Section A6.5 Chronic toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

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		1 REFERENCE
1.1	**Reference**	[redacted]
1.2	**Data protection**	No.
1.2.1	Data owner	Not applicable, published data.
1.2.2	Companies with letter of access	Not applicable, published data.
1.2.3	Criteria for data protection	No data protection claimed.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Not carried out to guideline B.30 in Annex V of Directive 67/548/EC
2.2	**GLP**	No. Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.
		3 MATERIALS AND METHODS
3.1	**Test material**	Amorphous silica, containing 3.8% of respirable dust. No other information available on the specification or purity of the test material.
3.1.1	Lot/Batch number	Not reported.
3.1.2	Specification	Not reported.
3.1.2.1	Description	Not reported.
3.1.2.2	Purity	Not reported.
3.1.2.3	Stability	Not reported. However, silicon dioxide is known to be a stable compound (melting point >1500°C).
3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Male.
3.2.5	Age/weight at study initiation	Average age of test group: 42 Average weight of test group: 79 kg. For more details refer to Table A6_5-1 at the end of this study summary.
3.2.6	Number of animals per group	Number of subjects in test group: 41 For more details refer to Table A6_5-1 at the end of this study summary.
3.2.7	Control animals	Yes. For details of test group, refer to Table A6_5-1 at the end of this study summary.
3.3	**Administration/ Exposure**	Inhalation.

Section A6.5 Chronic toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

3.3.1	Duration of treatment	Mean duration of exposure was 8 years (range 1-28 years)	
3.3.2	Frequency of exposure	Daily.	
3.3.3	Postexposure period	Not reported.	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration: Not reported.	
3.3.4.2		Analytical concentration: Total dust concentration ranged from 0 to 10.5 mg/m³ Respirable dust concentration ranged from 0 to 3.4 mg/m³.	
3.3.4.3	Particle size	Not reported.	
3.3.4.4	Type or preparation of particles	Dust	
3.3.4.5	Type of exposure	Whole body.	
3.3.4.6	Vehicle	No vehicle used: Test subjects were exposed to 100% silica dust.	
3.3.4.7	Concentration in vehicle	Not applicable. No vehicle used.	
3.3.4.8	Duration of exposure	8 h/day	
3.3.4.9	Controls	Sham exposure.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes: Prevalence of respiratory symptoms such as cough, phlegm, tightness of breath, wheezing, tightness, dyspnoea, asthma.	
3.4.1.2	Mortality	Not reported.	
3.4.2	Body weight	Not reported.	
3.4.3	Food consumption	Not reported.	
3.4.4	Water consumption	Not reported.	
3.4.5	Ophthalmoscopic examination	Not reported.	
3.4.6	Haematology	Yes.	
		Number of animals:	All test subjects.
		Time points:	End of study.
		Parameters:	Blood gas concentrations at rest and during exercise. Pulmonary function.
3.4.7	Clinical Chemistry	Not reported.	
3.4.8	Urinalysis	Not reported.	
3.5	**Sacrifice and pathology**		

Section A6.5 Chronic toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

3.5.1	Organ Weights	Not applicable.
3.5.2	Gross and histopathology	Not applicable.
3.5.3	Other examinations	Chest radiographs of test subjects were taken which would have found tumours and masses.
3.5.4	Statistics	Yes: Contingency tables (2 x 2) with γ^2 tests were used to determine whether relations shown between variables were statistically significant. Quantitative variables were compared by *t* test. Analysis of variance was used to examine the relation of respiratory symptoms, work exposure and smoking to pulmonary function.
3.6	**Further remarks**	Case studies from 3 workers were selected at random.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Refer to table A6_5-2 at the end of this study summary for details of respiratory symptoms reported, following exposure to amorphous silica.
4.1.2	Mortality	Not reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophtalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Refer to table A6_5-3 at the end of this study summary for details of blood gas concentrations reported, following exposure to amorphous silica.
4.5.2	Clinical chemistry	Not reported.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Not reported.
4.6.2	Gross and histopathology	Not reported.
4.7	**Other**	Macroscopic investigations: No difference was found between the chest radiographs of the control and test group.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The purpose of this study was to determine the pulmonary effects of occupational exposure to amorphous silica compared with a control group. In addition, blood gas concentrations, at rest and during exercise, were evaluated as possible indicators of changes in lung function as a result of exposure. Study population

