agar and minimal-glucose agar, composition: see 5.6.1 Plate incorporation assay
Procedure:	top agar and test substance mixed by rotation and poured on minimal-glucose agar 2 plates/sample incubation of plates at 37 degrees centigrade, manual counting of colonies after 2 d of incubation
Scientific version: Text version:	SOP MB 44/1 4.Dec.85

5.3 Dosing of Test Substance

Principle:	dilution of test substance stock solution with DMSO to the final concentration used in the study
Time:	at the day of mutagenicity assay
Sample material and quantity:	4 WSC-I suspension batches/test cigarette
Equipment:	brown glass vials, 8 ml, no. 224814, screw caps, no. 240409, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main
Chemicals:	DMSO, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure

Preparation of application suspension:	see TABLES B
	2 application suspensions/condensate batch
Storage:	in dark airtight vials at RT
Dosing:	see TABLE C

Scientific version:	SOP MB 43/2
Text version:	17.Dec.85

DATE OF MUTAGENICITY ASSAY	CIGARETTE	CONDENSATE BATCH NO.	STOCK SUSPENSION	APPLICATION SUSPENSION MIXTURE A PLUS B	
			DRY CONDENSATE CONCENTRATION	A CONDENSATE	B DMSO
(Oct.85)			(g/l)	(ml)	(ml)
9	SLOW-72	2366	21.60	0.579	4.42
		2372	21.89	0.571	4.43
	SLOW-77	2368	15.65	0.799	4.20
		2374	16.94	0.738	4.26
	2R1	2370	47.77	0.262	4.74
		2376	46.91	0.266	4.73
24	SLOW-72	2386	16.04	0.779	4.22
		2396	17.95	0.696	4.30
	SLOW-77	2388	15.40	0.812	4.19
		2398	15.51	0.806	4.19
	2R1	2390	42.68	0.293	4.71
		2400	44.18	0.283	4.72

TABLE B.1

PREPARATION OF MWSC-I/DMSO APPLICATION SUSPENSION

Remarks: 2 application suspensions/condensate batch

DATE OF MUTAGENICITY ASSAY	CIGARETTE	CONDENSATE BATCH NO.	STOCK SUSPENSION	APPLICATION SUSPENSION MIXTURE A PLUS B	
			DRY CONDENSATE CONCENTRATION	A CONDENSATE	B DMSO
(Oct.85)			(g/l)	(ml)	(ml)
9	SLOW-72	2367	30.25	0.413	4.59
		2373	31.39	0.398	4.60
	SLOW-77	2369	15.30	0.817	4.18
		2375	15.01	0.833	4.17
	2R1	2371	24.20	0.517	4.48
		2377	23.34	0.536	4.46
24	SLOW-72	2387	27.40	0.456	4.54
		2397	26.81	0.466	4.53
	SLOW-77	2389	17.71	0.706	4.29
		2399	13.19	0.948	4.05
	2R1	2391	21.66	0.577	4.42
		2401	25.25	0.495	4.51

TABLE B.2

PREPARATION OF SWSC-I/DMSO APPLICATION SUSPENSION

Remarks: 2 application suspensions/condensate batch

APPLICATION SUSPENSION

CONCENTRATION (g dry cond./l)	VOLUME PLATED (ul/plate)	DOSE (mg dry cond./plate)
2.5	0	0 (a)
	20	0.05
	40	0.10
	60	0.15

TABLE C

DOSING OF MWSC-I AND SWSC-I OF CIGARETTES

(a) solvent control: 60 ul DMSO/plate

5.4 Metabolic Promutagen Activation System (S9)

5.4.1 Animals

Species:	albino rat (Rattus norwegicus)
Strain and designation:	Sprague Dawley CRL:CD(SD)BR
Color:	white
Type of breeding:	outbred
Sex:	male
Microbiological conditions of breeding:	SPF until delivery
Breeder:	Charles River Wiga GmbH, D-8741 Sulzfeld 1
Transport containers:	special filter crates
INBIFO animal supply no.:	531
INBIFO animal study approval no.:	191
Number of animals	
Arrived:	21
Applied:	20
Date of shipment:	9.Nov.84

Body weight (grams) (a)	
At order:	180
1 day after arrival:	176.0 ± 2.2
At administration:	209.7 ± 2.0
On sacrifice:	201.4 ± 2.9
Age of animals (days)	
On arrival:	44 ± 1
At administration:	49 ± 1
On sacrifice:	54 ± 1
Acclimation period (days):	5
Text version:	27.Jun.85

5.4.2 Animal housing

Animal room:	INBIFO main laboratory building, area C (b), rooms R310 and R312
Construction and interior:	windowless floors, walls and ceilings coated with epoxy resins
Microbiological conditions:	conventional under defined laboratory conditions, rooms disinfected with 30 ml/l Kohrsolin prior to introduction of rats
Conditioning and ventilation:	100 0/0 fresh air, delivered from a 50 m high air inlet stack, approx. 15 changes/h

(a) mean ± SE
(b) area C only used for acute studies with small laboratory rodents

	filter: fine filter class C
Room temperature (degrees centigrade):	22 ± 2 (a)
Relative humidity (0/0):	55 ± 10 (a)

Light

Time cycle:	L/D 12 : 12, L 06.00 to 18.00 standard time
Source:	"daylight" fluorescent lamps: Lumilux W11, Osram GmbH, D-8000 München 1
Intensity in cages:	approx. 20 to 100 Lux
Cages:	type 3, polycarbonate (Makrolon), base area: 39 cm x 23 cm, height: 15 cm
Cage lids:	stainless steel, wire mesh with overhead hoppers
Change of cages:	3 times/week
Litter:	wire grid, autoclaved granulated dust free wood underneath
	each batch screened for pesticides, PCB residues, aflatoxins and heavy metals (arsenic, cadmium, lead, mercury) and used only when the tolerance levels given by the German Futtermittelverordnung (Jun.76) not exceeded
	sterilization: 15 min at 134 degrees centigrade, 2.5E5 Pa (equiv. to 2.4 kg/cm2)

(a) At outside temperatures above 28 degrees centigrade and at extremely high relative humidity, these specifications may not always be maintained.

	replacement of bedding material: 3 times/week (Mo., We., Fr.) rats set on double wire grids when diet removed
Number of animals per cage:	2 rats, except 1 cage with 1 rat
Scientific version: Text version:	SOP AT 102/2 27.Jun.85

5.4.3 Diet and drinking water

Diet:	fortified diet, cylindrical pellets, 16 mm diameter, HERILAN MRH-HALTUNG (a), H. Eggersmann KG, D-3260 Rinteln sterilization: 5 min, 120 degrees centigrade, 1.2E5 Pa, drying: 15 min each batch screened for pesticides, PCB residues, aflatoxins and heavy metals (arsenic, cadmium, lead, mercury) and used only when the tolerance levels given by the German Futtermittelverordnung (Jun.76) not exceeded
Diet supply:	ad libitum from stainless steel hoppers in cage lid, diet removed approx. 12 h before sacrifice
Water:	autoclaved tap water sterilization: 15 min, 120 degrees centigrade, 1.0E5 Pa

(a) random samples of all autoclaved batches of diet microbiologically investigated

Water supply:	ad libitum from 250-ml DIN glass bottles, with stainless steel sipper tubes, water changed 3 times/week (Tu., Th., Sa.)
Scientific version:	SOP AT 21/5, AT 26/2, MB 1/2 (a)
Text version:	4.Oct.85

5.4.4 Administration of Aroclor 1254

Principle:	intraperitoneal injection
Time:	5 d prior to sacrifice
Sample material and quantity:	20 rats
Equipment:	disposable syringes: 2 ml Luer-Lok conus, sterile, no. 461702/9, cannula: Luer Nr. 14, sterile, B. Braun Melsungen AG, D-3508 Melsungen teflon filter, type SM 11807, 25 mm diameter, pore size 0.2 um, Sartorius GmbH, D-3400 Göttingen
Chemicals:	Arochlor 1254, Dr. S. u. I. Ehrenstorfer, D-8900 Augsburg cereal seed oil: Mazola, Maizena GmbH, D-7100 Heilbronn

Procedure

Preparation:	Aroclor 1254 emulsified in sterile-filtered cereal seed oil by shaking until establishment of homogeneous emulsion at a concentration of 200 g/l

(a) refers only to sterilization of drinking water

Dose:	500 mg/kg BW, approx. 0.47 ml Aroclor emulsion/rat
Scientific version: Text version:	SOP MB 46/2 27.Jun.85

5.4.5 Preparation of tissue homogenate supernatant (S9 fraction)

Principle:	mechanical grinding and centrifugation of tissue under sterile condition
Time	
Sampling:	5 d after administration of Aroclor
Preparation:	on day of sampling
Sample material and quantity:	20 rat livers pooled
Final product:	Aroclor 1254-induced rat liver homogenate, batch no. 84-1
Equipment:	homogenizer: Potter-Elvehjem, glass, with motor-driven teflon pestle, B. Braun Melsungen AG, D-3508 Melsungen homogenizer drive: Multifix, model M 80, Alfred Schwinher, D-7070 Schwäbisch-Gmünd centrifuge: Sorvall RC-5B, rotor: SS-34, Du Pont Instruments, D-6350 Bad Nauheim freezer: no. 8218, Forma Scientific, via Labotect, D-3400 Göttingen

tubes:
polycarbonate, no. 3934,
Du Pont Instruments,
D-6350 Bad Nauheim

cryotubes with screw caps,
polypropylene, no. 363401 and
no. 363452,
Nunc GmbH,
D-6200 Wiesbaden

Chemicals:

potassium chloride, no. 4936,
E. Merck,
D-6100 Darmstadt 1

ethanol, denatured with ethyl
methyl ketone, no. 642,
Hofmann,
D-5000 Köln 21

potassium chloride solution, buffered:
150 mmol potassium chloride/l and
1 mmol phosphate buffer, pH 7.4

Procedure

Reference:

basically according to Garner et al. (1972) and Maron and Ames (1983), modified by INBIFO

Killing:

decapitation, followed by 30 s exsanguination

Removal of organs:

sterile dissection after soaking the fur with ethanol (700 ml/l), short submersion of removed organs in 150 mmol potassium chloride/l, pH 7.4, at 0 degrees centigrade, determination of organ wet weight

Homogenization:

after addition of 3 volumes of 150 mmol potassium chloride/l, pH 7.4, to the original organ wet weight (1 g equiv. to 1 ml), mechanical grinding in Potter-Elvehjem apparatus with motor-driven teflon pestle, approx. 400 rpm, 4 degrees centigrade

glassware and equipment in contact with homogenate precooled

Centrifugation:

1.6E4 m/s2 (= 1650 x g) for 5 min, subsequently 8.8E4 m/s2 (= 9000 x g) for 10 min, 4 degrees centigrade

Storage of supernatant:	in 0.25-ml, 0.5-ml, 2-ml and 5-ml aliquots at -75 degrees centigrade
Scientific version:	SOP MB 47/4
Text version:	29.Nov.85

5.4.6 Analyses of rat liver homogenates

5.4.6.1 Determination of protein concentration

Principle:	photometric determination of a dye complex formed between peptide bonds and the Biuret reagent
Time:	20.Nov.84 and 6.Nov.85
Sample material and quantity:	liver S9 homogenate, batch no. 84-1, S9 mixes, 10 ul
Results expressed in:	g/l
Equipment:	ABA-100 Bichromatic Analyzer, Abbott GmbH, Diagnostics Division, D-6236 Eschborn
Chemicals:	test set: Total Protein (Biuret method), no. 124281, standard proteins: Precimat, no. 125601, Precinorm U, no. 171735, Boehringer Mannheim GmbH, D-6800 Mannheim 31
Procedure:	according to Weichselbaum (1946), adapted by INBIFO to the bichromatic analyzer each sample determined in duplicates

	final concentration of components in assay mixture:
	sodium hydroxide 0.1 mol/l
	potassium sodium tartrate 15.7 mmol/l
	potassium iodide 14.7 mmol/l
	copper sulfate 5.9 mmol/l
	photometric determination: wavelength 1: 550 nm wavelength 2: 650 nm
	storage until determination: -75 degrees centigrade
	standard curve: see FIGURE E
Detection limit:	10 ug protein per assay equivalent to 1 g/l
Scientific version:	SOP BC 128/2
Text version:	20.Jan.86

5.4.6.2 Determination of aryl hydrocarbon monooxygenase (EC 1.14.14.2) activity

Principle:	fluorometric determination of 3- and 9-hydroxy-B(a)P formed during the incubation of B(a)P with tissue extracts and separated after incubation by HPLC
Time:	20.Nov.84 and 6.Nov.85
Sample material and quantity:	6.4 to 8.9 ug S9 protein diluted in SVT buffer, batch no. 84-1, S9 mixes, 10 ul
Results expressed in:	U/mg protein (1 U = 1 nmol 3- plus 9-hydroxy-B(a)P/h
Equipment:	high performance liquid chromatograph: model 1084 B, Hewlett-Packard GmbH, D-7500 Karlsruhe

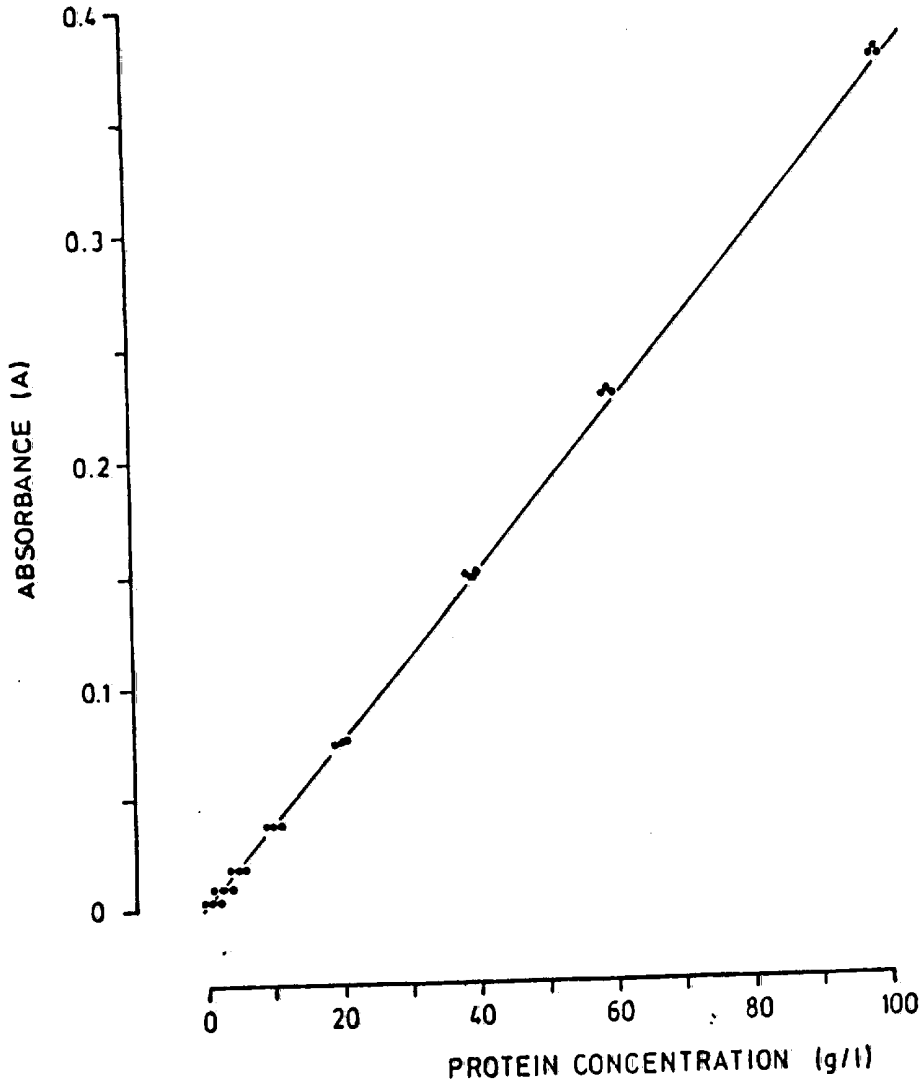


FIGURE E

PROTEIN STANDARD CURVE, BIURET METHOD

Remarks: Scientific version: SOP BC 128/2

column: RP-18, length: 100 mm, inner diameter: 4 mm, particle diameter: 5 um, no. 44026534,
Dr. H. Knauer KG,
D-6370 Oberursel

incubation vessels: no. 611-52,
Sovirel,
via Faust GmbH,
D-5000 Köln 90

injection vial: no. 70214,
capacity: 1 ml,
Machery-Nagel GmbH und Co. KG,
D-5160 Düren

spectrofluorometer: model 650-10S,
Perkin Elmer GmbH,
D-7770 Überlingen

shaking water bath: no. 3047,
Köttermann KG,
D-3165 Hänigsen

centrifuge: model J6,
rotor: JS-4.2,
Beckman Instruments GmbH,
D-8000 München 40

Chemicals:

magnesium chloride, no. 5833,
acetonitrile, no. 30,
sodium dihydrogen phosphate, no. 6346,
acetone, no. 14,
methanol, no. 6007,
hydrochloric acid, no. 9057,
saccharose, no. 7651,
EDTA, no. 8418,
E. Merck,
D-6100 Darmstadt 1

glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), no. 197726,
glucose-6-phosphate (G6P), no. 127027,
NADP, no. 128031,
NAD, no. 127981,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31

benzo(a)pyrene (B(a)P), no. 3176,
Carl Roth GmbH,
D-7500 Karlsruhe 21

1,2-benzanthracene, no. B 220-9,
Ega Chemie,
D-7924 Steinheim

bovine serum albumin (BSA), no.
A 4378,
tris(hydroxymethyl)aminomethane
(Tris), no. T 1503,
Sigma Chemie GmbH,
D-8028 Taufkirchen

9-hydroxybenzo(a)pyrene, no. 106,
3-hydroxybenzo(a)pyrene, no. 75,
IIT Research Institute,
Chicago, Illinois 60616, USA

Tris buffer:
250 mmol/l, adjusted to pH 7.6 with
hydrochloric acid

solution for termination of enzyme
reaction:
methanol containing 0.15 mg/l
benzanthracene (-20 degrees centigrade)

SVT buffer:
250 mmol saccharose/l, 5 mmol EDTA/l,
20 mmol Tris/l, pH 7.4, pH adjusted
with hydrochloric acid

Procedure

Enzyme incubation:

according to Van Cantfort et al. (1977),
modified by INBIFO

triplicate determination per sample

incubation time: 10 min

final concentration of components
in assay mixture:

B(a)P	0.08 mmol/l
NAD	0.43 mmol/l
NADP	0.37 mmol/l
G6P	2.50 mmol/l
BSA	0.80 g/l
Tris buffer, pH 7.6	50.00 mmol/l
magnesium chloride	5.00 mmol/l
G6PDH	1E3 U/l
total assay volume:	0.50 ml

Protein precipitation:	addition of solution for termination of enzyme reaction (1 ml/assay mixture, -20 degrees centigrade) with 2 s mixing. After storage at 0 degrees centigrade for 15 to 30 min centrifugation at 4.4E4 m/s2 (= 4500 x g) at 4 degrees centigrade for 10 min. Injection of supernatant into HPLC either immediately or after storage at -30 degrees centigrade for unlimited time
HPLC separation	
Injection volume:	20 ul
Oven and solvent temperature (degrees centigrade):	40
Solvent A:	10 mmol sodium dihydrogen phosphate/l, pH 4.8
Solvent B:	1000 ml acetonitrile/l
Flow:	0 to 10 min: from 0.5 ml/min to 1.1 ml/min 10 to 20 min: from 1.1 ml/min to 0.5 ml/min
Gradient:	0 to 10 min: elution gradient from 400 ml acetonitrile/l to 700 ml acetonitrile/l 10 to 15 min: elution gradient from 700 ml acetonitrile/l to 1000 ml acetonitrile/l 15 to 20 min: elution gradient from 1000 ml acetonitrile/l to 400 ml acetonitrile/l regeneration time between 2 analyses: 3 min
Column pressure (MPa):	up to 10
Detection:	fluorometric, excitation wavelength: 305 nm, emission wavelength: 430 nm, slit of excitation and emission monochromators: 15 nm

Calculation:	by HPLC microprocessor on the basis of external (3- plus 9-hydroxy-B(a)P) and internal (1,2-benzanthracene) standards
Scientific version:	SOP BC 1/13
Text version:	21.Jan.86