Section A6.5 **Chronic toxicity (Inhalation 2 of 2)**

Annex Point IIA, VI, 6.5 **Section 6: Toxicological and metabolic studies**

The study population was composed of workers at a large chemical plant engaged in the synthesis of amino acids and vitamins. 131 men worked in three 8h shifts for continuous production (4am-12 noon, 12 noon to 8pm and 8pm to 4 am). The shifts alternated each week.

The workers were divided into two groups according to exposure in their current job. Group E comprised 41 workers exposed or previously exposed to silica. The mean duration of exposure was 8 years (range 1 – 28 years). Group C was the control group and comprised of 90 workers of equivalent socio-economic state in the same plant. They were not exposed to appreciable air contaminants in the plant and were matched for age with group E. The data, except for the chest radiograph, were collected during a week in May 1988.

Questionnaire

A questionnaire was presented to participants by trained interviewers. It included questions about individual characteristics such as age, height, race, medical history and work history at the plant, and before employment at the plant. Non-smokers were defined as those persons smoking less than one cigarette a day, and ex-smokers as those who had stopped smoking completely at least six months before the study. Questions about respiratory symptoms were adapted from the questionnaire of the International Union Against Tuberculosis and Lung Disease.

Tests of pulmonary function

Measurement of pulmonary function was carried out at the work site, using a computerised pneumotachograph Fleisch No 3 (Spiromatic, MSR) which was calibrated daily. Forced expiration was assessed on a oscilloscope. At least three readings were obtained with the worker seated and wearing a noseclip. The curve producing the largest sum of forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) was selected for analysis. Other standard parameters of pulmonary function (forced expiratory flow $(FEF)_{25-75}$, FEF_{25}, FEF_{50} and FEF_{75}) were recorded from this best maximal expiratory flow volume curve. All values were adjusted for age and height using regressions on the whole sample and normalised (mean (SD) = 0(1)). In the figures normalised values are presented for subjects of mean population age (42, SD 8 years) and height (172 SD 7 cm).

Blood gas concentrations at rest and during exercise

Samples for blood gas analysis were taken from the earlobe after vasodilation with Finalgon (Boehringer Ingelheim) 10 minutes before the first incision. Arterial blood was collected and heparinised capillary tubes and immediately analysed by a trained technician with Corning 170 apparatus. Concentrations of blood gases were measured at rest after the questionnaire and after spirometry during standardised exercise on a treadmill (Gymroll 1000, Gillet). Heart rate was continuously monitored by electrocardiography (TEC 7100, MSR). The mean duration of exercise was about 7 minutes. When heart rate reached 130 beats per minute, samples for blood analysis were collected again and analysed by the same technician. Exercise was not performed by subjects with cardiac or rheumatic conditions (12 in group C and 10 in group E).

Chest Radiographs

Posteroanterior chest radiographs (8 x 8 cm) were obtained at a different time from the interview. They were read by three independent physicians according to the International Labour Office classification.

Assessment of Environmental Exposure

About thirty tonnes a day of precipitated amorphous silica containing 3.8% of respirable dust were used in the plant. Levels of dust exposure were appraised in the working area using a CIP10 individual sampler. The duration of each sampling was 340 minutes. 36 samplings were made while the workers were actually performing their jobs. Total dust concentrations ranged from 0 to 3.4 mg/m^3. An exposure index was calculated for each worker according to the quantity and the duration of exposure to amorphous silica. For each worker, time spent in the presence of amorphous silica was evaluated. This time was multiplied by three if it occurred before 1984. Three was chosen because dust measurements were three times greater before 1984, after which a better system of ventilation was established in the working area.

5.2 Results and discussion

Smoking habits and characteristics of the population did not differ significantly between the exposed and control groups, except for the percentage of shift workers (61% vs 87% in the exposed group. This was statistically significant with a *p* value of <0.01). Table A6_5-1 at the end of this study summary gives details of the characteristics of the control group and exposed subjects.

Table A6_5-2 at the end of this study summary shows the prevalence of respiratory symptoms.

Concentrations of blood gas at rest and during exercise were not significantly different between the exposed group and the control group (Table A6_5-2 at the end of this study summary), and no difference was found between the chest radiographs of the two groups.

All airflow values were lower in the exposed group than in the control (Table A6_5-4 at the end of this study summary) with significant differences for FEV_1 / FVC, FEF_{25-75}, FEF_{50} and FEF_{75}. There was no significant correlation, however between the exposure index and pulmonary function.

Results of the pulmonary function tests were compared according to the exposure to tobacco and to amorphous silica. The mean values of FEF_{25-75}, FEF_{50} and FEF_{75} were lower among the smokers and the exposed workers than among the non-smoking, non-exposed workers. These differences were significant between the smoking-exposed group and the non-smoking non-exposed group.

This study has shown that exposure to precipitated silica dust induces little respiratory impairment, which was increased by smoking. The test subject questionnaire, chest x ray films and concentrations of arterial blood gas were used to distinguish the two groups of workers (exposed or not). None of these methods were able to discriminate.

Arterial blood gas concentrations are used to study lung function. Several factors may explain the lack of difference between the exposed and non-exposed workers. Amorphous silica is less fibrogenic than crystalline silica, the dust is not highly respirable, and no pneumoconiosis was found on the chest radiographs of the exposed workers. Finally the exercise regime was have not been strenuous enough in the study.

Section A6.5 Chronic toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

		In conclusion, exposure to amorphous silica dust may induce a mild small airway disease, only shown by comparison to a control group. This obstruction to airflow is increased by cigarette smoking. Only flow volumes curves were different between groups. Arterial blood gases at rest and during standardised exercise, and chest radiographs were similar between groups.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Not reported.
5.3.3	Other	None.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that no post-mortem analysis could be carried out for obvious reasons. Also urinalysis was not measured. However, with the other measured parameters showing no effects, this does not appear to adversely affect the study. There are deficiencies in the reporting, including full reporting of method and results. However, as this study uses humans as the test subjects and the study was conducted over 8 years (which is longer than the 10% of expected life for a repeated-dose study (90 days)) and no adverse effects were shown suggesting that this length of time is adequate, it is deemed appropriate for adding information to the risk assessment. The test material used in this study is not identical to that as given in Section 2. However, it is considered sufficiently similar and adds to weight of evidence with regards to the inert nature of silicon dioxide. The study was performed with only a single dose range, so no NOAEL could be established. Despite the deficiencies in this study, it gives an indication of the level of silicon dioxide that can be tolerated without effect by humans.

Section A6.5 **Chronic toxicity (Inhalation 2 of 2)**

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Section 6: Toxicological and metabolic studies

Table A6_5-1. Characteristics of control workers and workers exposed to amorphous silica

	Group C (control)	Group E (exposed group)	*p* Value
Number of subjects	90	41	
Age (*y*, mean (SD))	42 (8)	42 (9)	NS
Height (cm, mean (SD))	174 (7)	174 (7)	NS
Weight (kg mean (SD))	77 (10)	79 (13)	NS
Shift workers (No (%))	55 (61)	36 (87)	<0.01
Smokers (No (%))	38 (42)	19 (46)	NS
Ex smokers (No (%))	23 (25)	9 (22)	NS
Non smokers (No (%))	29 (32)	13 (31)	NS

Table A6_5-2. Prevalence of respiratory symptoms in controls, and workers exposed to amorphous silica