5.4.6.3 Bacteriological examination of S9 fraction

Principle:	determination of bacterial contamination by growth on nutrient agar plates detection limited to aerobic cocci and rods
Time:	immediately after preparation of S9 fraction
Sample material and quantity:	liver S9 fraction, 0.1 ml/plate
Results expressed in:	CFU/ml
Equipment:	incubator: no. 3916, Forma Scientific, via Labotect, D-3400 Göttingen petri dishes: 100 mm x 15 mm, polystyrene, sterilized, colony counter: Colony Star 2, Funke-Gerber, D-1000 Berlin
Chemicals:	nutrient agar, standard 1, no. 7881, E. Merck, D-6100 Darmstadt 1
Procedure:	plating of 0.1 ml liver S9 fraction on nutrient agar plates in duplicates, plates incubated at 37 degrees centigrade

	manual counting of colonies after 1 d and 4 d of incubation
Scientific version: Text version	SOP MB 48/1 27.Jun.85

5.5 Tester Strain Bacteria

5.5.1 Species and source

Species:	Salmonella typhimurium LT-2 mutant strains TA98 and TA100
Genotypes	
hisD3052 or hisG46:	mutations in histidine operons, resulting in requirement for histidine (TA98: hisD3052, TA100: hisG46)
rfa:	deep rough, mutation in the lipopoly-saccharide barrier, making the bacteria cell more permeable and completely nonpathogenic
delta uvrB:	deletion of excision repair system, resulting in sensitivity to ultraviolet light
pKM101:	resistance transfer system, so-called R factor plasmid
Sensitivity	
TA98:	to mutagens causing frameshift mutation
TA100:	to mutagens causing base-pair substitution
Source:	kindly provided by Prof. Dr. Bruce N. Ames, University of California, Berkeley CA., USA

Receipt at INBIFO:	Jul.79
Text version:	17.Dec.85

5.5.2 Cultivation

Principle:	cultivation of tester strain bacteria in nutrient broth to an early stationary growth phase
Time:	approx. 12 h before use in the mutagenicity assay
Sample material and quantity:	Salmonella typhimurium strains from stock culture stored at -196 degrees centigrade
Equipment:	incubator shaker: model G 24, New Brunswick Scientific, via Biotronik, D-4000 Düsseldorf culture flask: Erlenmeyer flask, 100 ml, with long neck, used with stainless steel caps, Schott, D-6500 Mainz cryotubes: polypropylene, 2 ml, with screw caps, no. 985731, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main
Chemicals:	sodium chloride, no. 6400, DMSO, for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1

Difco-nutrient broth, pH 6.8 ± 0.2,
composition (g/l):

beef extract	3.0
peptone	5.0
sodium chloride (added separately)	5.0

no. 0003-01,
Difco Laboratories,
via Otto Nordwald KG,
D-2000 Hamburg 50

saline solution, 9 g sodium chloride/l

Procedure

Inoculation:	addition of 10 ul of the thawed and 10-fold diluted stock culture to 30 ml nutrient broth in the culture flask
Cultivation:	cultures incubated in a shaking incubator at 37 degrees centigrade at 200 rpm
	cultures grown for 12 h to obtain an early stationary growth phase
Centrifugation:	no centrifugation and washing of bacterial cells
Storage of stock culture:	at -196 degrees centigrade in liquid nitrogen in 0.1 ml aliquots of tester strain suspension culture with 87.5 ml DMSO/l

Scientific version: SOP MB 50/3
Text version: 29.Nov.85

5.5.3 Determination of cell suspension density

Principle:	photometric determination of the amount of light of 565 nm wavelength scattered by diluted culture suspension
Time:	at the start and at the end of the mutagenicity assay
Sample material and quantity:	bacteria suspension culture, 0.5 ml each strain
Results expressed in:	absorbance unit (A)
Equipment:	photometer: DB-GT, Beckman Instruments GmbH, D-8000 München 40 disposable plastic cuvets, no. TN 4000-12, Nunc GmbH, D-6200 Wiesbaden 12
Chemicals:	sodium chloride, no. 6400, E. Merck, D-6100 Darmstadt 1

Procedure

Dilution:	0.5 ml bacteria suspension culture plus 2.0 ml sodium chloride solution (9 g/l)
Photometric determination:	wavelength: 565 nm blank: sodium chloride solution (9 g/l) absorbance calculation for the undiluted culture
Scientific version:	SOP MB 51/4
Text version:	24.Jul.85

5.5.4 Determination of number of viable bacteria

Principle:	spreading of bacteria with top agar plating technique and counting of colony forming units
Time:	at the start and at the end of the mutagenicity assay
Sample material and quantity:	bacteria suspension culture, approx. 0.1 ml of each strain
Results expressed in:	CFU/plate
Equipment:	automatic colony counter: model no. 880, Artek System Corporation, via Fisher Scientific, D-8000 München manual colony counter: Colony Star 2, Funke-Gerber, D-1000 Berlin incubator: no. 3916, Forma Scientific, via Labotect, D-3400 Göttingen petri dishes: no. 10239, 100 mm x 15 mm, polystyrene, sterilized, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1
Chemicals:	sodium chloride, no. 6400, E. Merck, D-6100 Darmstadt 1 L-histidine hydrochloride-1-hydrate, no. H 8125, biotin, no. B 4501, Sigma Chemie GmbH, D-8028 Taufkirchen histidine/biotin solution no. 2: 19.10 g histidine hydrochloride-1-hydrate and 244 mg biotin dissolved in 1 l distilled water, sterilized by filtration

	minimal-glucose agar and top agar, composition: see 5.6.1 Plate incorporation assay
Procedure:	aliquots of bacteria suspension culture diluted 1E6-fold in sodium chloride (9 g/l). 0.1 ml of this dilution mixed with 2.0 ml top agar and 0.1 ml histidine/biotin solution and plated on minimal-glucose agar plates incubation: 48 h at 37 degrees centigrade counting: automatic or manual counting of CFU
Scientific version: Text version:	SOP MB 53/3 10.Mar.86

5.5.5 Analyses of tester strain properties

Principle:	tester strain checked for: (1) auxotrophy in the form of histidine requirement (2) absence or presence of lipopolysaccharide barrier in the form of sensitivity or resistance to crystal violet (3) absence or presence of R factor in the form of sensitivity or resistance to ampicillin (4) lack of excision repair system in the form of sensitivity to ultraviolet light
Time:	prior to and after the study
Sample material and quantity:	bacteria suspension culture, approx. 0.5 ml of each strain

Equipment:

ultraviolet light source:
Astralux F, no. 15136, 890 W,
Astralux-Werke,
A-1000 Wien

filter paper disks: no. 95354,
ampicillin sensitivity disk,
10 ug/disk, no. 93332,
diameter: 6 mm,
Becton Dickinson GmbH,
D-6900 Heidelberg 1

petri dishes: no. 1029, 100 mm x
15 mm, polystyrene,
Falcon,
via Becton Dickinson GmbH,
D-6900 Heidelberg 1

incubator: no. 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingen

Chemicals:

L-histidine hydrochloride-1-hydrate,
no. H 8125,
biotin, no. B 4051,
Sigma Chemie GmbH,
D-8028 Taufkirchen

crystal violet, no. 1407,
nutrient agar, standard 1, no. 7881,
E. Merck,
D-6100 Darmstadt 1

minimal-glucose agar and top agar,
composition: see 5.6.1 Plate incorporation assay

Procedure

Reference:

basically according to Ames et al. (1975) and Maron and Ames (1983)

Histidine requirement:

tester strain bacteria streaked on minimal-glucose agar plate without and with 0.1 ml of histidine and biotin solution (0.1 mol L-histidine hydrochloride-1-hydrate and 0.5 mmol biotin/l)

	incubation at 37 degrees centigrade for 18 to 24 h
	plates checked for growth
Crystal violet sensitivity:	10 ul crystal violet solution (1 g/l) applied to filter paper disk, placed onto complete nutrient agar plate, with tester strain bacteria plated
	incubation at 37 degrees centigrade for 12 to 16 h
	plates checked for inhibition or growth zone around the disk
Ampicillin resistance:	ampicillin disk (10 ug) applied onto complete nutrient agar with tester strain bacteria plated
	incubation at 37 degrees centigrade for 18 to 24 h
	plates checked for growth or inhibition zone around ampicillin disk
Sensitivity to ultraviolet light:	tester strain bacteria to be tested streaked across nutrient agar plates and half of the streak irradiated for 30 s with ultraviolet light at a distance of 33 cm
	incubation at 37 degrees centigrade for 18 to 24 h
	plate checked for growth inhibition
Scientific version:	SOP MB 54/4
Text version:	27.Jun.85

5.6 Mutagenicity Assay

5.6.1 Plate incorporation assay

Principle:	mixture of test substance and tester strain bacteria with or without the metabolic activation system spread on minimal-glucose agar plates containing minimal amounts of histidine and biotin using the top agar plating technique after incubation at 37 degrees centigrade counting of revertants (see FIGURE F)
Time of top agar plating:	see 5.1 Chronology
Sample material and quantity:	(1) test substances: see 5.3 Dosing of Test Substance (2) positive controls (diagnostic mutagens): MMS (0.01 l/l DMSO, 0.5 ul/plate), daunomycin (0.12 g/l H2O, 6 ug/plate), 2-AA and 2-AF (0.04 g/l DMSO, 2 ug/plate)
Results expressed in:	revertants/plate and increase in the number of revertants/mg dry condensate
Equipment:	medium autoclave: cultmatic 800 with Pretagar as controller, Best, via E. Schütt jr., D-3400 Göttingen petri dish filler: automatic dose dishes and stacking unit, no. 8030, pbi pool bioanalysis italiana, via Labora, D-6395 Weilrod-Winden petri dishes: no. 1029, 100 mm x 15 mm, test tubes: no. 3033, 16 mm x 125 mm, polystyrene, sterile, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1

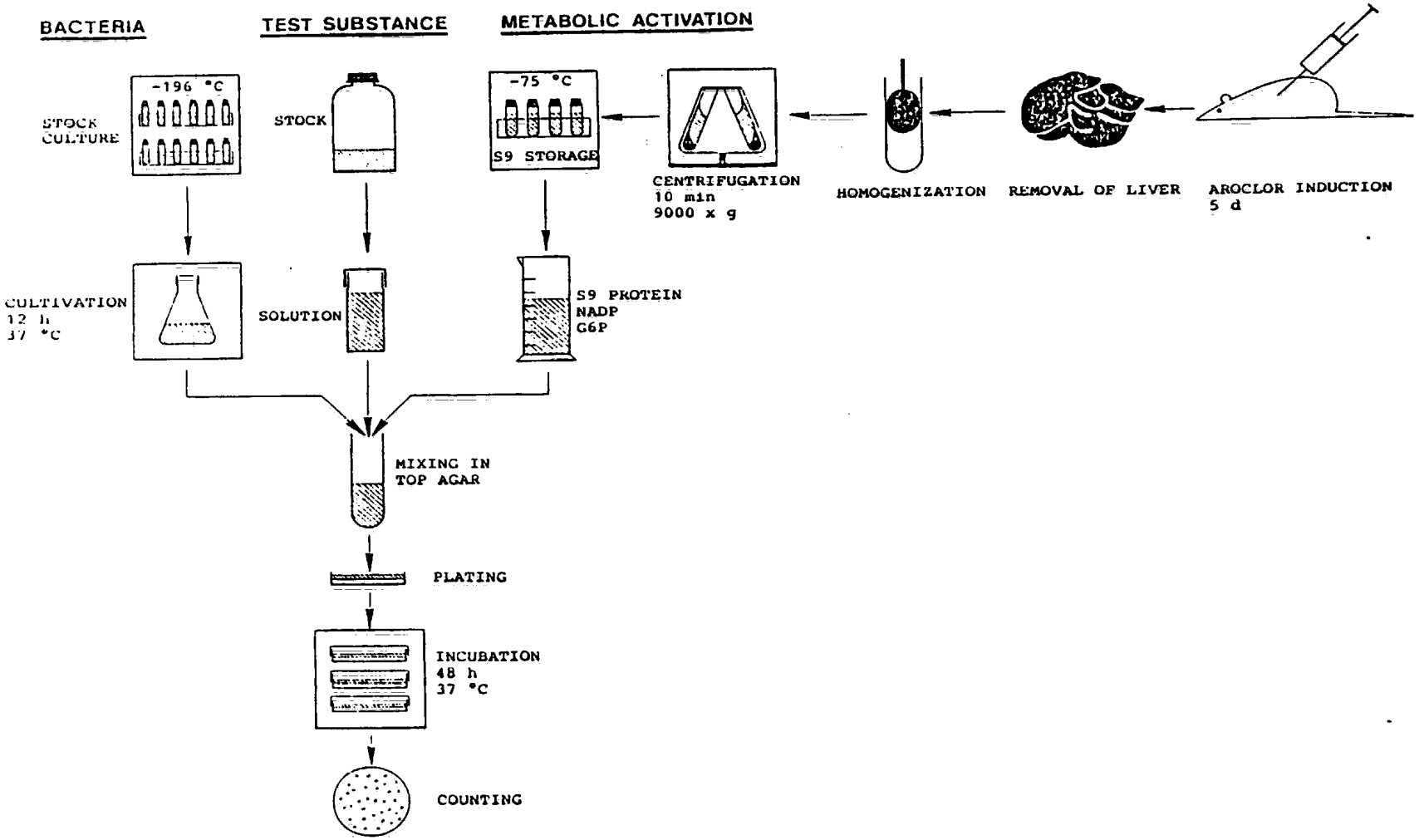


FIGURE F

FLOW CHART OF THE PLATE INCORPORATION MUTAGENICITY ASSAY

disposable membrane filter unit:
Millex, 0.45 um pore size,
no. SLHA 025BS (for S9 mix),
filter unit: sterifil,
no. XX1104710,
membrane filter, 0.2 um pore size,
no. GSWP 04700 (for salt solution),
prefilter, no. AP2504200 and membrane
filter, no. HAWG04700, 0.45 um pore
size (for glucose solution),
Millipore GmbH,
D-6078 Neu-Isenburg

disposable membrane filter unit:
0.2 um pore size, no. FP 030/3
(for NADP and G6P),
Schleicher und Schüll GmbH,
D-3354 Dassel

incubator: no. 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingen

whirlmix: no. 34524-200,
Cenco Instrumenten,
NL-4800 Breda

thermostat: aluminum bloc thermo-
stat, no. 2092,
Gebr. Liebisch,
D-4800 Bielefeld

manual colony counter: Colony Star 2,
Funke-Gerber,
D-1000 Berlin

automatic colony counter connected
to teletype: model no. 880,
Artek System Corporation,
via Fisher Scientific,
D-8000 München

automatic pipettes:
(1) refilling syringes: Cornwall syringe,
maximal volume: 2 ml (for top agar),
Becton Dickinson,
E. Schütt,
D-3400 Göttingen

(2) bottle-top dispenser:
"dispensette", maximal volume: 2 ml
(for S9 mix),
Brand GmbH und Co.,
D-6980 Wertheim

"distrivar", maximal volume: 0.5 ml
(for bacteria suspension),
Gilson,
via Abimed,
D-4000 Düsseldorf

(3) adjustable pipettes:
P 20, P 200 and P 1000,
maximal volumes: 0.02, 0.2 and 1 ml
(for test substance),
Gilson,
via Abimed,
D-4000 Düsseldorf

Finnpipette digital,
maximal volume: 0.04 ml
(for test substance),
Justor DG pipette,
maximal volume: 5 ml (for solvent),
LKB Instrument GmbH,
D-8032 Gräfelfin

Chemicals:

glucose-6-phosphate-disodium,
no. 127647,
NADP-disodium, no. 128058,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31

agar, no. 0140-01,
Difco Laboratories,
via Biotest Seruminstitut GmbH,
D-6050 Offenbach

citric acid-1-hydrate, no. 244,
DMSO, no. 2950,
D(+)-glucose-1-hydrate, no. 8342,
magnesium chloride-6-hydrate, no. 5833,
magnesium sulfate-7-hydrate, no. 5886,
sodium ammonia hydrogen phosphate-
4-hydrate, no. 6682,
sodium chloride, no. 6400,
sodium dihydrogen phosphate-1-hydrate,
no. 6346,
disodium hydrogen phosphate-2-hydrate,
no. 6580,

potassium chloride, no. 4936,
dipotassium hydrogen phosphate-3-hydrate,
no. 5099,
E. Merck,
D-6100 Darmstadt 1

2-aminoanthracene, no. A 1381,
2-aminofluorene, no. A 9031,
daunomycin, no. D 4885,
methyl methanesulfonate, no. M 4016,
D(+)-biotin, no. B 4501,
L-histidine-hydrochloride-1-hydrate,
no. H 8125,
Sigma Chemie GmbH,
D-8028 Taufkirchen

nitrogen, .GE.99.999 0/0 (v/v),
Linde AG,
via Elbert,
D-5000 Köln

Minimal-glucose agar:

composition (g/l):

glucose-1-hydrate	20.0
magnesium sulfate-7-hydrate	0.2
dipotassium hydrogen phosphate-3-hydrate	13.1
citric acid-1-hydrate	2.0
sodium ammonia hydrogen phosphate-4-hydrate	3.5
agar	15.0

glucose solution prepared separately 10-fold concentrated and filter-sterilized, salts 10-fold concentrated and agar solutions sterilized separately at 121 degrees centigrade (1.0E5 Pa) for 15 min

automatically filling into petri dishes, approx. 30 ml molten agar/plate, excess water on the solid agar plates removed by exposure of the covered plates at 37 degrees centigrade for 3 d, subsequently stored at RT

Top agar:

composition (g/l):

L-histidine-hydrochloride-1-hydrate	0.0087
biotin	0.011
sodium chloride	4.5
agar	5.5

histidine-biotin solution prepared separately as 10-fold concentrated solution (no. 1), filter-sterilized and stored at 4 degrees centigrade

agar-sodium chloride solution sterilized at 121 degrees centigrade (1.0E5 Pa) for 15 min and stored at RT

prior to use remolting of the agar-sodium chloride solution by boiling in a water bath for approx. 20 min, addition of histidine-biotin solution (no. 1) after cooling down to approx. 45 degrees centigrade

S9 mix (a):

composition (g/l and mmol/l):

sodium phosphate buffer, pH 7.4	-	100.0
magnesium-chloride-6-hydrate	3.25	16.0
potassium chloride	4.92	66.0
glucose-6-phosphate-disodium	1.68	5.0
NADP-disodium	3.22	4.0
S9 protein (prior to filtration)	10.0	-

S9 mix filter-sterilized prior to use and stored at 0 degrees centigrade under nitrogen atmosphere during the mutagenicity assay

dilution of S9 mix with sodium phosphate buffer (0.1 mol/l) to adjust other S9 protein concentrations with a fixed S9 protein/cofactor ratio (according to Zeiger et al., 1979)

sodium phosphate buffer pH 7.4, 0.1 mol/l (prepared as a 2-fold concentrated solution, 5.24 g sodium dihydrogen phosphate and 28.8 g disodium hydrogen phosphate/l), sterilized at 121 degrees centigrade (1.0E5 Pa) for 15 min and stored at 4 degrees centigrade

(a) protein concentration and AHM activity determined from each S9 mix

	magnesium-potassium chloride prepared as 25-fold concentrated solution, sterilized at 121 degrees centigrade (1.0E5 Pa) for 15 min and stored at 4 degrees centigrade glucose-6-phosphate prepared as 200-fold and NADP as 25-fold concentrated solutions, filter-sterilized and stored at -75 degrees centigrade
Tester strain bacteria:	Salmonella typhimurium strains TA98 and TA100, 12-h cultures
Procedure	
Reference:	basically according to Ames et al. (1975) and Maron and Ames (1983)
Dosing of test substance:	see 5.3 Dosing of Test Substance
Plating mixture preparation:	components added in the following order: (1) 2.0 ml top agar, 45 degrees centigrade (2) test substance, stored at RT (3) 0.1 ml tester strain suspension culture, containing approx. 1E8 CFU, stored at RT (a) (4) 0.5 ml metabolic activation system or buffer solution stored at 0 degrees centigrade under nitrogen atmosphere
Top agar plating:	components mixed by rotating the test tube gently on a whirlmix, then poured on minimal-glucose agar plates and spread evenly on the surface by wobbling, mixing, pouring and spreading of the top agar occured within 20 s, plates allowed to harden for 3 to 6 min and then transferred to the dark incubator
Incubation:	48 h at 37 degrees centigrade in the dark

(a) Temperature may arise to approx. 30 degrees centigrade during mutagenicity assay.