	Group C (control) No (%)	Group E (exposed group) No (%)	*p* Value
Morning cough	8 (8.9)	7 (17)	NS
Usual cough	8 (8.9)	5 (12.2)	<0.01
Attack of coughing	15 (16.7)	5 (12.2)	NS
Phlegm	5 (5.6)	4 (9.8)	NS
Shortness of breath	3 (3.3)	2 (4.9)	NS
Wheezing	12 (13.3)	2 (4.9)	<0.001
Tightness	9 (10)	3 (7.3)	<0.05
Dsyspnoea grade 1	19 (21.1)	16 (39)	<0.001
Dsyspnoea grade 2	0 (0)	1 (2.4)	NS
Statement about breathing	==	==	==
Good	78 (86.7)	35 (85.4)	NS
Medium	9 (10)	2 (4.9)	NS
Bad	3 (3.3)	4 (9.8)	NS
Asthma	2 (2.2)	4 (9.8)	<0.001

Section A6.5 **Chronic toxicity (Inhalation 2 of 2)**

Annex Point IIA, VI, 6.5 **Section 6: Toxicological and metabolic studies**

Table A6_5-3. Blood gas concentrations at rest and during exercise in control workers and workers exposed to amorphous silica

	Group C (control) Mean (SD)	Group E (exposed group) Mean (SD)	*p* Value
PaO_2 at rest (mmHg)	78.1 (6.7)	77.2 (6.7)	NS
$PaCO_2$ at rest (mmHg)	38 (2.2)	38.7 (2.4)	NS
PaO_2 during exercise (mmHg)	78 (8.7)	79.3 (7.2)	NS
$PaCO_2$ during exercise (mmHg)	40 (2.8)	40.1 (3.3)	NS
Heart rate at rest (beats/minute)	83 (12.6)	80 (12.5)	NS
Heart rate during exercise (beats/minute)	126 (5.4)	126 (7.6)	NS
Duration of exercise (min)	6.2 (1.8)	6.9 (1.8)	NS

Table A6_5-4. Pulmonary function in controls and workers exposed to amorphous silica

	Group C (control) Mean (SD)	Group E (exposed group) Mean (SD)	*p* Value
FVC (l)	4.8 (0.8)	4.8 (0.8)	NS
FEV_1 (l/s)	3.9 (0.6)	3.8 (0.6)	NS
FEF_{25-75} (l/s)	4.2 (1.2)	3.6 (1.2)	<0.01
PF (l/s)	10.7 (1.6)	10.3 (1.5)	NS
FEF_{25} (l/s)	8.7 (1.8)	8.2 (1.6)	NS
FEF_{50} (l/s)	5.1 (1.5)	4.5 (1.4)	<0.03
FEF_{75} (l/s)	1.8 (0.7)	1.4 (0.5)	<0.008
FEV_1 / FVC (%)	82.1 (5.6)	79.2 (5.7)	<0.007

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Section 6: Toxicological and metabolic studies

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.5 Chronic toxicity (Oral 1 of 1)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

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1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	No.
1.2.1	Data owner	Not applicable, published data.
1.2.2	Companies with letter of access	Not applicable, published data.
1.2.3	Criteria for data protection	No data protection claimed.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to guideline B.30 in Annex V of Directive 67/548/EC.
2.2	**GLP**	No. Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.