Labeling of the petri dishes:	individual plate no., project no., name of test substance, batch no., type of test substance, tester strain, absence/presence of metabolic activation system, test substance doses, date of top agar plating
Counting of revertants:	manual and/or automatical counting immediately or after storage at 4 degrees centigrade for not longer than 48 h, plates brought to RT automatic counting: standardized by 5100 mm2 plate aperture area without discrimination of colony size, each plate counted 3 times, rotation of the plate for 120 degrees between each count, data recorded on punched tape or floppy disk, highest count used for the computer calculation of revertants
Scientific version:	SOP MB 55/3
Text version:	21.Jan.86

5.6.2 Reversion assay

Principle:	analysis of growth of individual colonies on minimal-glucose agar without histidine
Time:	see 5.1 Chronology
Sample material and quantity:	20 individual colonies from 1 mutagenicity assay plate with the highest nontoxic dose of test substance
Results expressed in:	number of colonies grown (histidine prototrophs/20 colonies inoculated)

Equipment:	incubator: no. 3916, Forma Scientific, via Labotect, D-3400 Göttingen
Chemicals:	biotin, no. B4501, Sigma Chemie GmbH, D-8028 Taufkirchen minimal-glucose agar, composition: see 5.6.1 Plate incorporation assay biotin solution: 0.12 g biotin/l
Procedure:	minimal amount of colonies to be assayed streaked on minimal-glucose agar plate with 0.2 ml of biotin solution incubation of plates at 37 degrees centigrade for 24 to 48 h counting of colonies grown after incubation
Scientific version: Text version:	SOP MB 59/2 7.Oct.85

5.6.3 Statistical evaluation

Primary data (revertants/ plate):	calculation of mean and RSD from all plates for each test cigarette also calculated separately for each substudy, data not corrected for automatical counting data stored and calculated in data base management system ORACLE on a VAX 750 computer
Specific mutagenicity:	equivalent to regression coefficient "a" of the linear dose-response curve $y = ax + b$

	increase in the number of revertants per mg dry condensate
Relative difference:	absolute difference between 2 values (A and B) divided by the mean of them $\frac{\lvert A - B \rvert}{(A + B)/2}$
Statistical significance of the difference of the specific mutagenicity of 2 independent substudies:	statistical significance reached at the level of significance equal to 0.017 with a relative difference of the specific mutagenicities by 25 percent (a)
Statistical significance of the difference of the specific mutagenicity of WSC of 2 test cigarettes:	statistical significance reached at the level of significance equal to 0.05 with a relative difference of the specific mutagenicities by 16 percent (a)
Scientific version:	SOP MB 60/1
Text version:	29.Nov.85

(a) In the basic biometric INBIFO study P 0268/2029 with strain TA98 the mainstream WSC-I of 1 test cigarette was assayed according to the INBIFO standard procedure (same procedure as in the present study: 2 independent substudies, 4 doses and 64 plates/test cigarette). For the lack of a basic biometric study with strain TA100, the limit of the relative difference for 2 test cigarettes or 2 substudies is set to 0.16 or 0.25 respectively following the biometric study with TA98.

6 STORAGE OF MATERIALS AND RECORDS

Test substance

Test cigarettes:	no storage of test cigarettes after completion of the study
Condensates:	5 ml of each condensate preparation transferred at day of cigarette condensate preparation into brown glass vial with screw cap and stored at -75 degrees centigrade for at least 1 year the remaining volume of each condensate preparation stored at 4 degrees centigrade for approx. 1 month
Protocol, records and evaluation sheets:	stored in our archives for at least 5 years, they can be claimed by the client
Scientific version:	SOP MB 61/1, QA 10/1
Text version:	21.Jan.86

7 QUALITY ASSURANCE

Position of QA unit in organizational structure of INBIFO:	organizational unit separate from and independent of the staff engaged in the management and conduct of the study, direct assignment to general manager
Responsibility:	assurance that all studies are conducted in a manner that will guarantee the quality and integrity of all data generated therein, that the report of a study accurately describes the assays carried out and the results obtained and that the testing facility on the whole with all operating procedures complies with the standards set forth in the Good Laboratory Practice Regulations (a)
Records to be maintained:	master schedule sheet (computerized), copies of protocols with amendments, records of changes and special instructions of all studies, standard operating procedures, copies of all reports delivered to the clients, tables with QA inspection dates, records showing the results of the QA inspection and the actions to solve possible existing problems, tables for all animals that died or were killed in moribund state with their previous history and findings at dissection, organization structure chart, list of staff with their qualifications, list with the abbreviated signatures of the staff

(a) Federal Register, December 22, 1978, Part 2, pp. 59986-60025

Main organizational aids:	IBM PC XT, digital Mini Minc computer system, graphic printer, Vydec 1800 text processing system, planning board (time table for each study), microfiche reader
Special measures:	reporting of all findings and problems, actions recommended and taken to solve existing problems to general manager and study director
Staff	
Head:	E. Römer, Biologist (Diplombiologe)
Assistant:	N. Dax, Biologist (Diplombiologe)
	E. Jansen, Technician (Biologie-laborant)
	M. Woiwode, Secretary (Diplom-Agrar-biologe)
Date of inspection for this study:	9. and 24.Oct.85
Scientific version:	SOP QA 1/5, QA 2/2, QA 4/2, QA 5/4, QA 6/2
Text version:	4.Dec.85

8 RESULTS AND DISCUSSION

8.1 Text

8.1.1 Whole smoke condensate

8.1.1.1 Yield of dry condensate, water, nicotine and catechol

The yield of dry condensate and nicotine in the MWSC-I as well as the puff count were equal to the specification provided by the supplier (see 4.2 Supplier's Specification and TABLES 1). The yield of water in MWSC-I was found to be higher than the supplier's analyses and showed the highest day-to-day variations. The yield of dry condensate in the MWSC-I of cigarette SLOW-77 was approx. 85 percent and in the SWSC-I approx. 55 percent of that of cigarette SLOW-72 (see TABLES 1 and 2).

It should be noted that conditioned SLOW-77 cigarettes appeared to be "wet" in substudy 1, but no influence of this wetness was observed on condensate yield and composition (see also footnote (b) on PAGE 4-1).

8.1.1.2 Bacteriological examination of SWSC-I application suspension

The SWSC-I/DMSO application suspensions of all test cigarettes plated on minimal-glucose agar were found to be free from bacterial contaminants interfering with the mutagenicity assay (see TABLE 3).

8.1.2 Properties and responses of the tester strains

8.1.2.1 Properties

Suspension cultures from frozen stock cultures of Salmonella typhimurium strains TA98 and TA100 checked for

(1) the auxotrophy as histidine requirement,
(2) the absence of the lipopolysaccharide barrier as sensitivity to crystal violet,
(3) the presence of the ampicillin resistance carrying extrachromosomal R factor and
(4) the lack of an excision repair system (uvrB) as sensitivity to ultraviolet light

were found to respond in a characteristic manner as recommended in the basic "method papers" by Ames et al. (1975) and Maron and Ames (1983) (see TABLES 4.1 and 5.1).

8.1.2.2 Spontaneous reversion

The number of spontaneous TA98 revertants per plate was found to be 26.9 ± 1.3 (a) in the absence and 47.4 ± 2.3 (a) in the presence of S9 protein, when approx. 1.0E8 viable bacteria were added to each plate (see TABLES 4.2 to 4.4).

The number of spontaneous TA100 revertants per plate was found to be 150.3 ± 3.3 (a) in the absence and 148.4 ± 6.2 (a) in the presence of S9 protein, when approx. 1.5E8 viable bacteria were added to each plate (see TABLES 5.2 to 5.4).

The results of the spontaneous reversion were in agreement with previous results and with the findings by Maron and Ames (1983).

(a) mean ± SE, N: 16

8.1.2.3 Mutagenicity of diagnostic mutagens

The mutagenic response of the strain-specific positive control substances daunomycin (TA98) and MMS (TA100) were found to be in accordance with the responses published by Babudri et al. (1984) and Maron and Ames (1983). The mutagenic response to the mutagens 2-AA and 2-AF indicated sufficient metabolic activity of the S9 protein. These results were in accordance with those published by Maron and Ames (1983) and Zeiger et al. (1979) (see TABLES 4.5 and 5.5).

8.1.3 Properties of S9 protein

The S9 protein was prepared from an Aroclor 1254-induced rat liver homogenate. The specific AHM activity of the S9 protein batch used in the mutagenicity assays was found to be 171 units per milligram protein (see TABLE 6.1) and was in the expected range.

During the preparation of S9 mix, the S9 protein was diluted to 4 grams/liter and the S9 mix was sterile-filtered. The final amount of S9 protein per plate was assayed for each mutagenicity assay and was approx. 1.7 milligrams (see TABLE 6.2). The AHM activity of the S9 mix was found to be 50 percent lower than the activity of the original S9 protein batch determined previously. Nevertheless the mutagenicity of the diagnostic mutagens indicated sufficient metabolic activation (see 8.1.2.3 Mutagenicity of diagnostic mutagens).

8.1.4 Mutagenicity of whole smoke condensate

8.1.4.1 Dose response

WSC-I of the test cigarettes was assayed at the doses 0, 0.05, 0.10 and 0.15 milligrams dry condensate per plate. An approx. linear increase in the number of revertants was obtained (see TABLES 7 to 10 and FIGURES 1 and 2).

8.1.4.2 Reversion assay

180 out of 180 (100 percent) colonies of strain TA98 or of strain TA100 from mutagenicity assay plates, to which the highest dose of MWSC-I or SWSC-I had been added, were found to be histidine prototrophs (revertants) in the subsequent reversion assay on plates without histidine (a) (see TABLES 11).

8.1.4.3 Reproducibility

The mutagenic activity of WSC-I of the cigarettes obtained in substudy 1 was statistically not different from that obtained in substudy 2 with the exception of MWSC-I of cigarette SLOW-72 for base-pair substitution. The mutagenicity of MWSC-I of this cigarette showed a relative difference greater than the limit of 0.25 between both substudies (see TABLES 12 to 15).

(a) A trace amount of histidine was added to the top agar in the plate incorporation assay to allow the bacteria on the plate to undergo several divisions which are in many cases necessary for mutagenesis to occur. In case of massive cell death during exposure of the bacteria to a test substance more histidine is available to the individual surviving bacteria, and they undergo more cell divisions forming small colonies ("false revertants") which can be mistaken for revertants (Ames et al., 1975).

The specific mutagenicity of MWSC-I and SWSC-I of the standard reference cigarette 2R1 was found to be in the expected range for inducing frameshift mutation as well as base-pair substitution when compared with all previous INBIFO data obtained with 2R1-WSC-I.

8.1.4.4 Specific mutagenicity of mainstream whole smoke condensate

The mean activity of MWSC-I to induce frameshift mutation in strain TA98 was found to be 1752 revertants per milligram dry condensate for the cigarette SLOW-72 and 2046 for SLOW-77 (see TABLE 16.1 and FIGURE 3). The mutagenicity of MWSC-I of SLOW-77 was higher than that of the reference cigarette SLOW-72. The difference was statistically not significant but approached the borderline (see TABLE 18).

The mean activity of MWSC-I to induce base-pair substitution in strain TA100 was found to be 1060 revertants per milligram dry condensate for the cigarette SLOW-72 and 1250 for SLOW-77 (see TABLE 16.2 and FIGURE 3). The mutagenicity of MWSC-I of SLOW-77 was higher than that of the reference cigarette SLOW-72. Due to the difference between substudies 1 and 2, the evaluation of MWSC-I of cigarette SLOW-72 has to be taken with reservation (see TABLE 18).

8.1.4.5 Specific mutagenicity of sidestream whole smoke condensate

The mean activity of SWSC-I to induce frameshift mutation in strain TA98 was found to be 1367 revertants per milligram dry condensate for the cigarette SLOW-72 and 2156 for SLOW-77 (see TABLE 17.1 and FIGURE 4). The mutagenicity of SWSC-I of SLOW-77

was higher than that of the reference cigarette SLOW-72 and this difference was statistically significant (see TABLE 18 and FIGURE 4).

The mean activity of SWSC-I to induce base-pair substitution in strain TA100 was found to be 1312 revertants per milligram dry condensate for the cigarette SLOW-72 and 2043 for SLOW-77 (see TABLE 17.2 and FIGURE 4). The mutagenicity of SWSC-I of SLOW-77 was higher than that of the reference cigarette SLOW-72 and this difference was statistically significant (see TABLE 18 and FIGURE 4).

8.1.4.6 Total mutagenicity of mainstream and sidestream whole smoke condensate

For MWSC-I, the total mutagenicity was found to be practically the same for both test cigarettes with respect to frameshift mutation as well as to base-pair substitution (see TABLES 19 and 21). The dry condensate yield was approx. 15 percent lower for cigarette SLOW-77. Due to the difference between substudies 1 and 2 with respect to base-pair substitution, the evaluation of MWSC-I of cigarette SLOW-72 has to be taken with reservation.

For SWSC-I, the total mutagenicity of cigarette SLOW-72 was found to be higher than that of cigarette SLOW-77 which showed an approx. 45 percent lower yield of dry condensate (see TABLE 20). With respect to frameshift mutation the difference approached the significance limit, and with respect to base-pair substitution the difference was statistically significant (see TABLE 21).

8.1.5 Comment

Based upon the specific and total mutagenicity of their condensates, the cigarettes SLOW-72 and SLOW-77 are considered to be equal with regard to MWSC-I. With regard to SWSC-I the specific mutagenicity of SLOW-72 is considered to be lower, but the total mutagenicity of SLOW-72 is considered to be higher than that of SLOW-77.

8.2 Tables and Figures

CIGARETTE	DATE OF WSC-I PREPARATION	BATCH NO. / STAT. PARAMETER	CONCENTRATION MOIST CONDENSATE	WATER	DRY CONDENSATE	NICOTINE	CATECHOL
	(Oct.85)		(g/l)	(g/l)	(g/l)	(g/l)	(mg/l)
SLOW-72	1	2366	28.76	7.16	21.60	1.61	76.6
	2	2372	28.84	6.95	21.89	1.60	81.4
	17	2386	18.84	2.80	16.04	1.24	62.4
	18	2396	22.12	4.17	17.95	1.37	68.0
		M	24.64	5.27	19.37	1.46	72.1
		SE	2.49	1.07	1.43	0.09	4.3
		RSD (0/0)	20.2	40.6	14.7	12.4	11.8
SLOW-77	1	2368 (a)	21.72	6.07	15.65	1.06	49.9
	2	2374 (a)	26.44	9.50	16.94	1.05	50.2
	17	2388	21.84	6.44	15.40	0.92	43.5
	18	2398	21.84	6.33	15.51	1.11	53.0
		M	22.96	7.09	15.88	1.04	49.2
		SE	1.16	0.81	0.36	0.04	2.0
		RSD (0/0)	10.1	22.8	4.5	7.8	8.2
2R1	1	2370	59.40	11.63	47.77	3.04	203.6
	2	2376	57.24	10.33	46.91	2.94	203.8
	17	2390	50.48	7.80	42.68	3.02	187.2
	18	2400	53.92	9.74	44.18	3.08	197.9
		M	55.26	9.88	45.39	3.02	198.1
		SE	1.95	0.80	1.18	0.03	3.9
		RSD (0/0)	7.1	16.1	5.2	2.0	3.9

TABLE 1.1

ANALYTICAL PARAMETERS OF CIGARETTES, MWSC-I,
MOIST CONDENSATE, WATER, DRY CONDENSATE, NICOTINE AND CATECHOL CONCENTRATION
IN WSC-I/DMSO APPLICATION SUSPENSION

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

CIGARETTE	DATE OF WSC-I PREPARATION (Oct.85)	BATCH NO. ----- STAT. PARAMETER	CONCENTRATION (g/kg) NICOTINE	CATECHOL
SLOW-72	1	2366	74.54	3.55
	2	2372	73.09	3.72
	17	2386	76.31	3.89
	18	2396	76.32	3.79
		M	75.07	3.74
		SE	0.78	0.07
		RSD (0/0)	2.1	3.8
SLOW-77	1	2368 (a)	67.73	3.19
	2	2374 (a)	61.98	2.96
	17	2388	59.74	2.83
	18	2398	71.57	3.42
		M	65.26	3.10
		SE	2.70	0.13
		RSD (0/0)	8.3	8.4
2R1	1	2370	63.64	4.26
	2	2376	62.67	4.34
	17	2390	70.76	4.39
	18	2400	69.72	4.48
		M	66.70	4.37
		SE	2.07	0.05
		RSD (0/0)	6.2	2.1

TABLE 1.2

ANALYTICAL PARAMETERS OF CIGARETTES, MWSC-I,
NICOTINE AND CATECHOL CONCENTRATION IN DRY CONDENSATE

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

CIGARETTE	DATE OF CONDENSATE PREPARATION (Oct.85)	WSC-I BATCH NO. ----- STAT. PARAMETER	YIELD (mg/cig.) DRY CONDENSATE	WATER	NICOTINE	CATECHOL	PUFF COUNT (puff/cig.)
SLOW-72	1	2366	17.71	5.87	1.32	0.063	9.2
	2	2372	17.71	5.62	1.29	0.066	10.0
	17	2386	13.46	2.35	1.04	0.052	10.2
	18	2396	15.16	3.52	1.16	0.057	10.3
		M	16.01	4.34	1.20	0.060	9.9
		SE	1.04	0.85	0.07	0.003	0.3
		RSD (0/0)	13.0	39.0	10.7	10.4	5.0
SLOW-77	1	2368 (a)	13.73	5.33	0.93	0.044	8.6
	2	2374 (a)	14.26	8.00	0.88	0.042	9.2
	17	2388	12.88	5.39	0.77	0.036	8.3
	18	2398	13.01	5.31	0.93	0.044	8.3
		M	13.47	6.01	0.88	0.042	8.6
		SE	0.32	0.67	0.04	0.002	0.2
		RSD (0/0)	4.8	22.1	8.6	9.0	4.9
2R1	1	2370	40.08	9.76	2.55	0.171	11.4
	2	2376	39.49	8.70	2.48	0.172	11.3
	17	2390	36.42	6.66	2.58	0.160	11.5
	18	2400	38.75	8.54	2.70	0.174	11.4
		M	38.69	8.42	2.58	0.169	11.4
		SE	0.80	0.65	0.05	0.003	0.04
		RSD (0/0)	4.1	15.3	3.6	3.7	0.7

TABLE 1.3

ANALYTICAL PARAMETERS OF CIGARETTES, MWSC-I,
DRY CONDENSATE, WATER, NICOTINE AND CATECHOL YIELD AND PUFF COUNT

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

CIGARETTE	DATE OF WSC-I PREPARATION (Oct.85)	BATCH NO. / STAT. PARAMETER	CONCENTRATION MOIST CONDENSATE (g/l)	WATER (g/l)	DRY CONDENSATE (g/l)	NICOTINE (g/l)	CATECHOL (mg/l)
SLOW-72	1	2367	38.12	7.87	30.25	2.76	137.1
	2	2373	37.92	6.53	31.39	3.08	125.8
	17	2387	32.48	5.08	27.40	2.61	115.2
	18	2397	31.24	4.43	26.81	2.90	113.3
		M	34.94	5.98	28.96	2.84	122.9
		SE	1.80	0.77	1.10	0.10	5.5
		RSD (0/0)	10.3	25.7	7.6	7.1	8.9
SLOW-77	1	2369 (a)	24.72	9.42	15.30	1.67	38.6
	2	2375 (a)	36.48	21.47	15.01	1.85	38.6
	17	2389	24.32	6.61	17.71	2.48	24.9
	18	2399	26.16	12.97	13.19	2.09	23.0
		M	27.92	12.62	15.30	2.02	31.3
		SE	2.88	3.23	0.93	0.18	4.3
		RSD (0/0)	20.6	51.1	12.1	27.3	27.2
2R1	1	2371	30.36	6.16	24.20	2.53	92.1
	2	2377	26.24	2.90	23.34	2.25	96.0
	17	2391	23.68	2.02	21.66	2.11	83.1
	18	2401	29.08	3.83	25.25	3.02	97.8
		M	27.34	3.73	23.61	2.48	92.3
		SE	1.49	0.89	0.76	0.20	3.3
		RSD (0/0)	10.9	47.8	6.4	16.2	7.1

TABLE 2.1

ANALYTICAL PARAMETERS OF CIGARETTES, SWSC-I,
MOIST CONDENSATE, WATER, DRY CONDENSATE, NICOTINE AND CATECHOL CONCENTRATION
IN WSC-I/DMSO APPLICATION SUSPENSION

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

CIGARETTE	DATE OF WSC-I PREPARATION (Oct.85)	BATCH NO. ----- STAT. PARAMETER	CONCENTRATION (g/kg) NICOTINE	CATECHOL
SLOW-72	1	2367	91.24	4.53
	2	2373	98.12	4.01
	17	2387	95.26	4.20
	18	2397	108.17	4.23
		M	98.20	4.24
		SE	3.61	0.11
		RSD (0/0)	7.4	5.1
SLOW-77	1	2369 (a)	109.15	2.52
	2	2375 (a)	123.25	2.57
	17	2389	140.03	1.41
	18	2399	158.45	1.74
		M	132.72	2.06
		SE	10.65	0.29
		RSD (0/0)	16.0	28.0
2R1	1	2371	104.55	3.81
	2	2377	96.40	4.11
	17	2391	97.42	3.84
	18	2401	119.60	3.87
		M	104.49	3.91
		SE	5.35	0.07
		RSD (0/0)	10.2	3.5

TABLE 2.2

ANALYTICAL PARAMETERS OF CIGARETTES, SWSC-I,
NICOTINE AND CATECHOL CONCENTRATION IN DRY CONDENSATE

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

CIGARETTE	DATE OF CONDENSATE PREPARATION (Oct.85)	WSC-I BATCH NO. ----- STAT. PARAMETER	YIELD (mg/cig.) DRY CONDENSATE	WATER	NICOTINE	CATECHOL
SLOW-72	1	2367	24.80	0.45	2.26	0.112
	2	2373	25.40	5.28	2.49	0.102
	17	2387	22.99	4.26	2.19	0.097
	18	2397	22.64	3.74	2.45	0.096
		M	23.96	4.93	2.35	0.102
		SE	0.68	0.60	0.07	0.004
		RSD (0/0)	5.6	24.3	6.2	7.2
SLOW-77	1	2369 (a)	13.42	8.26	1.47	0.034
	2	2375 (a)	12.64	18.07	1.56	0.032
	17	2389	14.81	5.53	2.07	0.021
	18	2399	11.07	10.88	1.75	0.019
		M	12.99	10.69	1.71	0.027
		SE	0.78	2.69	0.13	0.004
		RSD (0/0)	12.0	50.4	15.5	28.1
2R1	1	2371	20.30	5.17	2.12	0.077
	2	2377	19.65	2.44	1.89	0.081
	17	2391	18.48	1.72	1.80	0.071
	18	2401	22.15	3.36	2.65	0.086
		M	20.15	3.17	2.12	0.079
		SE	0.77	0.75	0.19	0.003
		RSD (0/0)	7.6	47.0	18.0	8.0

TABLE 2.3

ANALYTICAL PARAMETERS OF CIGARETTES, SWSC-I,
DRY CONDENSATE, WATER, NICOTINE AND CATECHOL YIELD AND PUFF COUNT

(a) condensate batches prepared from cigarettes showing some wetness on cigarette paper

DATE OF ASSAY (Oct.85)	SWSC-I OF CIGARETTE	BATCH NO.	NUMBER OF BACTERIA (CFU/plate) PLATE 1	2
9	SLOW-72	2366	0	0
	SLOW-77	2369	0	0
	2R1	2370	0	0
24	SLOW-72	2386	0	0
	SLOW-77	2388	0	0
	2R1	2390	0	0

TABLE 3

BACTERIOLOGICAL EXAMINATION OF SWSC-I APPLICATION SUSPENSION

Remarks: 0.15 mg dry condensate/plate

PARAMETER	RESPONSE	
	RECOMMENDED	DETERMINED
histidine requirement		
growth without histidine	0	0
growth with histidine	+	+
crystal violet sensitivity	+	+
ampicillin resistance	+	+
sensitivity to ultraviolet light	+	+

TABLE 4.1

SALMONELLA TYPHIMURIUM STRAIN TA98,
PHENOTYPIC CHARACTERISTICS

Remarks: dates of determinations: 25.Sep.85 and 12.Nov.85

DATE OF ASSAY	DETERMINATION NO.	ABSORBANCE	VIABILITY (CFU/plate)				NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (CFU/plate)	
			PLATE				MEAN (1E6)	RSD (0/0)
(Oct.85)			1	2	3	4		
9	1	2.36	134	129	137	151	137.8	6.8
	2	2.36	72	53	43	119	71.8	47.0
24	1	2.30	120	100	143	152	128.8	18.2
	2	2.46	37	73	37	35	45.5	40.3
9 and 24	1 and 2	-	-	-	-	-	95.9 ± 11.2 (a)	-

TABLE 4.2

SALMONELLA TYPHIMURIUM STRAIN TA98,
ABSORBANCE, VIABILITY AND NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 16

DATE OF ASSAY (Oct.85)	DETERMINATION NO.	SPONTANEOUS REVERTANTS (revertants/plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
9	1	27	26	24	23	25.0	7.3
	2	26	22	19	30	24.3	19.7
24	1	30	24	40	31	31.3	21.1
	2	31	30	28	19	27.0	20.3
9 and 24	1 and 2	-	-	-	-	26.9 ± 1.3 (a)	-

TABLE 4.3

SALMONELLA TYPHIMURIUM STRAIN TA98,
SPONTANEOUS REVERTANTS IN THE ABSENCE OF S9 PROTEIN

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 16

DATE OF ASSAY	DETERMI-NATION NO.	SPONTANEOUS REVERTANTS (revertants/plate)					
		PLATE				MEAN	RSD (0/0)
(Oct.85)		1	2	3	4		
9	1	-	44	44	42	43.3	2.7
	2	51	53	59	32	48.8	23.9
24	1	55	53	58	52	54.5	4.9
	2	54	29	39	46	42.0	25.3
9 and 24	1 and 2	-	-	-	-	47.4 ± 2.3 (a)	-

TABLE 4.4

SALMONELLA TYPHIMURIUM STRAIN TA98,
SPONTANEOUS REVERTANTS IN THE PRESENCE OF S9 PROTEIN

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 15

PARAMETER	DIAGNOSTIC MUTAGEN			
	MMS	DAUNOMYCIN	2-AA PLUS S9 PROTEIN (a)	2-AF PLUS S9 PROTEIN (a)
dose (mg/plate)	0.647	0.006	0.002	0.002
mutagenicity (rev./plate)				
date				
9.Oct.85	3	1178	1489	328
24.Oct.85	4	1185	1374	274
summarized response				
INBIFO	0	+++	+++	++
reference	0, Maron and Ames (1983)	+++, Babudri et al. (1984)	+++, Zeiger et al. (1979) (b)	++, Maron and Ames (1983) (c)

TABLE 4.5

SALMONELLA TYPHIMURIUM STRAIN TA98,
MUTAGENICITY OF DIAGNOSTIC MUTAGENS

Remarks: determinations performed on 2 plates/diagnostic mutagen in the plate incorporation assay, number of colonies counted automatically and corrected for spontaneous revertants

0: .LT.20 revertants/plate
+: 20 to 100 revertants/plate
++: 100 to 400 revertants/plate
+++: .GT.400 revertants/plate
0 or +: variable response, .LT.20 to 100 revertants/plate

(a) 1.7 mg S9 protein/plate
(b) 2.35 mg S9 protein/plate
(c) approx. 2 mg S9 protein/plate

PARAMETER	RESPONSE	
	RECOMMENDED	DETERMINED
histidine requirement		
growth without histidine	0	0
growth with histidine	+	+
crystal violet sensitivity	+	+
ampicillin resistance	+	+
sensitivity to ultraviolet light	+	+

TABLE 5.1

SALMONELLA TYPHIMURIUM STRAIN TA100,
PHENOTYPIC CHARACTERISTICS

Remarks: dates of determinations: 25.Sep.85 and 13.Nov.85

DATE OF ASSAY (Oct.85)	DETERMINATION NO.	ABSORBANCE	VIABILITY (CFU/plate) PLATE 1	2	3	4	NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (CFU/plate) MEAN (1E6)	RSD (0/0)
9	1	2.37	157	160	151	170	159.5	5.0
	2	2.55	153	132	113	143	135.3	12.7
24	1	2.44	144	138	151	113	136.5	12.1
	2	2.52	148	150	137	160	148.8	6.3
9 and 24	1 and 2	-	-	-	-	-	145.0 ± 3.9 (a)	-

TABLE 5.2

SALMONELLA TYPHIMURIUM STRAIN TA100,
ABSORBANCE, VIABILITY AND NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 16

DATE OF ASSAY (Oct.85)	DETERMINATION NO.	SPONTANEOUS REVERTANTS (revertants/plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
9	1	164	167	174	159	166.0	3.8
	2	143	135	148	126	138.0	7.0
24	1	142	155	144	150	147.8	4.0
	2	157	149	159	132	149.3	8.2
9 and 24	1 and 2	-	-	-	-	150.3 ± 3.3 (a)	-

TABLE 5.3

SALMONELLA TYPHIMURIUM STRAIN TA100,
SPONTANEOUS REVERTANTS IN THE ABSENCE OF S9 PROTEIN

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 16

DATE OF ASSAY	DETERMI-NATION NO.	SPONTANEOUS REVERTANTS (revertants/plate)					
		PLATE				MEAN	RSD (0/0)
(Oct.85)		1	2	3	4		
9	1	178	135	169	196	169.5	15.1
	2	142	139	192	153	156.5	15.6
24	1	145	149	111	152	139.3	13.7
	2	121	143	127	122	128.3	7.9
9 and 24	1 and 2	-	-	-	-	148.4 ± 6.2 (a)	-

TABLE 5.4

SALMONELLA TYPHIMURIUM STRAIN TA100,
SPONTANEOUS REVERTANTS IN THE PRESENCE OF S9 PROTEIN

Remarks: determinations performed at the start (1) and at the end (2) of each mutagenicity assay, plates counted manually

(a) mean ± SE, N: 16

PARAMETER	DIAGNOSTIC MUTAGEN			
	MMS	DAUNOMYCIN	2-AA PLUS S9 PROTEIN (a)	2-AF PLUS S9 PROTEIN (a)
dose (mg/plate)	0.647	0.006	0.002	0.002
mutagenicity (rev./plate)				
date				
9.Oct.85	511	77	1980	181
24.Oct.85	570	73	2003	168
summarized response				
INBIFO	+++	+	+++	++
reference	+++, Maron and Ames (1983)	+, Maron and Ames (1983)	-	++, Maron and Ames (1983) (b)

TABLE 5.5

SALMONELLA TYPHIMURIUM STRAIN TA100,
MUTAGENICITY OF DIAGNOSTIC MUTAGENS

Remarks: determinations performed on 2 plates/diagnostic mutagen in the plate incorporation assay, number of colonies counted automatically and corrected for spontaneous revertants

0: .LT.20 revertants/plate
+: 20 to 100 revertants/plate
++: 100 to 400 revertants/plate
+++: .GT.400 revertants/plate
0 or +: variable response, .LT.20 to 100 revertants/plate

(a) 1.7 mg S9 protein/plate
(b) approx. 2 mg S9 protein/plate

PARAMETER	UNIT	DATA
batch no.	-	84-1
induced organ	-	liver
number of rats	-	20
date of Aroclor administration	-	14.Nov.84
date of sacrifice	-	19.Nov.84
body weight	g	201.4 ± 2.9
organ weight	g	12.8 ± 0.3
date of S9 preparation	-	19.Nov.84
protein concentration	g/l	42.4
specific AHM activity	U/mg protein	170.9
bacterial contamination (a)	CFU/ml	0
L-histidine concentration		
free	umol/l	-
bound	umol/l	-

TABLE 6.1

ANALYTICAL DATA OF S9 PROTEIN,
S9 FRACTION BATCH NO. 84-1

(a) results of unfiltered S9 fraction

DATE OF MUTAGENICITY ASSAY (Oct.85)	PROTEIN CONCENTRATION (g/l) UNFILTERED	FILTERED	AMOUNT (mg/plate)	SPECIFIC AHM ACTIVITY (U/mg protein)	BACTERIAL CONTAMINATION (CFU/ml)
9	4.0	3.3	1.7	86.5	0
24	4.0	3.3	1.7	85.2	0

TABLE 6.2

ANALYTICAL DATA OF S9 PROTEIN,
S9 MIXES USED IN THE MUTAGENICITY ASSAY

Remarks: S9 mixes stored at -75 degrees centigrade until determination
date of determination: 6.Nov.85

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2366	0.00	35	45	28	49	39.3	24.3
		0.05	119	120	105	97	110.3	10.1
		0.10	168	191	194	208	190.3	8.7
		0.15	322	303	276	313	303.5	6.6
	2372	0.00	40	46	36	34	39.0	13.6
		0.05	89	97	88	94	92.0	4.6
		0.10	184	210	179	187	190.0	7.2
		0.15	277	337	297	291	300.5	8.6
24-OCT-85	2386	0.00	45	53	46	49	48.3	7.4
		0.05	105	110	107	114	109.0	3.6
		0.10	209	210	195	220	208.5	4.9
		0.15	298	323	312	321	313.5	3.6
	2396	0.00	38	52	50	41	45.3	15.0
		0.05	118	105	121	101	111.3	8.8
		0.10	155	183	226	175	184.8	16.2
		0.15	286	311	295	328	305.0	6.1

TABLE 7.1

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2368	0.00	44	32	39	30	36.3	17.8
		0.05	96	105	101	96	99.5	4.4
		0.10	237	225	207	176	211.3	12.6
		0.15	349	368	327	369	353.3	5.6
	2374	0.00	34	46	39	31	37.5	17.5
		0.05	115	122	104	114	113.8	6.5
		0.10	217	211	197	206	207.8	4.1
		0.15	340	335	336	323	333.5	2.2
24-OCT-85	2388	0.00	50	51	47	41	47.3	9.5
		0.05	122	140	133	150	136.3	8.7
		0.10	230	199	210	219	214.5	6.1
		0.15	359	331	340	363	348.3	4.4
	2398	0.00	39	40	52	36	41.8	16.9
		0.05	107	94	114	117	108.0	9.5
		0.10	199	239	207	248	223.3	10.7
		0.15	377	348	362	347	358.5	3.9

TABLE 7.2

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2370	0.00	-	41	38	31	36.7	14.0
		0.05	85	82	86	120	93.3	19.2
		0.10	170	150	154	162	159.0	5.6
		0.15	245	259	263	220	246.8	7.9
	2376	0.00	50	30	30	52	40.5	30.0
		0.05	90	114	104	102	102.5	9.6
		0.10	194	157	138	-	163.0	17.5
		0.15	234	237	281	231	245.8	9.6
24-OCT-85	2390	0.00	49	49	53	51	50.5	3.8
		0.05	109	100	104	118	107.8	7.2
		0.10	162	195	192	169	179.5	9.2
		0.15	155	189	240	277	215.3	25.1
	2400	0.00	50	41	52	56	49.8	12.8
		0.05	123	110	94	111	109.5	10.9
		0.10	184	166	178	159	171.8	6.6
		0.15	260	273	251	317	275.3	10.6

TABLE 7.3

MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2366	0.00	124	134	95	116	117.3	14.1
		0.05	167	169	189	183	177.0	6.0
		0.10	207	195	223	205	207.5	5.6
		0.15	248	267	258	250	255.8	3.4
	2372	0.00	114	121	127	117	119.8	4.7
		0.05	159	181	156	162	164.5	6.9
		0.10	181	213	202	191	196.8	7.0
		0.15	246	209	235	268	239.5	10.2
24-OCT-85	2386	0.00	143	122	150	111	131.5	13.8
		0.05	188	205	196	187	194.0	4.3
		0.10	222	250	216	235	230.8	6.5
		0.15	312	342	323	340	329.3	4.3
	2396	0.00	110	144	116	133	125.8	12.4
		0.05	207	203	173	154	184.3	13.7
		0.10	233	224	241	241	234.8	3.4
		0.15	315	339	312	339	326.3	4.5

TABLE 8.1

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2368	0.00	107	130	120	106	115.8	9.9
		0.05	190	189	175	179	183.3	4.0
		0.10	247	189	219	165	205.0	17.4
		0.15	289	312	279	279	289.8	5.4
	2374	0.00	119	123	114	117	118.3	3.2
		0.05	186	177	175	128	166.5	15.7
		0.10	246	227	253	242	242.0	4.5
		0.15	294	306	313	290	300.8	3.5
24-OCT-85	2388	0.00	142	125	113	119	124.8	10.0
		0.05	174	192	195	169	182.5	7.1
		0.10	225	276	254	194	237.3	15.0
		0.15	301	330	327	326	321.0	4.2
	2398	0.00	106	129	119	120	118.5	8.0
		0.05	173	175	208	185	185.3	8.7
		0.10	230	224	247	253	238.5	5.8
		0.15	343	327	318	334	330.5	3.2

TABLE 8.2

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2370	0.00	128	121	114	111	118.5	6.4
		0.05	174	170	189	186	179.8	5.1
		0.10	202	170	185	154	177.8	11.6
		0.15	238	255	240	253	246.5	3.5
	2376	0.00	115	127	120	106	117.0	7.5
		0.05	152	143	197	174	166.5	14.5
		0.10	197	241	197	220	213.8	9.9
		0.15	280	258	236	238	253.0	8.1
24-OCT-85	2390	0.00	121	135	126	139	130.3	6.3
		0.05	169	190	168	179	176.5	5.8
		0.10	232	226	248	224	232.5	4.7
		0.15	297	272	302	278	287.3	5.0
	2400	0.00	139	138	121	127	131.3	6.7
		0.05	191	191	197	207	196.5	3.8
		0.10	242	238	230	277	246.8	8.4
		0.15	300	313	347	321	320.3	6.2

TABLE 8.3

MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2367	0.00	46	40	26	30	35.5	25.8
		0.05	95	99	93	96	95.8	2.6
		0.10	191	181	172	165	177.3	6.4
		0.15	268	212	279	260	254.8	11.6
	2373	0.00	44	43	32	43	40.5	14.0
		0.05	84	84	70	96	83.5	12.7
		0.10	155	157	151	154	154.3	1.6
		0.15	221	242	201	226	222.5	7.6
24-OCT-85	2387	0.00	35	49	48	47	44.8	14.6
		0.05	99	99	109	120	106.8	9.4
		0.10	159	182	156	167	166.0	7.0
		0.15	252	264	263	259	259.5	2.1
	2397	0.00	46	50	49	44	47.3	5.8
		0.05	106	113	105	91	103.8	8.9
		0.10	158	190	180	181	177.3	7.7
		0.15	219	253	261	257	247.5	7.8

TABLE 9.1

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2369	0.00	31	45	41	37	38.5	15.5
		0.05	115	110	76	113	103.5	17.8
		0.10	231	222	204	204	215.3	6.3
		0.15	330	328	327	375	340.0	6.9
	2375	0.00	40	45	47	28	40.0	21.3
		0.05	101	141	127	113	120.5	14.4
		0.10	210	222	241	278	237.8	12.5
		0.15	319	336	389	334	344.5	8.9
24-OCT-85	2389	0.00	42	41	37	42	40.5	5.9
		0.05	128	120	123	112	120.8	5.6
		0.10	221	215	208	198	210.5	4.7
		0.15	308	326	351	368	338.3	7.8
	2399	0.00	50	29	36	40	38.8	22.6
		0.05	117	135	126	161	134.8	14.1
		0.10	274	261	247	259	260.3	4.2
		0.15	423	431	415	429	424.5	1.7

TABLE 9.2

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2371	0.00	35	41	41	37	38.5	7.8
		0.05	86	89	79	89	85.8	5.5
		0.10	136	171	145	175	156.8	12.2
		0.15	209	196	227	233	216.3	7.8
	2377	0.00	48	40	39	42	42.3	9.5
		0.05	82	104	70	95	87.8	17.0
		0.10	145	147	167	179	159.5	10.3
		0.15	246	242	297	228	253.3	11.9
24-OCT-85	2391	0.00	42	53	57	51	50.8	12.5
		0.05	96	104	79	95	93.5	11.2
		0.10	194	186	152	182	178.5	10.3
		0.15	222	250	241	246	239.8	5.2
	2401	0.00	49	32	50	46	44.3	18.9
		0.05	91	104	85	88	92.0	9.1
		0.10	156	190	175	216	184.3	13.7
		0.15	270	258	243	252	255.8	4.4

TABLE 9.3

MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2367	0.00	171	136	138	142	146.8	11.1
		0.05	230	247	229	239	236.3	3.6
		0.10	285	310	262	309	291.5	7.8
		0.15	334	374	370	332	352.5	6.4
	2373	0.00	132	150	138	178	149.5	13.7
		0.05	215	209	225	212	215.3	3.2
		0.10	281	-	275	272	276.0	1.7
		0.15	341	345	303	327	329.0	5.8
24-OCT-85	2387	0.00	148	108	110	132	124.5	15.3
		0.05	210	232	202	192	209.0	8.1
		0.10	245	260	249	260	253.5	3.0
		0.15	349	351	319	296	328.8	8.0
	2397	0.00	147	126	128	129	132.5	7.4
		0.05	186	176	187	209	189.5	7.3
		0.10	267	269	306	278	280.0	6.4
		0.15	294	312	358	372	334.0	11.1

TABLE 10.1

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2369	0.00	137	150	168	139	148.5	9.6
		0.05	219	220	223	226	222.0	1.4
		0.10	319	358	304	301	320.5	8.2
		0.15	438	432	425	444	434.8	1.9
	2375	0.00	149	193	110	117	142.3	26.6
		0.05	283	241	232	215	242.8	11.9
		0.10	321	367	285	274	311.8	13.5
		0.15	480	473	449	491	473.3	3.8
24-OCT-85	2389	0.00	137	129	127	141	133.5	4.9
		0.05	235	182	204	217	209.5	10.6
		0.10	286	298	285	286	288.8	2.1
		0.15	457	441	361	424	420.8	10.0
	2399	0.00	123	121	122	100	116.5	9.5
		0.05	232	239	229	218	229.5	3.8
		0.10	321	357	352	331	340.3	5.0
		0.15	419	512	439	450	455.0	8.8