3 MATERIALS AND METHODS

3.1	**Test material**	SYLOID 244 (Fuji Davison Chemical Ltd, Japan).
3.1.1	Lot/Batch number	JC-2108
3.1.2	Specification	Deviating from specification given in section 2 (please see 'Deficiencies').
3.1.2.1	Description	Fine white powder chemical composition SiO_2 xH_2O
3.1.2.2	Purity (%)	Not reported.
3.1.2.3	Impurities (%)	Not reported.
3.1.2.4	Density	Not reported.
3.1.2.5	Particle Size	Not reported.
3.1.2.6	Stability	Not reported. However, silicon dioxide is known to be a stable compound (melting point >1500°C).
3.2	**Test Animals**	
3.2.1	Species	Mice; Rats
3.2.2	Strain	$B_6C_3F_1$ mice; Fisher rats
3.2.3	Source	Funabashifarm Animal Co. Ltd, Japan
3.2.4	Sex	160 Male; 160 female (mice and rats)
3.2.5	Age/weight at study initiation	Mice: 21.0-27.3g (male); 16.0-19.9g (female) – 5 weeks old Rats: 117-150g (male); 92.0-126.0g (female) – 5 weeks old
3.2.6	Number of animals per group	10 per dosage group (see Table 1)
3.2.7	Control animals	Yes (10 animals, 0g test material)

Section A6.5 Chronic toxicity (Oral 1 of 1)

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Section 6: Toxicological and metabolic studies

3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Mice: 93 weeks Rats: 103 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	Overnight
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	See below
3.3.4.3	Vehicle	Not reported
3.3.4.4	Concentration in vehicle	0, 1.25, 2.5 and 5%
3.3.4.5	Total volume applied	Mice: Mean: (M/F) – 1.25%: 38.45/37.02g; 2.5%: 79.78/72.46g; 5%: 160.23/157.59g Rats: Mean: (M/F) – 1.25%: 143.46/107.25g; 2.5%: 179.55/205.02g; 5%: 581.18/435.33g
3.3.4.6	Controls	Plain diet.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Unusual signs monitored ad hoc.
3.4.1.2	Mortality	Yes. Daily.
3.4.2	Body weight	Yes. Weekly.
3.4.3	Food consumption	Yes. Weekly.
3.4.4	Water consumption	Not reported.
3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Yes. Number of animals: All animals. Time points: 24- and 48-weeks. Parameters: Erythrocytes (RBC), haemoglobin (Hb), leukocytes (WBC) and haematocrit (Ht).
3.4.7	Clinical Chemistry	Yes. Number of animals: All animals. Time points: 24- and 48-weeks. Parameters: Aspartate transaminase (AST), alanine transaminase (ALT), serum inorganic phosphorus (IP), total protein (TP), albumin (ALB), lactic dehydrogenase (LDH), alkali phosphatase (ALP), total bilirubin (TB), total cholesterol (T-Cho), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), blood urea nitrogen (BUN), uric acid (UA), creatinine (Cre) and calcium (Ca).

Section A6.5 Chronic toxicity (Oral 1 of 1)

Annex Point IIA, VI, 6.5 **Section 6: Toxicological and metabolic studies**

3.4.8	Urinalysis	No.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. All animals. Organs: Heart, liver, spleen, kidneys and brain.
3.5.2	Gross and histopathology	Yes. All animals. Organs: Lungs, bronchus, heart, kidneys, liver, spleen, brain, stomach, colon, intestines, pancreas, adrenal glands, pituitary, thyroid, salivary glands, thymus, testes, prostate, bladder, ovaries, uterus, oviducts, femoral bones, mammary glands, skin and subcutis.
3.5.3	Other examinations	Heart, liver, spleen, kidneys and brain: examined microscopically.
3.5.4	Statistics	The mean and standard deviations of various measured parameters were calculated for each dose group. The significant difference between the control and the compound-treated groups was tested using Student's t-analysis variance test. Those means showing significant differences have been marked with asterisks ($P<0.05$;*, $P<0.01$; **). The chi-square test of significance ($P<0.05$) by Mantel-Hanszel was employed to compare the survival date exclusive of sacrificed specimens. Prevalence rates were cited as percentages of tumour groups and non-tumour groups in cases of post-mortem examination. The significance of differences between the two means of prevalence was tested by using Fisher'' exact test for fourfold tables. The percentages of the frequencies of tumour in specific tissues were analysed by using the following technique: The Cochran-Armitage test for linear trend in proportion with continuity correction.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Mice and rats: No effects.
4.1.2	Mortality	Mice and rats: No effects.
4.2	**Body weight gain**	Mice and rats: No significant effects (see Tables 2, 3, 7, 8 and Figs 1, 2, 5, 6).
4.3	**Food consumption and compound intake**	Mice and rats: No significant effects (see Tables 2, 3, 7, 8).
4.4	**Ophtalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Mice and rats: No evidence of dose-related alteration (see Tables 4-1, 4-2, 9-1, 9-2).
4.5.2	Clinical chemistry	Mice and rats: No evidence of dose-related alteration (see Tables 4-1, 4-2, 9-1, 9-2).
4.5.3	Urinalysis	Not reported.