TABLE 10.2

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2371	0.00	123	158	141	166	147.0	13.0
		0.05	210	229	217	227	220.8	4.0
		0.10	303	264	250	278	273.8	8.3
		0.15	348	372	332	336	347.0	5.2
	2377	0.00	102	112	127	138	119.8	13.3
		0.05	206	188	229	236	214.8	10.2
		0.10	228	266	304	299	274.3	12.8
		0.15	401	398	368	358	381.3	5.6
24-OCT-85	2391	0.00	120	130	100	131	120.3	12.0
		0.05	197	198	209	195	199.8	3.1
		0.10	234	247	291	231	250.8	11.1
		0.15	301	284	320	322	306.8	5.8
	2401	0.00	141	113	116	129	124.8	10.3
		0.05	213	157	203	210	195.8	13.4
		0.10	274	261	275	276	271.5	2.6
		0.15	352	310	354	367	345.8	7.2

TABLE 10.3

MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

TYPE OF WSC-I	CIGARETTE	NUMBER OF COLONIES/ MUTAGENICITY PLATE		NUMBER OF HISTIDINE PROTOTROPHS/ 20 COLONIES ASSAYED	
		SUBSTUDY 1	2	SUBSTUDY 1	2
mainstream	SLOW-72	303	323	20	20
	SLOW-77	368	331	20	20
	2R1	259	189	20	20
sidestream	SLOW-72	-	263	-	20
	SLOW-77	-	326	-	20
	2R1	-	250	-	20

TABLE 11.1

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF STRAIN TA98

Remarks: WSC-I dose: 0.15 ug/plate
dates of determinations: 14.Oct.85 and 29.Oct.85

TYPE OF WSC-I	CIGARETTE	NUMBER OF COLONIES/ MUTAGENICITY PLATE		NUMBER OF HISTIDINE PROTOTROPHS/ 20 COLONIES ASSAYED	
		SUBSTUDY 1	2	SUBSTUDY 1	2
mainstream	SLOW-72	267	342	20	20
	SLOW-77	312	330	20	20
	2R1	255	272	20	20
sidestream	SLOW-72	-	351	-	20
	SLOW-77	-	441	-	20
	2R1	-	284	-	20

TABLE 11.2

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF STRAIN TA100

Remarks: WSC-I dose: 0.15 ug/plate
dates of determinations: 14.Oct.85 and 29.Oct.85

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	39.1	18.2	2.5		
	0.05	8	101.1	12.4	4.4		
	0.10	8	190.1	7.4	5.0		
	0.15	8	302.0	7.1	7.6	1755	0.983
24-OCT-85	0.00	8	46.8	11.3	1.9		
	0.05	8	110.1	6.3	2.5		
	0.10	8	196.6	12.4	8.6		
	0.15	8	309.3	4.8	5.3	1748	0.982
09-OCT-85 24-OCT-85	0.00	16	42.9	16.8	1.8		
	0.05	16	105.6	10.3	2.7		
	0.10	16	193.4	10.1	4.9		
	0.15	16	305.6	6.0	4.5	1752	0.982

TABLE 12.1

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.00. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	36.9	16.4	2.1		
	0.05	8	106.6	8.9	3.3		
	0.10	8	209.5	8.7	6.5		
	0.15	8	343.4	5.1	6.1	2045	0.984
24-OCT-85	0.00	8	44.5	14.0	2.2		
	0.05	8	122.1	14.9	6.4		
	0.10	8	218.9	8.4	6.5		
	0.15	8	353.4	4.2	5.2	2047	0.985
09-OCT-85	0.00	16	40.7	17.5	1.8		
24-OCT-85	0.05	16	114.4	14.1	4.0		
	0.10	16	214.2	8.6	4.6		
	0.15	16	348.4	4.7	4.1	2046	0.983

TABLE 12.2

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.00. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	7	38.9	24.0	3.5		
	0.05	8	97.9	14.6	5.0		
	0.10	7	160.7	11.0	6.7		
	0.15	8	246.3	8.1	7.1	1377	0.978
24-OCT-85	0.00	8	50.1	8.7	1.5		
	0.05	8	108.6	8.6	3.3		
	0.10	8	175.6	7.8	4.9		
	0.15	8	245.3	21.0	18.2	1305	0.944
09-OCT-85	0.00	15	44.9	20.0	2.3		
24-OCT-85	0.05	16	103.3	12.5	3.2		
	0.10	15	168.7	10.1	4.4		
	0.15	16	245.8	15.3	9.4	1339	0.959

TABLE 12.3

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.05. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	118.5	9.7	4.1		
	0.05	8	170.8	7.1	4.3		
	0.10	8	202.1	6.5	4.6		
	0.15	8	247.6	7.7	6.8	837	0.958
24-OCT-85	0.00	8	128.6	12.4	5.6		
	0.05	8	189.1	9.6	6.4		
	0.10	8	232.8	4.9	4.0		
	0.15	8	327.8	4.1	4.8	1282	0.969
09-OCT-85 24-OCT-85	0.00	16	123.6	11.7	3.6		
	0.05	16	179.9	9.8	4.4		
	0.10	16	217.4	9.1	4.9		
	0.15	16	287.7	15.4	11.1	1060	0.911

TABLE 13.1

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.42. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	117.0	6.8	2.8		
	0.05	8	174.9	11.4	7.0		
	0.10	8	223.5	14.1	11.1		
	0.15	8	295.3	4.6	4.8	1167	0.958
24-OCT-85	0.00	8	121.6	8.9	3.8		
	0.05	8	183.9	7.4	4.8		
	0.10	8	237.9	10.5	8.8		
	0.15	8	325.8	3.8	4.3	1333	0.974
09-OCT-85 24-OCT-85	0.00	16	119.3	7.9	2.4		
	0.05	16	179.4	9.5	4.3		
	0.10	16	230.7	12.3	7.1		
	0.15	16	310.5	6.5	5.0	1250	0.960

TABLE 13.2

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.13. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	117.8	6.5	2.7		
	0.05	8	173.1	10.6	6.5		
	0.10	8	195.8	13.9	9.6		
	0.15	8	249.8	6.0	5.3	837	0.929
24-OCT-85	0.00	8	130.8	6.0	2.8		
	0.05	8	186.5	7.3	4.8		
	0.10	8	239.6	7.2	6.1		
	0.15	8	303.8	7.9	8.4	1144	0.971
09-OCT-85	0.00	16	124.3	8.1	2.5		
24-OCT-85	0.05	16	179.8	9.5	4.3		
	0.10	16	217.7	14.5	7.9		
	0.15	16	276.8	12.2	8.5	991	0.913

TABLE 13.3

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.31. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	38.0	19.8	2.7		
	0.05	8	89.6	10.8	3.4		
	0.10	8	165.8	8.7	5.1		
	0.15	8	238.6	11.8	10.0	1356	0.976
24-OCT-85	0.00	8	46.0	10.5	1.7		
	0.05	8	105.3	8.6	3.2		
	0.10	8	171.6	7.7	4.7		
	0.15	8	253.5	5.8	5.2	1378	0.988
09-OCT-85	0.00	16	42.0	17.6	1.8		
24-OCT-85	0.05	16	97.4	12.5	3.0		
	0.10	16	168.7	8.1	3.4		
	0.15	16	246.1	9.3	5.7	1367	0.980

TABLE 14.1

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.02. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	39.3	17.5	2.4		
	0.05	8	112.0	16.9	6.7		
	0.10	8	226.5	10.8	8.7		
	0.15	8	342.3	7.4	9.0	2047	0.982
24-OCT-85	0.00	8	39.6	15.2	2.1		
	0.05	8	127.8	11.9	5.4		
	0.10	8	235.4	12.0	10.0		
	0.15	8	381.4	13.0	17.5	2266	0.971
09-OCT-85	0.00	16	39.4	15.8	1.6		
24-OCT-85	0.05	16	119.9	15.4	4.6		
	0.10	16	230.9	11.3	6.5		
	0.15	16	361.8	11.9	10.8	2156	0.972

TABLE 14.2

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.10. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	40.4	9.5	1.4		
	0.05	8	86.8	11.8	3.6		
	0.10	8	158.1	10.5	5.9		
	0.15	8	234.8	12.8	10.6	1309	0.969
24-OCT-85	0.00	8	47.5	16.2	2.7		
	0.05	8	92.8	9.5	3.1		
	0.10	8	181.4	11.4	7.3		
	0.15	8	247.8	5.6	4.9	1379	0.980
09-OCT-85	0.00	16	43.9	15.8	1.7		
24-OCT-85	0.05	16	89.8	10.9	2.4		
	0.10	16	169.8	12.8	5.4		
	0.15	16	241.3	9.8	5.9	1344	0.971

TABLE 14.3

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.05. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	148.1	11.6	6.1		
	0.05	8	225.8	5.9	4.7		
	0.10	7	284.9	6.4	6.9		
	0.15	8	340.8	6.8	8.1	1273	0.970
24-OCT-85	0.00	8	128.5	11.4	5.2		
	0.05	8	199.3	8.9	6.3		
	0.10	8	266.8	7.2	6.8		
	0.15	8	331.4	9.0	10.5	1352	0.967
09-OCT-85 24-OCT-85	0.00	16	138.3	13.3	4.6		
	0.05	16	212.5	9.6	5.1		
	0.10	15	275.2	7.4	5.3		
	0.15	16	336.1	7.8	6.6	1312	0.961

TABLE 15.1

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.06. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	145.4	18.4	9.4		
	0.05	8	232.4	9.5	7.8		
	0.10	8	316.1	10.3	11.6		
	0.15	8	454.0	5.3	8.6	2019	0.969
24-OCT-85	0.00	8	125.0	9.9	4.4		
	0.05	8	219.5	8.6	6.7		
	0.10	8	314.5	9.5	10.6		
	0.15	8	437.9	9.6	14.9	2067	0.973
09-OCT-85	0.00	16	135.2	16.8	5.7		
24-OCT-85	0.05	16	225.9	9.3	5.2		
	0.10	16	315.3	9.6	7.6		
	0.15	16	445.9	7.7	8.6	2043	0.969

TABLE 15.2

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.02. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	133.4	16.4	7.7		
	0.05	8	217.8	7.3	5.6		
	0.10	8	274.0	10.0	9.7		
	0.15	8	364.1	7.1	9.2	1497	0.965
24-OCT-85	0.00	8	122.5	10.5	4.5		
	0.05	8	197.8	9.0	6.3		
	0.10	8	261.1	8.3	7.7		
	0.15	8	326.3	8.9	10.2	1349	0.967
09-OCT-85	0.00	16	127.9	14.2	4.6		
24-OCT-85	0.05	16	207.8	9.3	4.8		
	0.10	16	267.6	9.3	6.2		
	0.15	16	345.2	9.5	8.2	1423	0.957

TABLE 15.3

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.10. Deviations .GT.0.25 are considered statistically significant.

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1752
SLOW-77	2046

TABLE 16.1

SPECIFIC MUTAGENICITY OF MWSC-I, STRAIN TA98

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	(1060)
SLOW-77	1250

TABLE 16.2

SPECIFIC MUTAGENICITY OF MWSC-I, STRAIN TA100

Remarks: Mutagenicity data in brackets are those, which showed a statistically significant difference between both substudies.

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1367
SLOW-77	2156

TABLE 17.1

SPECIFIC MUTAGENICITY OF SWSC-I, STRAIN TA98

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1312
SLOW-77	2043

TABLE 17.2

SPECIFIC MUTAGENICITY OF SWSC-I, STRAIN TA100

MUTAGENIC EFFECT	CONDENSATE	RELATIVE DIFFERENCE (a)	STATISTICAL SIGNIFICANCE (b)
frameshift mutation	MWSC-I	-0.15	0
	SWSC-I	-0.45	2
base-pair substitution	MWSC-I	(-0.16)	(0)
	SWSC-I	-0.44	2

TABLE 18

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEAN SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTES SLOW-72 AND SLOW-77

Remarks: 0: no statistically significant difference
1: mutagenicity of cigarette SLOW-72 higher than the mutagenicity of cigarette SLOW-77
2: mutagenicity of cigarette SLOW-72 lower than the mutagenicity of cigarette SLOW-77
The mutagenicity of MWSC-I of cigarette SLOW-72 with respect to base-pair substitution showed a difference between both substudies greater than the limit of 0.25. Therefore the comparison with cigarette SLOW-77 has to be taken with reservation.

(a) difference between the data obtained from 2 test cigarettes divided by the mean of them
(b) relative difference .GT.0.16

CIGARETTE	DRY CONDENSATE YIELD	MUTAGENICITY	
		SPECIFIC	TOTAL
	(mg/cig.)	(rev./mg)	(rev./cig.)
SLOW-72	16.01	1752	28050
SLOW-77	13.47	2046	27560

TABLE 19.1

TOTAL MUTAGENICITY OF MWSC-I, STRAIN TA98

Remarks: dry condensate yield see TABLE 1.3,
specific mutagenicity see TABLE 16.1

CIGARETTE	DRY CONDENSATE YIELD	MUTAGENICITY	
		SPECIFIC	TOTAL
	(mg/cig.)	(rev./mg)	(rev./cig.)
SLOW-72	16.01	(1060)	(16971)
SLOW-77	13.47	1250	16838

TABLE 19.2

TOTAL MUTAGENICITY OF MWSC-I, STRAIN TA100

Remarks: Mutagenicity data in brackets are those, which showed a statistically significant difference between both substudies.
dry condensate yield see TABLE 1.3,
specific mutagenicity see TABLE 16.2

CIGARETTE	DRY CONDENSATE YIELD	MUTAGENICITY	
		SPECIFIC	TOTAL
	(mg/cig.)	(rev./mg)	(rev./cig.)
SLOW-72	23.96	1367	32753
SLOW-77	12.99	2156	28006

TABLE 20.1

TOTAL MUTAGENICITY OF SWSC-I, STRAIN TA98

Remarks: dry condensate yield see TABLE 2.3,
specific mutagenicity see TABLE 17.1

CIGARETTE	DRY CONDENSATE YIELD	MUTAGENICITY	
		SPECIFIC	TOTAL
	(mg/cig.)	(rev./mg)	(rev./cig.)
SLOW-72	23.96	1312	31436
SLOW-77	12.99	2043	26539

TABLE 20.2

TOTAL MUTAGENICITY OF SWSC-I, STRAIN TA100

Remarks: dry condensate yield see TABLE 2.3,
specific mutagenicity see TABLE 17.2

MUTAGENIC EFFECT	CONDENSATE	RELATIVE DIFFERENCE (a)	STATISTICAL SIGNIFICANCE (b)
frameshift mutation	MWSC-I	0.02	0
	SWSC-I	0.16	0
base-pair substitution	MWSC-I	(0.01)	(0)
	SWSC-I	0.17	1

TABLE 21

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE TOTAL MUTAGENICITY OF WSC-I OF CIGARETTES SLOW-72 AND SLOW-77

Remarks: 0: no statistically significant difference
1: mutagenicity of cigarette SLOW-72 higher than the mutagenicity of cigarette SLOW-77
2: mutagenicity of cigarette SLOW-72 lower than the mutagenicity of cigarette SLOW-77
The mutagenicity of MWSC-I of cigarette SLOW-72 with respect to base-pair substitution showed a difference between both substudies greater than the limit of 0.25. Therefore the comparison with cigarette SLOW-77 has to be taken with reservation.

(a) difference between the data obtained from 2 test cigarettes divided by the mean of them
(b) relative difference .GT.0.16

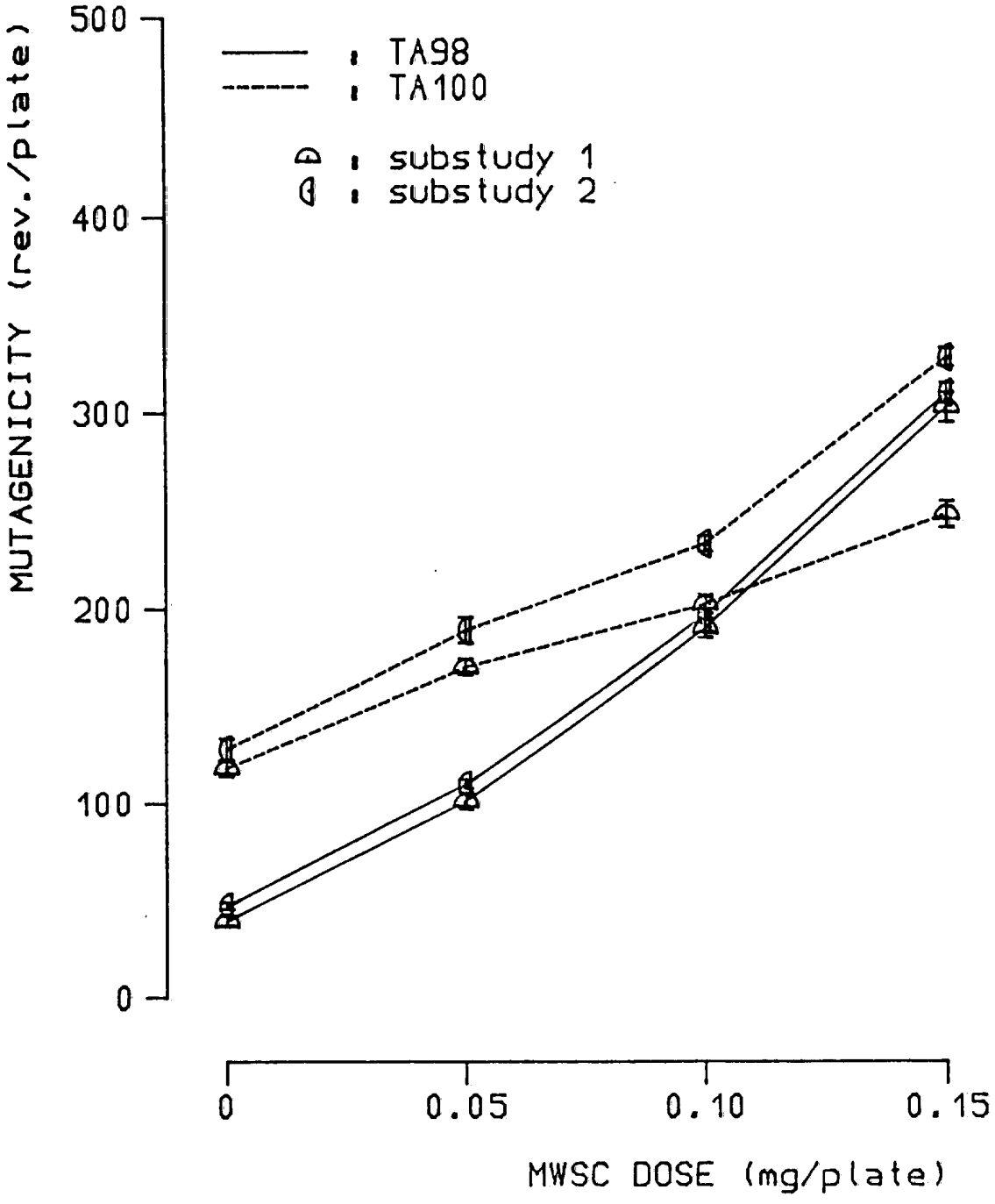


FIGURE 1.1

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72,
STRAINS TA98 AND TA100
(see TABLES 12.1 and 13.1)