Section A6.5 Chronic toxicity (Oral 1 of 1)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Mice: Sporadic effects (atrophy or hypertrophy of organs) found sporadically. However, these were not sex- or dose-related (see Tables 5-1, 5-2, 10-1, 10-2). Rats: No evidence of dose-related alteration
4.6.2	Gross and histopathology	Mice and rats: Positive dose-related trends were not statistically significant.
4.7	**Other**	Mice: Non-neoplastic lesions were observed in the subcutis, lungs, kidneys and liver in the treated groups but these were considered to be of no toxicological significance.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	320 mice (160 of each sex) and 320 rats (160 of each sex) were used. The animals were housed in wire-mesh cages (mice: 5 animals/cage; rats: 2 animals/cage) and prior to initiation of treatment were acclimatised to the laboratory environment for 1 week (mice; 2 weeks (rat). Tap water was available ad libitum. Animal quarters were air-conditioned with thermostats set to maintain 23±1°C room temperature continuously and 50±10% humidity; artificial fluorescent lighting was provided daily for a continuous 14-hour period. Animals were separated according to sex and by standard randomization 5 mice were put in one cage and 2 rats per cage. Mice and rats were dined into dosage groups of 10 animals each. The test materials which were prepared weekly were administered orally each day at the prescribed dosage levels.
5.2	**Results and discussion**	The repeated oral administration of the test substance produced no significant treatment-related effects in mice and rats.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Not reported.
5.3.3	Other	None.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that urinalysis parameters were not measured. However, with other measured parameters showing no significant effects, this does not appear to adversely affect the study. There are deficiencies in the reporting as no NOAEL value is stated. The test material used in this study is not identical to that as given in Section 2. It is however acknowledged to be a synthetic amorphous silica (the same as the substance that is being supported) therefore it is felt that this is highly relevant. Despite the deficiencies in this study, it gives an indication of the level

Section A6.5 — **Chronic toxicity (Oral 1 of 1)**

Annex Point IIA, VI, 6.5 — **Section 6: Toxicological and metabolic studies**

of silicon dioxide that can be tolerated without effect by mammals.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 1: Experimental Design of Chronic Toxicity Test with Carcinogen Observation of SYLOID

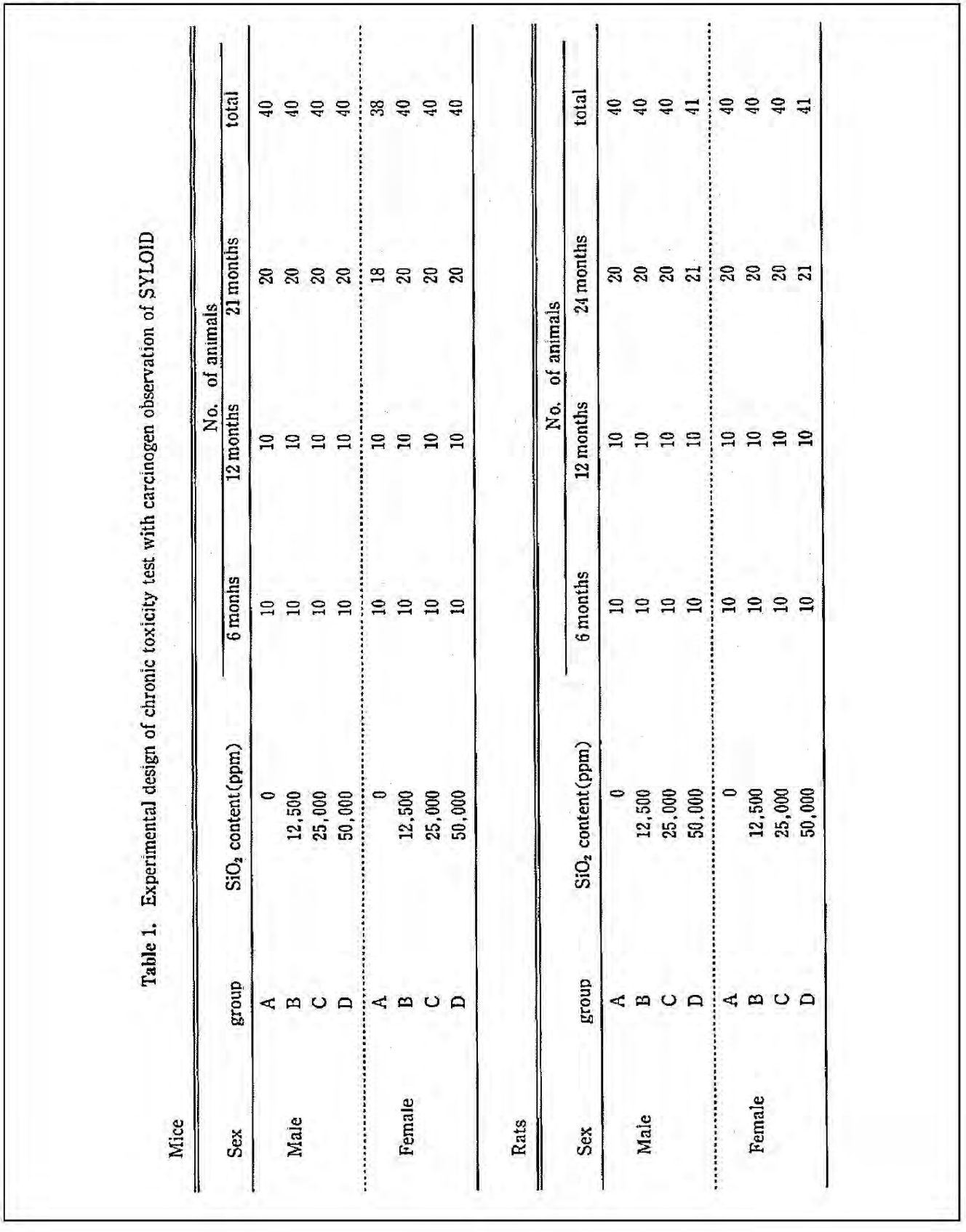

Table 1. Experimental design of chronic toxicity test with carcinogen observation of SYLOID

Mice

Sex	group	SiO_2 content(ppm)	No. of animals			
			6 months	12 months	21 months	total
Male	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	20	40
Female	A	0	10	10	18	38
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	20	40

Rats

Sex	group	SiO_2 content(ppm)	No. of animals			
			6 months	12 months	24 months	total
Male	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	21	41
Female	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	21	41

Table 2: Group Mean Body Weights, Food Intake and Intake of SYLOID for Male Mice in Lifespan Study