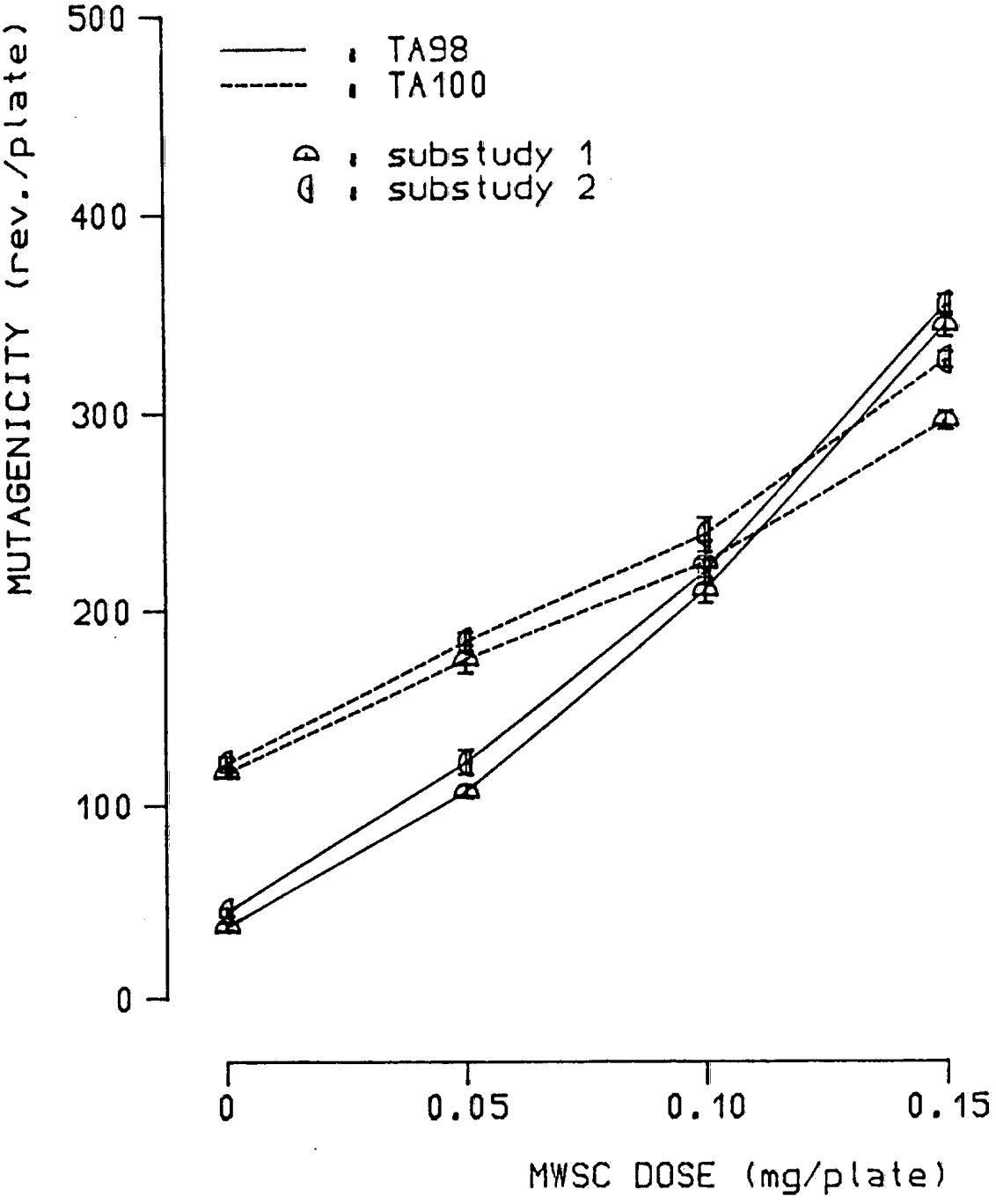


FIGURE 1.2

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77,
STRAINS TA98 AND TA100
(see TABLES 12.2 and 13.2)

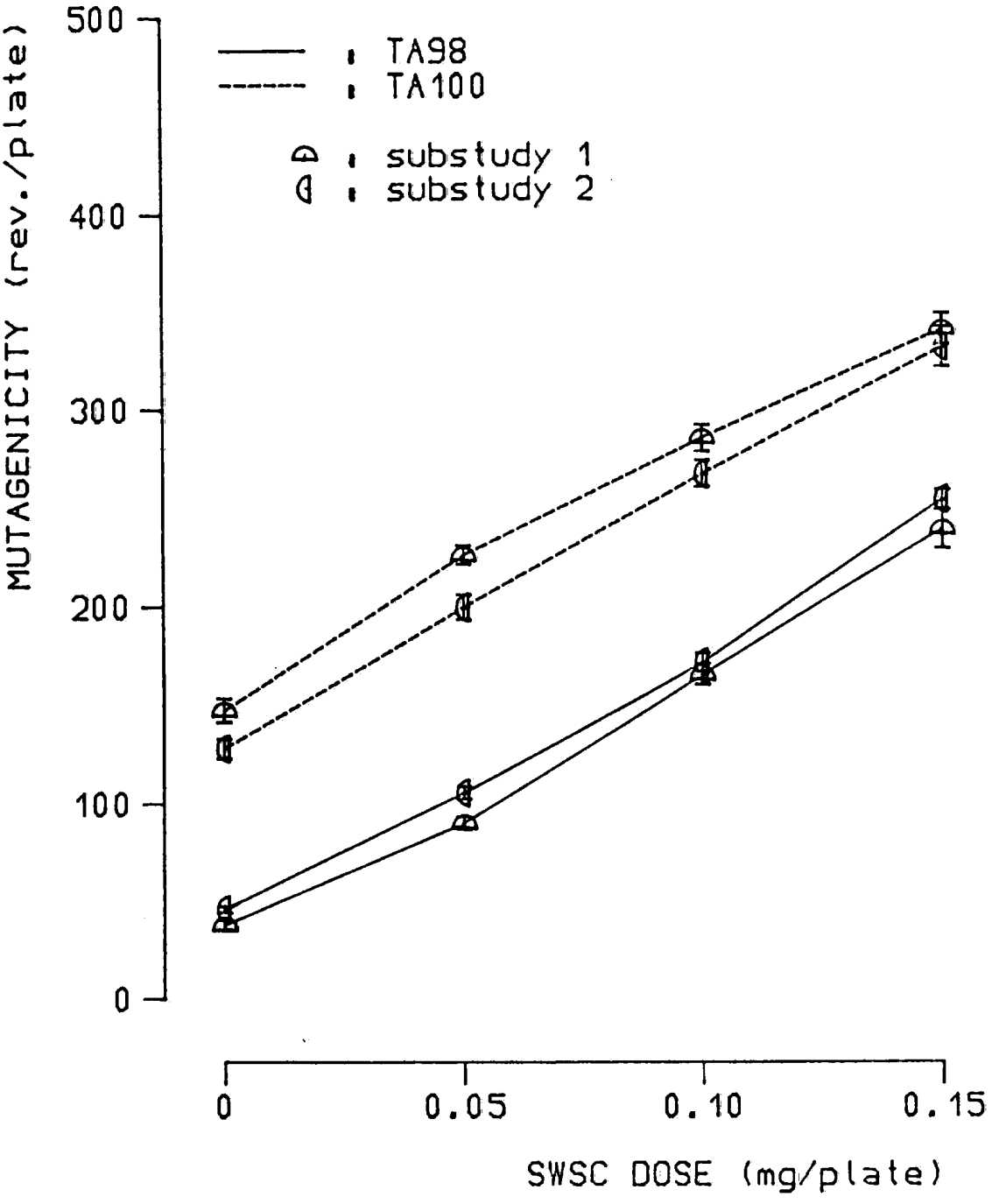


FIGURE 2.1

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72,
STRAINS TA98 AND TA100
(see TABLES 14.1 and 15.1)

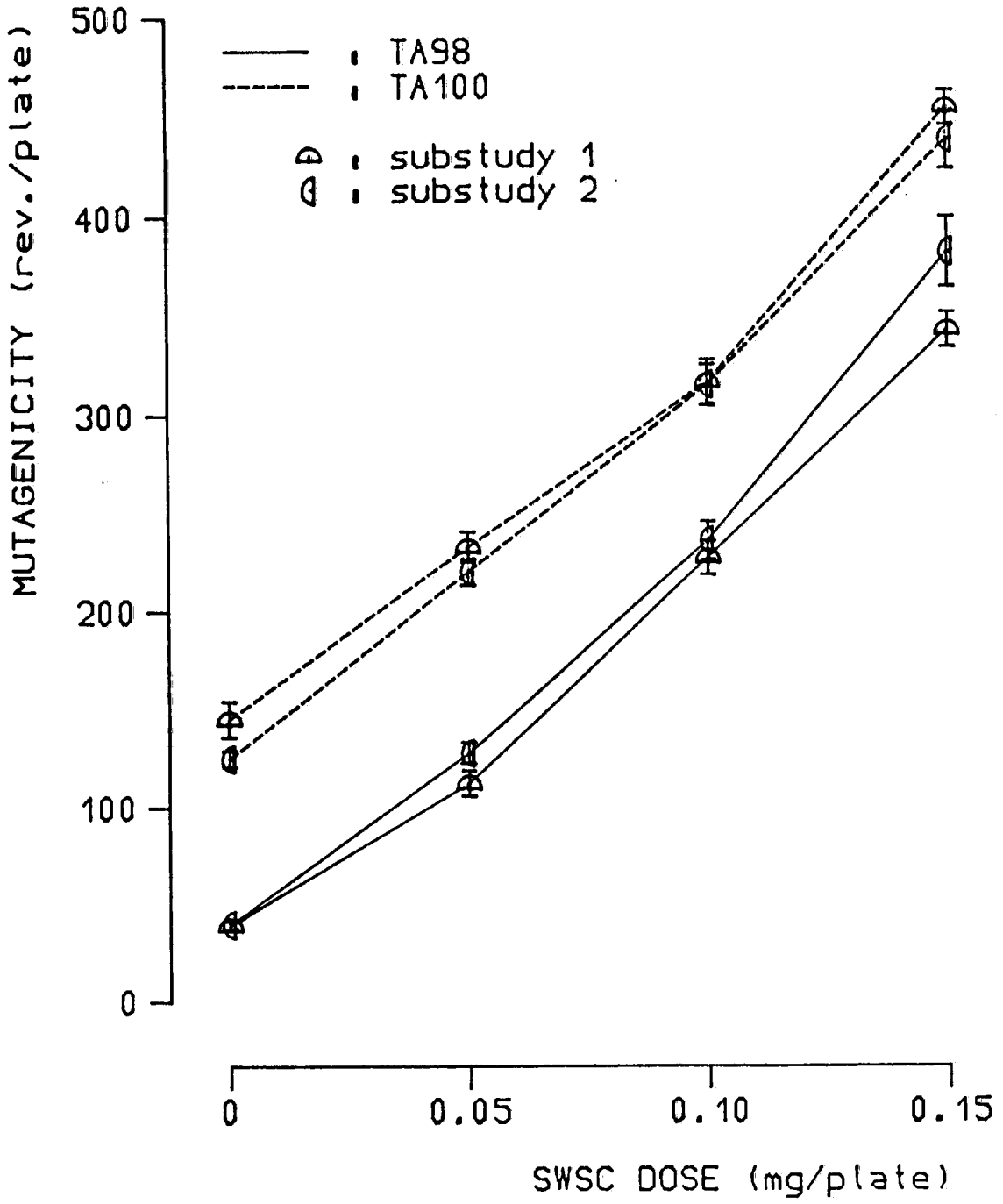


FIGURE 2.2

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77,
STRAINS TA98 AND TA100
(see TABLES 14.2 and 15.2)

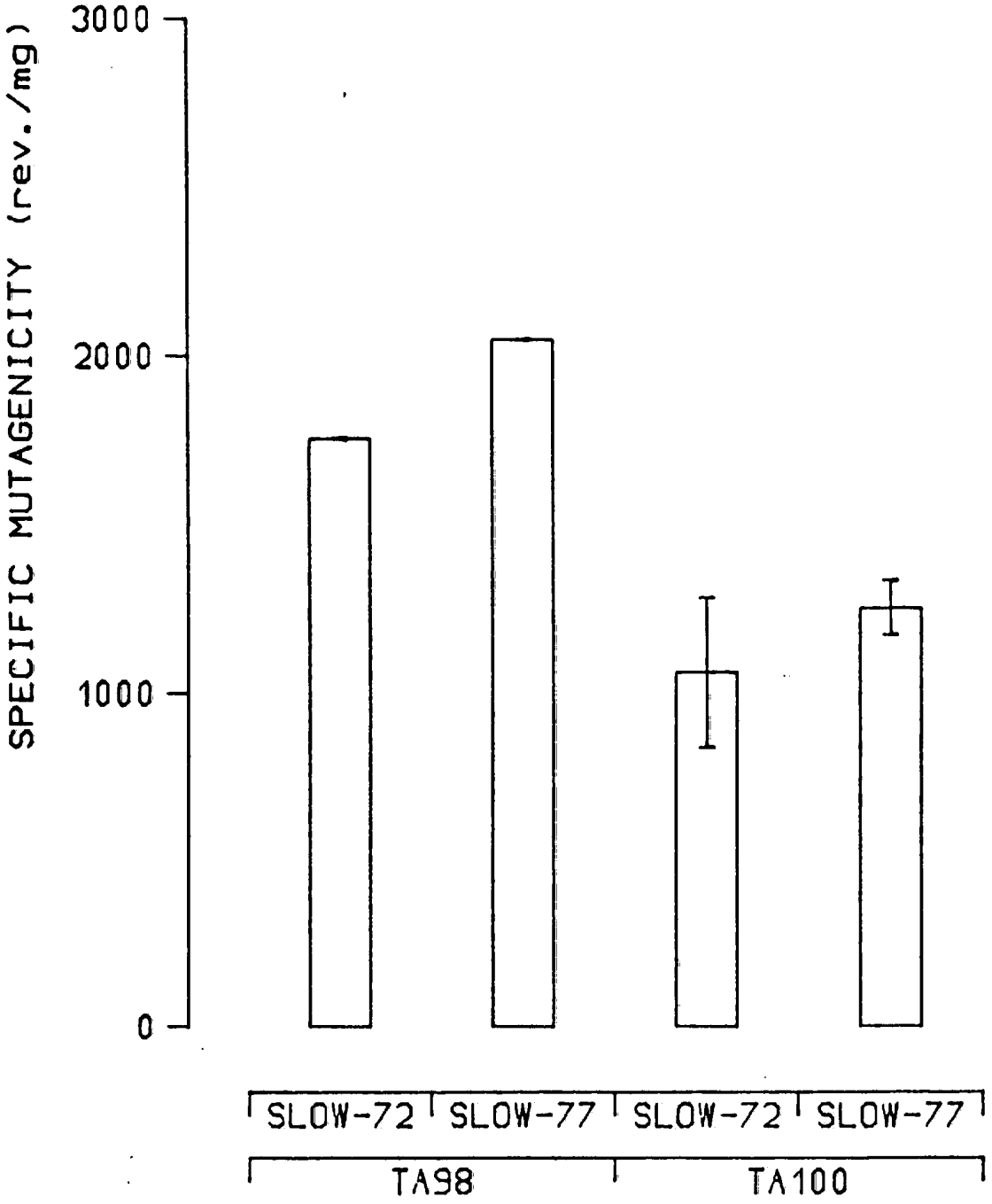


FIGURE 3

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTES
(see TABLES 12 and 13)

Remarks: means and single values of both substudies

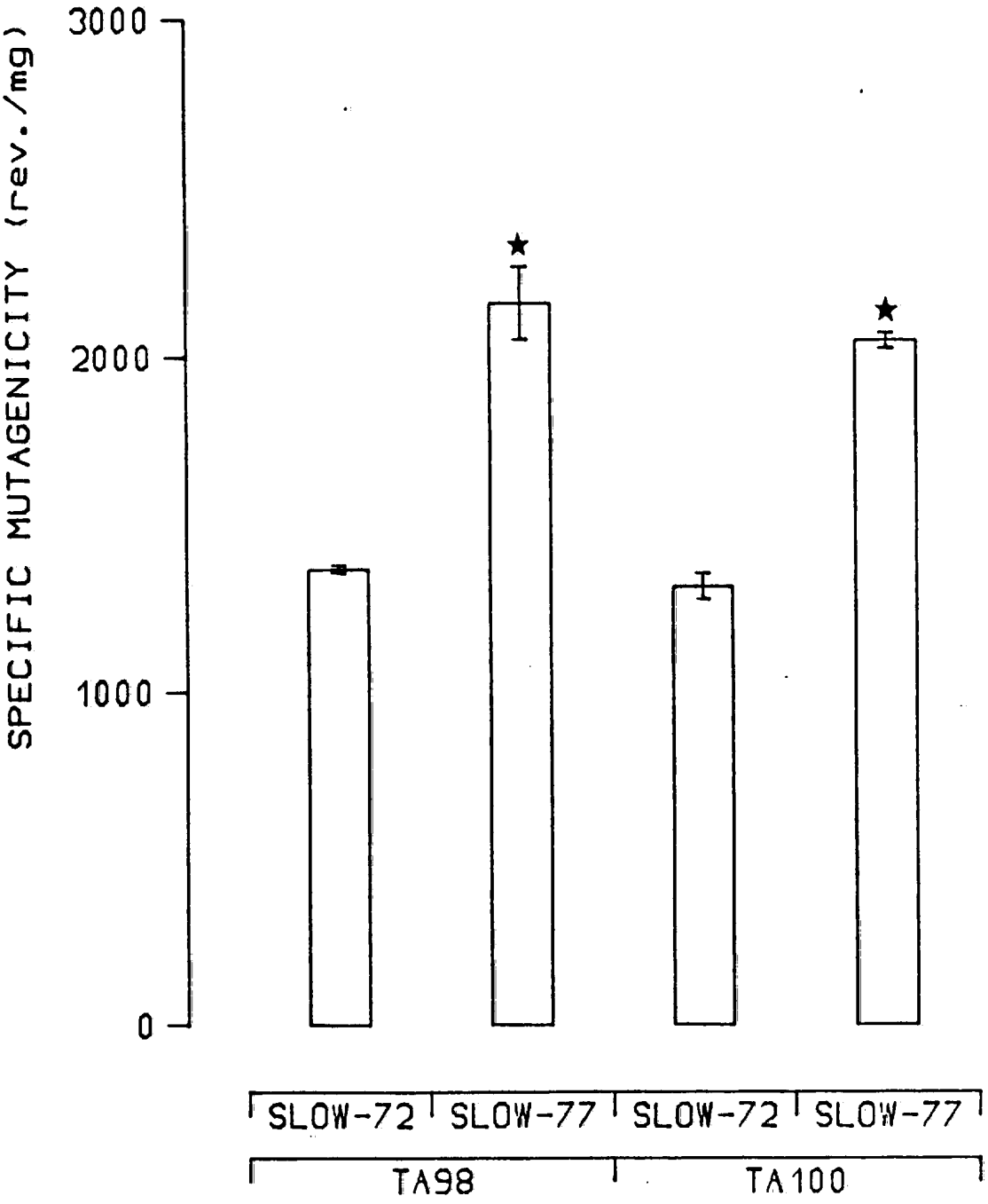


FIGURE 4

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTES
(see TABLES 14 and 15)

Remarks: means and single values of both substudies
★: indicating statistically significant difference between SLOW-72 and -77

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END OF REPORT

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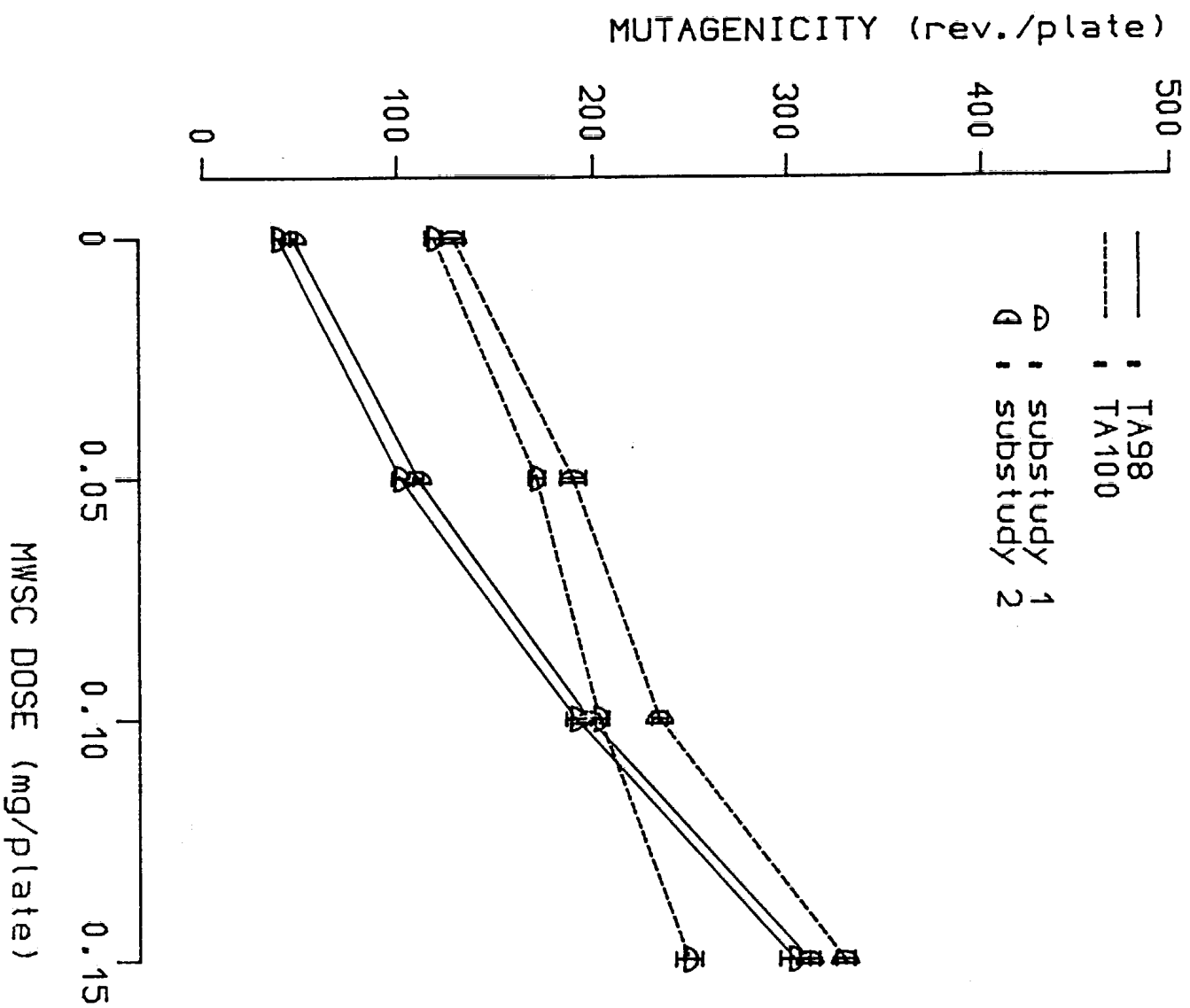
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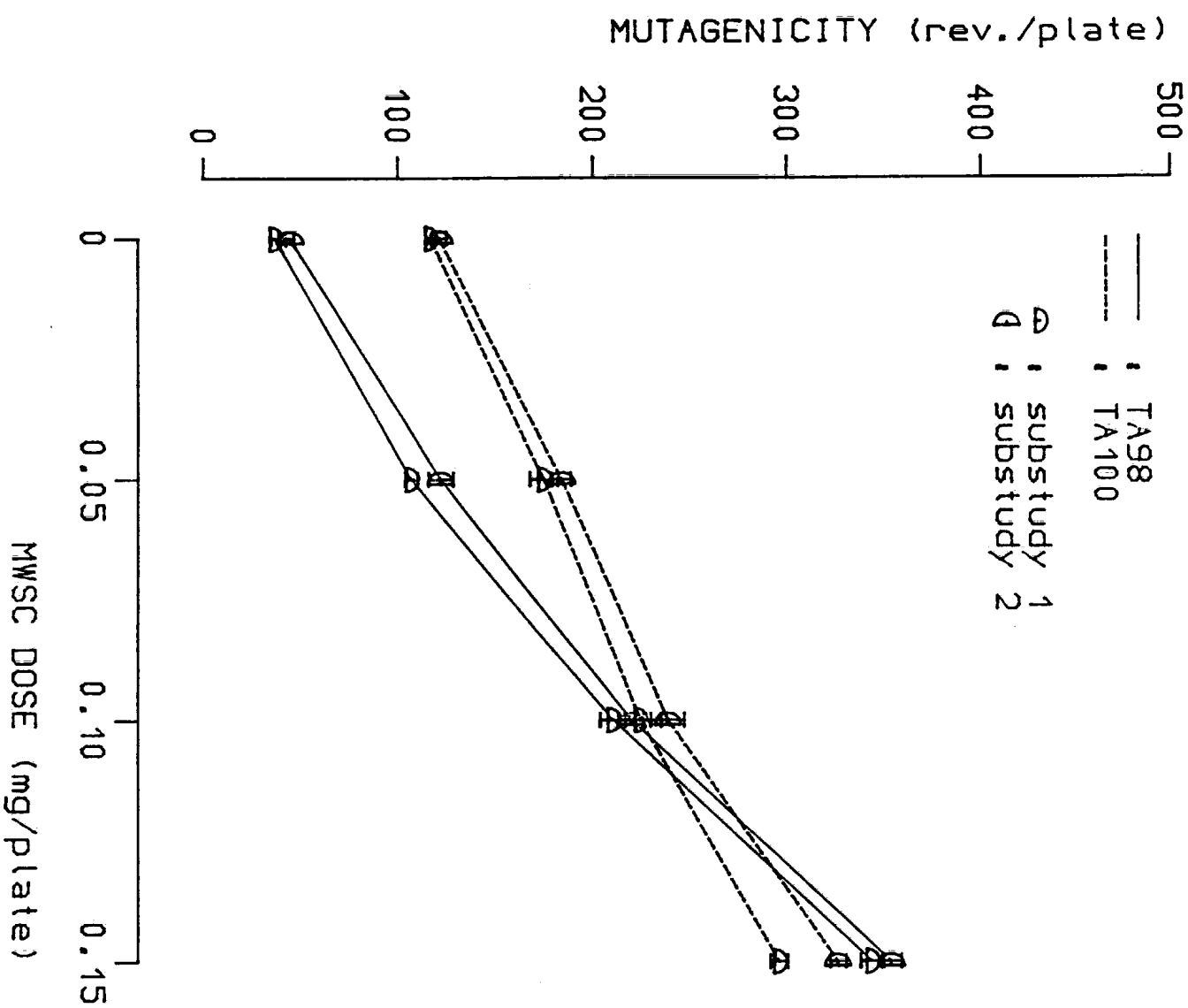
100
200
300
400
500
MWSC DOSE (mg/plate)
0
0.05
0.10
0.15
TA98
TA100
substudy 1
substudy 2

P 0268/2132, H04775, V69 F17 V68 F17, SLOW-77



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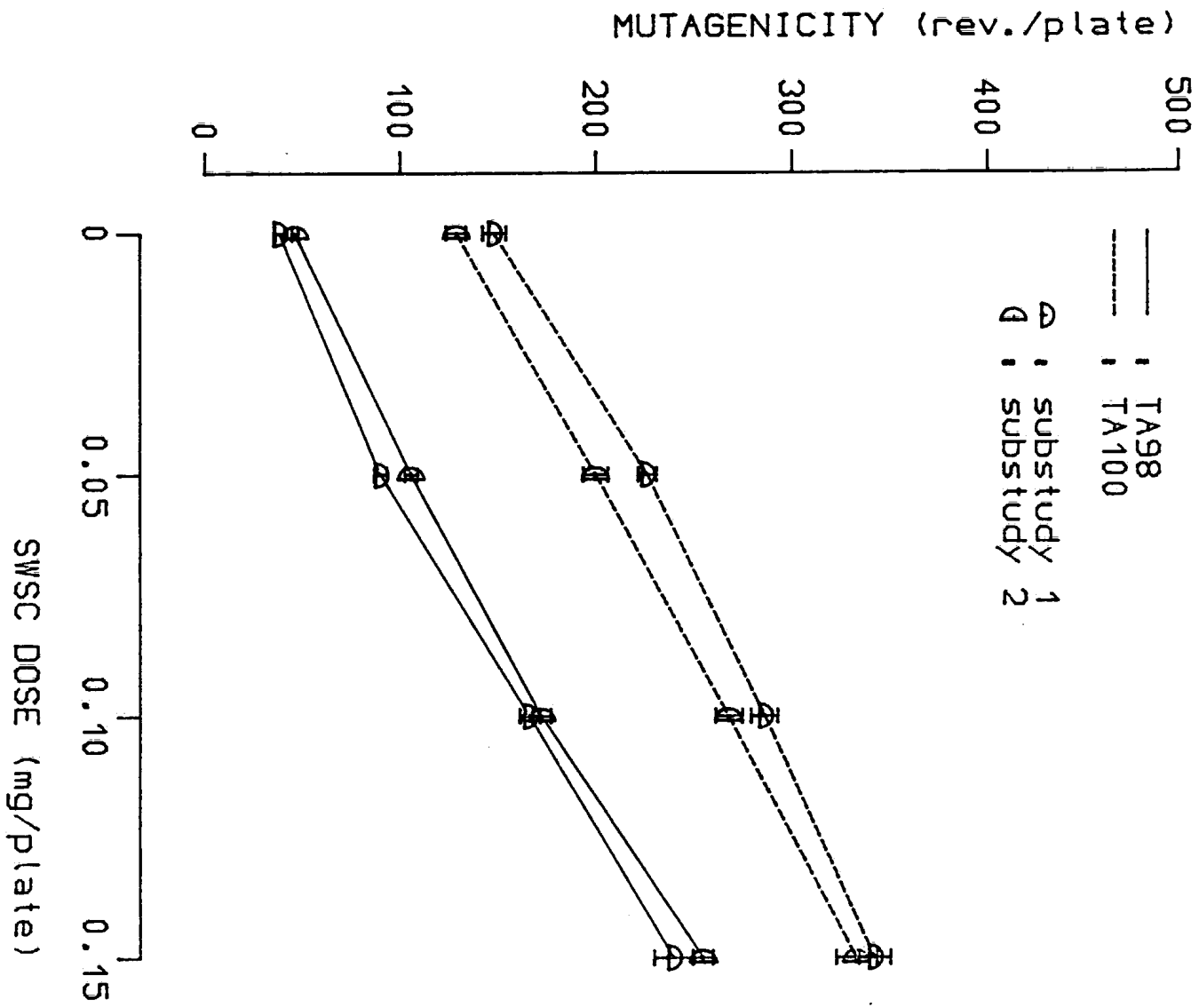

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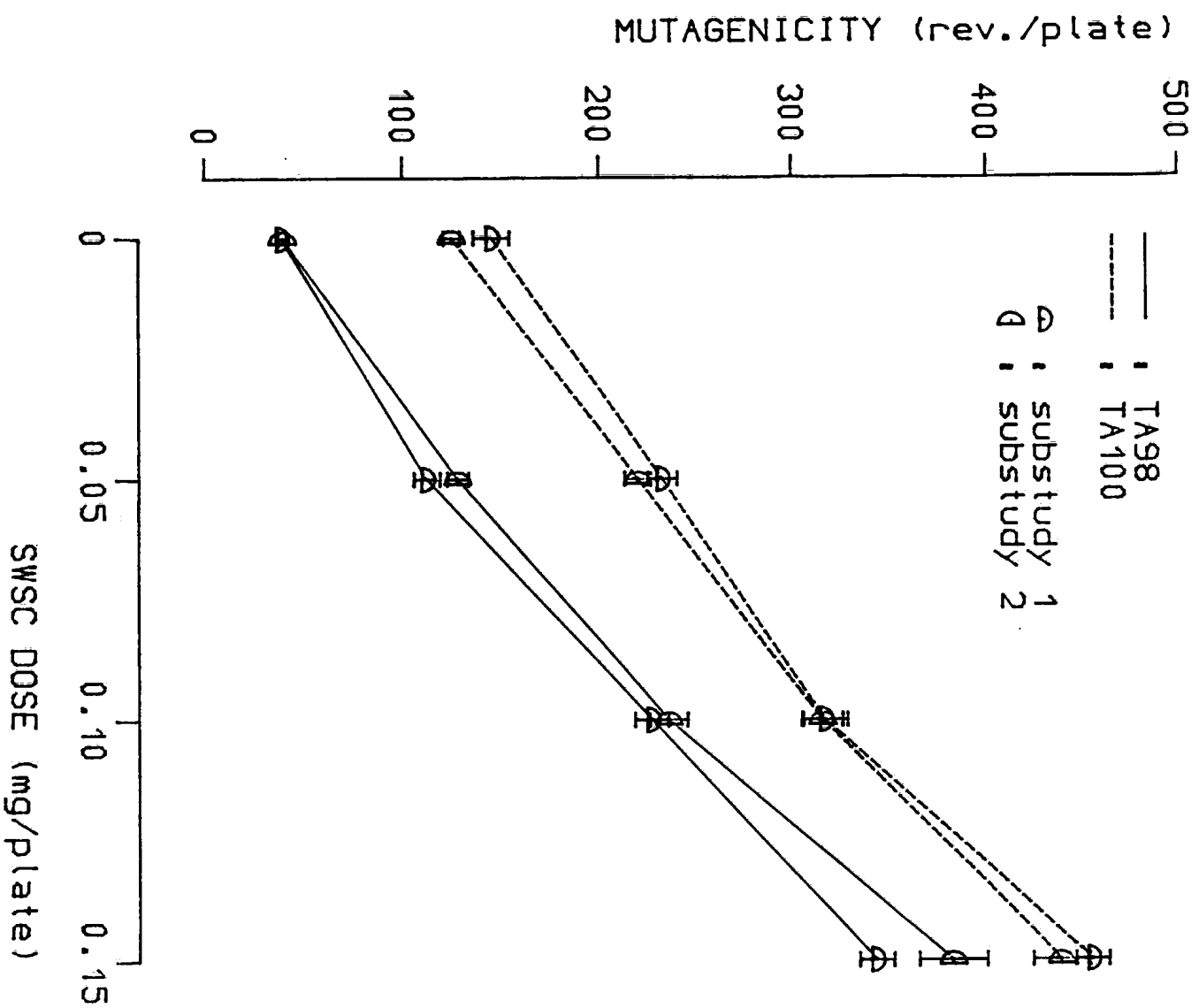
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100
200
300
400
500
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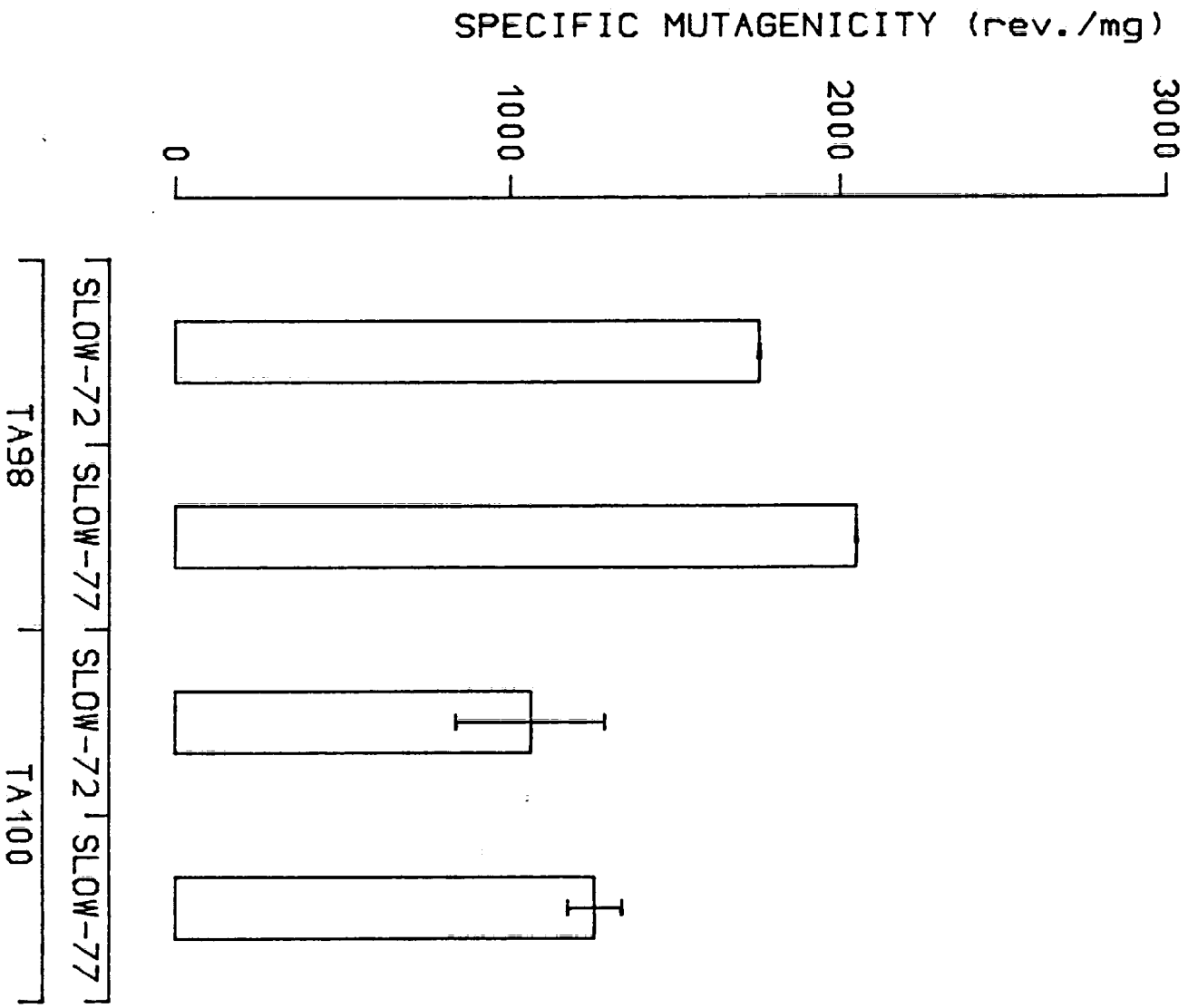
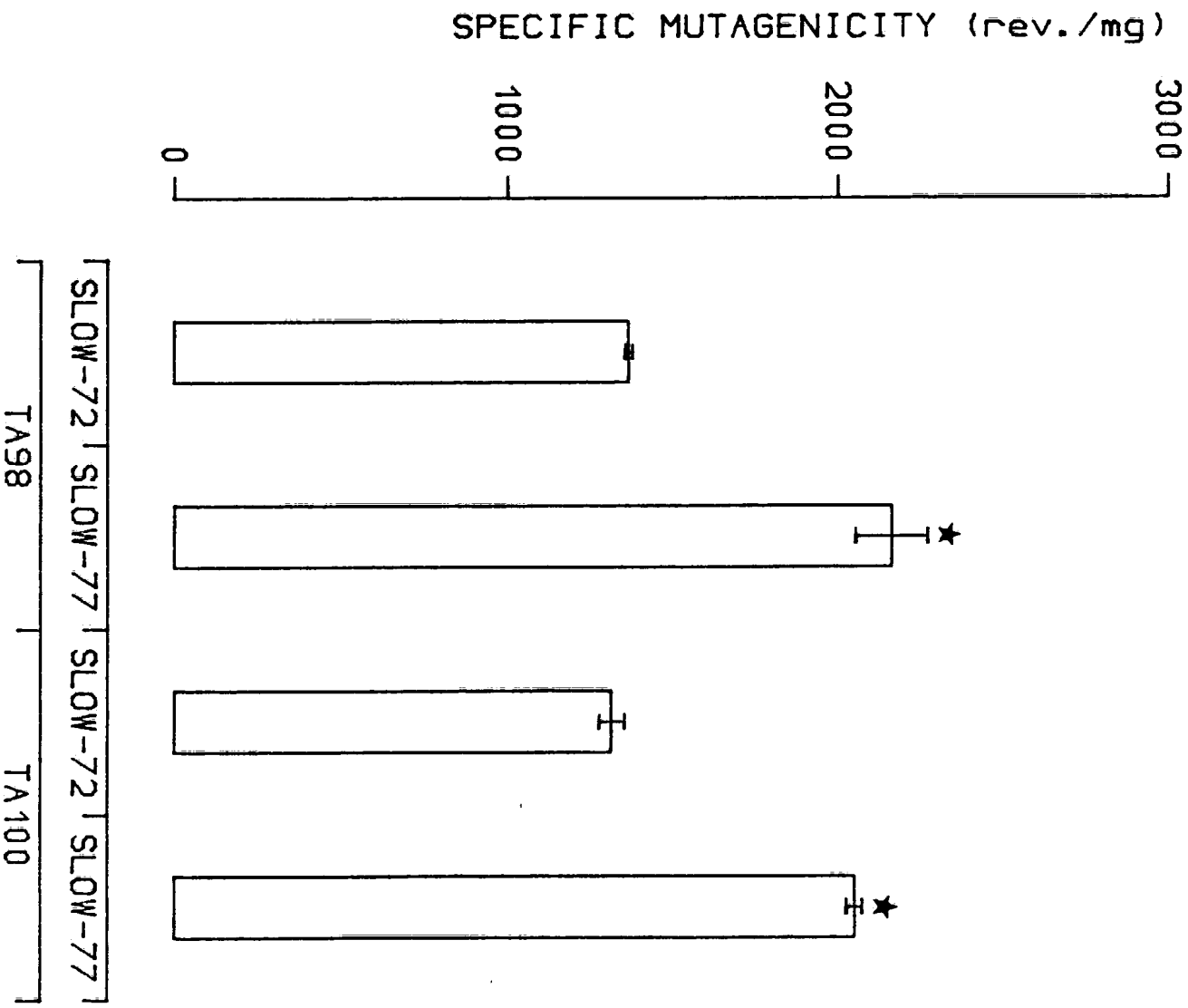
0.10
0.15
TA98
TA100
substudy 1
substudy 2

2026009491


SPECIFIC MUTAGENICITY (rev./mg)
0
1000
2000
3000
TA98
TA100
SLOW-72
SLOW-77
SLOW-72
SLOW-77

P 0268/2132, H04753, V84 F16 V83 F16, SWSC-I



DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2366	0.00	35	45	28	49	39.3	24.3
		0.05	119	120	105	97	110.3	10.1
		0.10	168	191	194	208	190.3	8.7
		0.15	322	303	276	313	303.5	6.6
	2372	0.00	40	46	36	34	39.0	13.6
		0.05	89	97	88	94	92.0	4.6
		0.10	184	210	179	187	190.0	7.2
		0.15	277	337	297	291	300.5	8.6
24-OCT-85	2386	0.00	45	53	46	49	48.3	7.4
		0.05	105	110	107	114	109.0	3.6
		0.10	209	210	195	220	208.5	4.9
		0.15	298	323	312	321	313.5	3.6
	2396	0.00	38	52	50	41	45.3	15.0
		0.05	118	105	121	101	111.3	8.8
		0.10	155	183	226	175	184.8	16.2
		0.15	286	311	295	328	305.0	6.1

TABLE 7.1

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2368	0.00	44	32	39	30	36.3	17.8
		0.05	96	105	101	96	99.5	4.4
		0.10	237	225	207	176	211.3	12.6
		0.15	349	368	327	369	353.3	5.6
	2374	0.00	34	46	39	31	37.5	17.5
		0.05	115	122	104	114	113.8	6.5
		0.10	217	211	197	206	207.8	4.1
		0.15	340	335	336	323	333.5	2.2
24-OCT-85	2388	0.00	50	51	47	41	47.3	9.5
		0.05	122	140	133	150	136.3	8.7
		0.10	230	199	210	219	214.5	6.1
		0.15	359	331	340	363	348.3	4.4
	2398	0.00	39	40	52	36	41.8	16.9
		0.05	107	94	114	117	108.0	9.5
		0.10	199	239	207	248	223.3	10.7
		0.15	377	348	362	347	358.5	3.9

TABLE 7.2

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2370	0.00	-	41	38	31	36.7	14.0
		0.05	85	82	86	120	93.3	19.2
		0.10	170	150	154	162	159.0	5.6
		0.15	245	259	263	220	246.8	7.9
	2376	0.00	50	30	30	52	40.5	30.0
		0.05	90	114	104	102	102.5	9.6
		0.10	194	157	138	-	163.0	17.5
		0.15	234	237	281	231	245.8	9.6
24-OCT-85	2390	0.00	49	49	53	51	50.5	3.8
		0.05	109	100	104	118	107.8	7.2
		0.10	162	195	192	169	179.5	9.2
		0.15	155	189	240	277	215.3	25.1
	2400	0.00	50	41	52	56	49.8	12.8
		0.05	123	110	94	111	109.5	10.9
		0.10	184	166	178	159	171.8	6.6
		0.15	260	273	251	317	275.3	10.6

TABLE 7.3

MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2366	0.00	124	134	95	116	117.3	14.1
		0.05	167	169	189	183	177.0	6.0
		0.10	207	195	223	205	207.5	5.6
		0.15	248	267	258	250	255.8	3.4
	2372	0.00	114	121	127	117	119.8	4.7
		0.05	159	181	156	162	164.5	6.9
		0.10	181	213	202	191	196.8	7.0
		0.15	246	209	235	268	239.5	10.2
24-OCT-85	2386	0.00	143	122	150	111	131.5	13.8
		0.05	188	205	196	187	194.0	4.3
		0.10	222	250	216	235	230.8	6.5
		0.15	312	342	323	340	329.3	4.3
	2396	0.00	110	144	116	133	125.8	12.4
		0.05	207	203	173	154	184.3	13.7
		0.10	233	224	241	241	234.8	3.4
		0.15	315	339	312	339	326.3	4.5

TABLE 8.1

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2368	0.00	107	130	120	106	115.8	9.9
		0.05	190	189	175	179	183.3	4.0
		0.10	247	189	219	165	205.0	17.4
		0.15	289	312	279	279	289.8	5.4
	2374	0.00	119	123	114	117	118.3	3.2
		0.05	186	177	175	128	166.5	15.7
		0.10	246	227	253	242	242.0	4.5
		0.15	294	306	313	290	300.8	3.5
24-OCT-85	2388	0.00	142	125	113	119	124.8	10.0
		0.05	174	192	195	169	182.5	7.1
		0.10	225	276	254	194	237.3	15.0
		0.15	301	330	327	326	321.0	4.2
	2398	0.00	106	129	119	120	118.5	8.0
		0.05	173	175	208	185	185.3	8.7
		0.10	230	224	247	253	238.5	5.8
		0.15	343	327	318	334	330.5	3.2

TABLE 8.2

MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CONDENSATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2370	0.00	128	121	114	111	118.5	6.4
		0.05	174	170	189	186	179.8	5.1
		0.10	202	170	185	154	177.8	11.6
		0.15	238	255	240	253	246.5	3.5
	2376	0.00	115	127	120	106	117.0	7.5
		0.05	152	143	197	174	166.5	14.5
		0.10	197	241	197	220	213.8	9.9
		0.15	280	258	236	238	253.0	8.1
24-OCT-85	2390	0.00	121	135	126	139	130.3	6.3
		0.05	169	190	168	179	176.5	5.8
		0.10	232	226	248	224	232.5	4.7
		0.15	297	272	302	278	287.3	5.0
	2400	0.00	139	138	121	127	131.3	6.7
		0.05	191	191	197	207	196.5	3.8
		0.10	242	238	230	277	246.8	8.4
		0.15	300	313	347	321	320.3	6.2

TABLE 8.3

MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2367	0.00	46	40	26	30	35.5	25.8
		0.05	95	99	93	96	95.8	2.6
		0.10	191	181	172	165	177.3	6.4
		0.15	268	212	279	260	254.8	11.6
	2373	0.00	44	43	32	43	40.5	14.0
		0.05	84	84	70	96	83.5	12.7
		0.10	155	157	151	154	154.3	1.6
		0.15	221	242	201	226	222.5	7.6
24-OCT-85	2387	0.00	35	49	48	47	44.8	14.6
		0.05	99	99	109	120	106.8	9.4
		0.10	159	182	156	167	166.0	7.0
		0.15	252	264	263	259	259.5	2.1
	2397	0.00	46	50	49	44	47.3	5.8
		0.05	106	113	105	91	103.8	8.9
		0.10	158	190	180	181	177.3	7.7
		0.15	219	253	261	257	247.5	7.8

TABLE 9.1

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2369	0.00	31	45	41	37	38.5	15.5
		0.05	115	110	76	113	103.5	17.8
		0.10	231	222	204	204	215.3	6.3
		0.15	330	328	327	375	340.0	6.9
	2375	0.00	40	45	47	28	40.0	21.3
		0.05	101	141	127	113	120.5	14.4
		0.10	210	222	241	278	237.8	12.5
		0.15	319	336	389	334	344.5	8.9
24-OCT-85	2389	0.00	42	41	37	42	40.5	5.9
		0.05	128	120	123	112	120.8	5.6
		0.10	221	215	208	198	210.5	4.7
		0.15	308	326	351	368	338.3	7.8
	2399	0.00	50	29	36	40	38.8	22.6
		0.05	117	135	126	161	134.8	14.1
		0.10	274	261	247	259	260.3	4.2
		0.15	423	431	415	429	424.5	1.7

TABLE 9.2

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2371	0.00	35	41	41	37	38.5	7.8
		0.05	86	89	79	89	85.8	5.5
		0.10	136	171	145	175	156.8	12.2
		0.15	209	196	227	233	216.3	7.8
	2377	0.00	48	40	39	42	42.3	9.5
		0.05	82	104	70	95	87.8	17.0
		0.10	145	147	167	179	159.5	10.3
		0.15	246	242	297	228	253.3	11.9
24-OCT-85	2391	0.00	42	53	57	51	50.8	12.5
		0.05	96	104	79	95	93.5	11.2
		0.10	194	186	152	182	178.5	10.3
		0.15	222	250	241	246	239.8	5.2
	2401	0.00	49	32	50	46	44.3	18.9
		0.05	91	104	85	88	92.0	9.1
		0.10	156	190	175	216	184.3	13.7
		0.15	270	258	243	252	255.8	4.4

TABLE 9.3

MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2367	0.00	171	136	138	142	146.8	11.1
		0.05	230	247	229	239	236.3	3.6
		0.10	285	310	262	309	291.5	7.8
		0.15	334	374	370	332	352.5	6.4
	2373	0.00	132	150	138	178	149.5	13.7
		0.05	215	209	225	212	215.3	3.2
		0.10	281	-	275	272	276.0	1.7
		0.15	341	345	303	327	329.0	5.8
24-OCT-85	2387	0.00	148	108	110	132	124.5	15.3
		0.05	210	232	202	192	209.0	8.1
		0.10	245	260	249	260	253.5	3.0
		0.15	349	351	319	296	328.8	8.0
	2397	0.00	147	126	128	129	132.5	7.4
		0.05	186	176	187	209	189.5	7.3
		0.10	267	269	306	278	280.0	6.4
		0.15	294	312	358	372	334.0	11.1

TABLE 10.1

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2369	0.00	137	150	168	139	148.5	9.6
		0.05	219	220	223	226	222.0	1.4
		0.10	319	358	304	301	320.5	8.2
		0.15	438	432	425	444	434.8	1.9
	2375	0.00	149	193	110	117	142.3	26.6
		0.05	283	241	232	215	242.8	11.9
		0.10	321	367	285	274	311.8	13.5
		0.15	480	473	449	491	473.3	3.8
24-OCT-85	2389	0.00	137	129	127	141	133.5	4.9
		0.05	235	182	204	217	209.5	10.6
		0.10	286	298	285	286	288.8	2.1
		0.15	457	441	361	424	420.8	10.0
	2399	0.00	123	121	122	100	116.5	9.5
		0.05	232	239	229	218	229.5	3.8
		0.10	321	357	352	331	340.3	5.0
		0.15	419	512	439	450	455.0	8.8

TABLE 10.2

MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	CON-DEN-SATE BATCH	DOSE (mg/plate)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	RSD (0/0)
09-OCT-85	2371	0.00	123	158	141	166	147.0	13.0
		0.05	210	229	217	227	220.8	4.0
		0.10	303	264	250	278	273.8	8.3
		0.15	348	372	332	336	347.0	5.2
	2377	0.00	102	112	127	138	119.8	13.3
		0.05	206	188	229	236	214.8	10.2
		0.10	228	266	304	299	274.3	12.8
		0.15	401	398	368	358	381.3	5.6
24-OCT-85	2391	0.00	120	130	100	131	120.3	12.0
		0.05	197	198	209	195	199.8	3.1
		0.10	234	247	291	231	250.8	11.1
		0.15	301	284	320	322	306.8	5.8
	2401	0.00	141	113	116	129	124.8	10.3
		0.05	213	157	203	210	195.8	13.4
		0.10	274	261	275	276	271.5	2.6
		0.15	352	310	354	367	345.8	7.2

TABLE 10.3

MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	39.1	18.2	2.5		
	0.05	8	101.1	12.4	4.4		
	0.10	8	190.1	7.4	5.0		
	0.15	8	302.0	7.1	7.6	1755	0.983
24-OCT-85	0.00	8	46.8	11.3	1.9		
	0.05	8	110.1	6.3	2.5		
	0.10	8	196.6	12.4	8.6		
	0.15	8	309.3	4.8	5.3	1748	0.982
09-OCT-85	0.00	16	42.9	16.8	1.8		
24-OCT-85	0.05	16	105.6	10.3	2.7		
	0.10	16	193.4	10.1	4.9		
	0.15	16	305.6	6.0	4.5	1752	0.982

TABLE 12.1

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.00. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	36.9	16.4	2.1		
	0.05	8	106.6	8.9	3.3		
	0.10	8	209.5	8.7	6.5		
	0.15	8	343.4	5.1	6.1	2045	0.984
24-OCT-85	0.00	8	44.5	14.0	2.2		
	0.05	8	122.1	14.9	6.4		
	0.10	8	218.9	8.4	6.5		
	0.15	8	353.4	4.2	5.2	2047	0.985
09-OCT-85	0.00	16	40.7	17.5	1.8		
24-OCT-85	0.05	16	114.4	14.1	4.0		
	0.10	16	214.2	8.6	4.6		
	0.15	16	348.4	4.7	4.1	2046	0.983

TABLE 12.2

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.00. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	7	38.9	24.0	3.5		
	0.05	8	97.9	14.6	5.0		
	0.10	7	160.7	11.0	6.7		
	0.15	8	246.3	8.1	7.1	1377	0.978
24-OCT-85	0.00	8	50.1	8.7	1.5		
	0.05	8	108.6	8.6	3.3		
	0.10	8	175.6	7.8	4.9		
	0.15	8	245.3	21.0	18.2	1305	0.944
09-OCT-85	0.00	15	44.9	20.0	2.3		
24-OCT-85	0.05	16	103.3	12.5	3.2		
	0.10	15	168.7	10.1	4.4		
	0.15	16	245.8	15.3	9.4	1339	0.959

TABLE 12.3

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.05. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	118.5	9.7	4.1		
	0.05	8	170.8	7.1	4.3		
	0.10	8	202.1	6.5	4.6		
	0.15	8	247.6	7.7	6.8	837	0.958
24-OCT-85	0.00	8	128.6	12.4	5.6		
	0.05	8	189.1	9.6	6.4		
	0.10	8	232.8	4.9	4.0		
	0.15	8	327.8	4.1	4.8	1282	0.969
09-OCT-85	0.00	16	123.6	11.7	3.6		
24-OCT-85	0.05	16	179.9	9.8	4.4		
	0.10	16	217.4	9.1	4.9		
	0.15	16	287.7	15.4	11.1	1060	0.911

TABLE 13.1

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.42. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	117.0	6.8	2.8		
	0.05	8	174.9	11.4	7.0		
	0.10	8	223.5	14.1	11.1		
	0.15	8	295.3	4.6	4.8	1167	0.958
24-OCT-85	0.00	8	121.6	8.9	3.8		
	0.05	8	183.9	7.4	4.8		
	0.10	8	237.9	10.5	8.8		
	0.15	8	325.8	3.8	4.3	1333	0.974
09-OCT-85	0.00	16	119.3	7.9	2.4		
24-OCT-85	0.05	16	179.4	9.5	4.3		
	0.10	16	230.7	12.3	7.1		
	0.15	16	310.5	6.5	5.0	1250	0.960

TABLE 13.2

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.13. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	117.8	6.5	2.7		
	0.05	8	173.1	10.6	6.5		
	0.10	8	195.8	13.9	9.6		
	0.15	8	249.8	6.0	5.3	837	0.929
24-OCT-85	0.00	8	130.8	6.0	2.8		
	0.05	8	186.5	7.3	4.8		
	0.10	8	239.6	7.2	6.1		
	0.15	8	303.8	7.9	8.4	1144	0.971
09-OCT-85	0.00	16	124.3	8.1	2.5		
24-OCT-85	0.05	16	179.8	9.5	4.3		
	0.10	16	217.7	14.5	7.9		
	0.15	16	276.8	12.2	8.5	991	0.913

TABLE 13.3

SPECIFIC MUTAGENICITY OF MWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.31. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	38.0	19.8	2.7		
	0.05	8	89.6	10.8	3.4		
	0.10	8	165.8	8.7	5.1		
	0.15	8	238.6	11.8	10.0	1356	0.976
24-OCT-85	0.00	8	46.0	10.5	1.7		
	0.05	8	105.3	8.6	3.2		
	0.10	8	171.6	7.7	4.7		
	0.15	8	253.5	5.8	5.2	1378	0.988
09-OCT-85	0.00	16	42.0	17.6	1.8		
24-OCT-85	0.05	16	97.4	12.5	3.0		
	0.10	16	168.7	8.1	3.4		
	0.15	16	246.1	9.3	5.7	1367	0.980

TABLE 14.1

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.02. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	39.3	17.5	2.4		
	0.05	8	112.0	16.9	6.7		
	0.10	8	226.5	10.8	8.7		
	0.15	8	342.3	7.4	9.0	2047	0.982
24-OCT-85	0.00	8	39.6	15.2	2.1		
	0.05	8	127.8	11.9	5.4		
	0.10	8	235.4	12.0	10.0		
	0.15	8	381.4	13.0	17.5	2266	0.971
09-OCT-85 24-OCT-85	0.00	16	39.4	15.8	1.6		
	0.05	16	119.9	15.4	4.6		
	0.10	16	230.9	11.3	6.5		
	0.15	16	361.8	11.9	10.8	2156	0.972

TABLE 14.2

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.10. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	40.4	9.5	1.4		
	0.05	8	86.8	11.8	3.6		
	0.10	8	158.1	10.5	5.9		
	0.15	8	234.8	12.8	10.6	1309	0.969
24-OCT-85	0.00	8	47.5	16.2	2.7		
	0.05	8	92.8	9.5	3.1		
	0.10	8	181.4	11.4	7.3		
	0.15	8	247.8	5.6	4.9	1379	0.980
09-OCT-85 24-OCT-85	0.00	16	43.9	15.8	1.7		
	0.05	16	89.8	10.9	2.4		
	0.10	16	169.8	12.8	5.4		
	0.15	16	241.3	9.8	5.9	1344	0.971

TABLE 14.3

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA98

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.05. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	148.1	11.6	6.1		
	0.05	8	225.8	5.9	4.7		
	0.10	7	284.9	6.4	6.9		
	0.15	8	340.8	6.8	8.1	1273	0.970
24-OCT-85	0.00	8	128.5	11.4	5.2		
	0.05	8	199.3	8.9	6.3		
	0.10	8	266.8	7.2	6.8		
	0.15	8	331.4	9.0	10.5	1352	0.967
09-OCT-85 24-OCT-85	0.00	16	138.3	13.3	4.6		
	0.05	16	212.5	9.6	5.1		
	0.10	15	275.2	7.4	5.3		
	0.15	16	336.1	7.8	6.6	1312	0.961

TABLE 15.1

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-72
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.06. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	145.4	18.4	9.4		
	0.05	8	232.4	9.5	7.8		
	0.10	8	316.1	10.3	11.6		
	0.15	8	454.0	5.3	8.6	2019	0.969
24-OCT-85	0.00	8	125.0	9.9	4.4		
	0.05	8	219.5	8.6	6.7		
	0.10	8	314.5	9.5	10.6		
	0.15	8	437.9	9.6	14.9	2067	0.973
09-OCT-85 24-OCT-85	0.00	16	135.2	16.8	5.7		
	0.05	16	225.9	9.3	5.2		
	0.10	16	315.3	9.6	7.6		
	0.15	16	445.9	7.7	8.6	2043	0.969

TABLE 15.2

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE SLOW-77
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.02. Deviations .GT.0.25 are considered statistically significant.

DATE OF ASSAY	DOSE (mg/plate)	MUTAGENICITY (rev./plate) N	M	RSD (0/0)	SE	REGR. COEFF. (rev./mg)	CORREL. COEFF. r
09-OCT-85	0.00	8	133.4	16.4	7.7		
	0.05	8	217.8	7.3	5.6		
	0.10	8	274.0	10.0	9.7		
	0.15	8	364.1	7.1	9.2	1497	0.965
24-OCT-85	0.00	8	122.5	10.5	4.5		
	0.05	8	197.8	9.0	6.3		
	0.10	8	261.1	8.3	7.7		
	0.15	8	326.3	8.9	10.2	1349	0.967
09-OCT-85 24-OCT-85	0.00	16	127.9	14.2	4.6		
	0.05	16	207.8	9.3	4.8		
	0.10	16	267.6	9.3	6.2		
	0.15	16	345.2	9.5	8.2	1423	0.957

TABLE 15.3

SPECIFIC MUTAGENICITY OF SWSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA100

Remarks: Difference of regression coefficients (specific mutagenicity) of both assays relative to their mean is 0.10. Deviations .GT.0.25 are considered statistically significant.

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1752
SLOW-77	2046

TABLE 16.1

SPECIFIC MUTAGENICITY OF MWSC-I, STRAIN TA98

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	(1060)
SLOW-77	1250

TABLE 16.2

SPECIFIC MUTAGENICITY OF MWSC-I, STRAIN TA100

Remarks: Mutagenicity data in brackets are those, which showed a statistically significant difference between both substudies.

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1367
SLOW-77	2156

TABLE 17.1

SPECIFIC MUTAGENICITY OF SWSC-I, STRAIN TA98

CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg)
SLOW-72	1312
SLOW-77	2043

TABLE 17.2

SPECIFIC MUTAGENICITY OF SWSC-I, STRAIN TA100

PROJET SLOw
PROTOTYPE 072C1

CIG. PAPER 30-1000-

PROD. DATE 22.08.85

PROJET SLOW
PROTOTYPE 077C1

CIG. PAPER 30-0061-D

PROD. DATE 22.08.85