Table 2. Group mean body weights, food intake, and intake of SYLOID for male mice in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	93
0 (Control)	Body weight (g)	23.9±1.2	30.5±2.0	42.7±3.5	48.0±3.4	50.4±3.4	46.7±4.9	44.2±6.3
	Food intake (g/day)		5.0±0.55	5.2±0.54	4.7±0.74	4.7±0.21	4.2±0.29	3.8±0.70
1.25	Body weight (g)	24.8±1.1**	31.9±2.3**	42.9±3.8	48.9±4.0	50.2±4.0	45.0±3.7	40.5±6.1
	Food intake (g/day)		4.4±0.19*	5.6±0.61	4.9±0.18	4.8±0.05	4.3±0.62	3.7±0.73
	Daily intake of SYLOID(g/kg/day)		1.88	1.63	1.23	1.20	1.22	1.24
	Cumulative dose of SYLOID (g/mouse)		1.90	6.84	13.42	21.90	34.35	38.45
2.5	Body weight (g)	24.1±1.5	31.3±2.6	40.8±7.2	46.5±4.7	47.7±5.2*	44.0±6.1	41.5±6.3
	Food intake (g/day)		4.4±0.36*	5.9±0.74*	4.7±0.28	4.9±0.34	4.9±0.24**	4.7±0.34
	Daily intake of SYLOID (g/kg/day)		3.51	3.61	2.58	2.52	2.67	2.89
	Cumulative dose of SYLOID (g/mouse)		3.63	13.73	26.42	42.88	69.62	79.78
5.0	Body weight (g)	24.3±1.1	30.7±1.8	39.2±2.8**	44.1±3.9**	47.4±4.0**	44.5±4.2	43.4±5.4
	Food intake (g/day)		4.6±0.21	5.3±0.42	4.8±0.23	4.9±0.18	5.2±0.26**	4.7±0.48
	Daily intake of SYLOID (g/kg/day)		7.49	7.14	5.44	5.27	6.07	5.53
	Cumulative dose of SYLOID (g/mouse)		7.64	26.77	52.68	86.59	139.15	160.23

*; Significantly different ($P<0.05$) **; Significantly different($P<0.01$)

Table 3: Group Mean Body Weights, Food Intake and Intake of SYLOID for Female Mice in Lifespan Study

Table 3. Group mean body weights, food intake, and intake of SYLOID for female mice in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	93
0 (Control)	Body weight (g)	18.3±0.8	23.8±1.8	35.0±4.3	42.9±5.3	50.9±5.3	54.8±4.4	53.3±7.6
	Food intake (g/day)		4.3±0.59	7.4±1.80	4.0±0.40	4.4±0.64	4.3±0.83	3.4±0.49
1.25	Body weight (g)	18.5±1.0	23.0±1.7**	35.0±4.4	42.7±5.6	48.9±5.4	55.3±4.5	54.7±6.1
	Food intake (g/day)		3.6±0.26**	7.1±1.46	3.7±0.27	3.9±0.24	4.7±0.39	4.1±0.36
	Daily intake of SYLOID (g/kg/day)		2.17	2.57	1.17	1.02	1.08	1.91
	Cumulative dose of SYLOID (g/mouse)		1.49	6.87	12.64	19.71	32.26	37.02
2.5	Body weight (g)	18.9±0.8**	23.4±1.5	35.3±3.2	42.6±4.5	49.0±4.8	56.1±4.7	54.8±6.5
	Food intake (g/day)		3.6±0.28**	7.1±1.94	3.4±0.36*	4.1±0.16	4.3±0.14	4.1±0.15*
	Daily intake of SYLOID (g/kg/day)		3.85	4.90	1.86	2.04	1.96	1.82
	Cumulative dose of SYLOID (g/mouse)		2.89	13.73	25.40	39.07	63.34	72.46
5.0	Body weight(g)	18.8±0.9	23.3±1.5	33.8±3.8	39.5±5.8*	47.1±6.6*	55.7±4.3	55.7±5.8
	Food intake (g/day)		3.9±0.38	8.1±0.94	3.8±0.38	4.3±0.41	4.8±0.05	4.4±0.41*
	Daily intake of SYLOID (g/kg/day)		8.58	13.31	4.81	4.67	4.31	3.95
	Cumulative dose of SYLOID (g/mouse)		6.10	29.97	56.75	86.56	138.79	157.59

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Fig. 1: Growth Curves for Male Mice by Dose

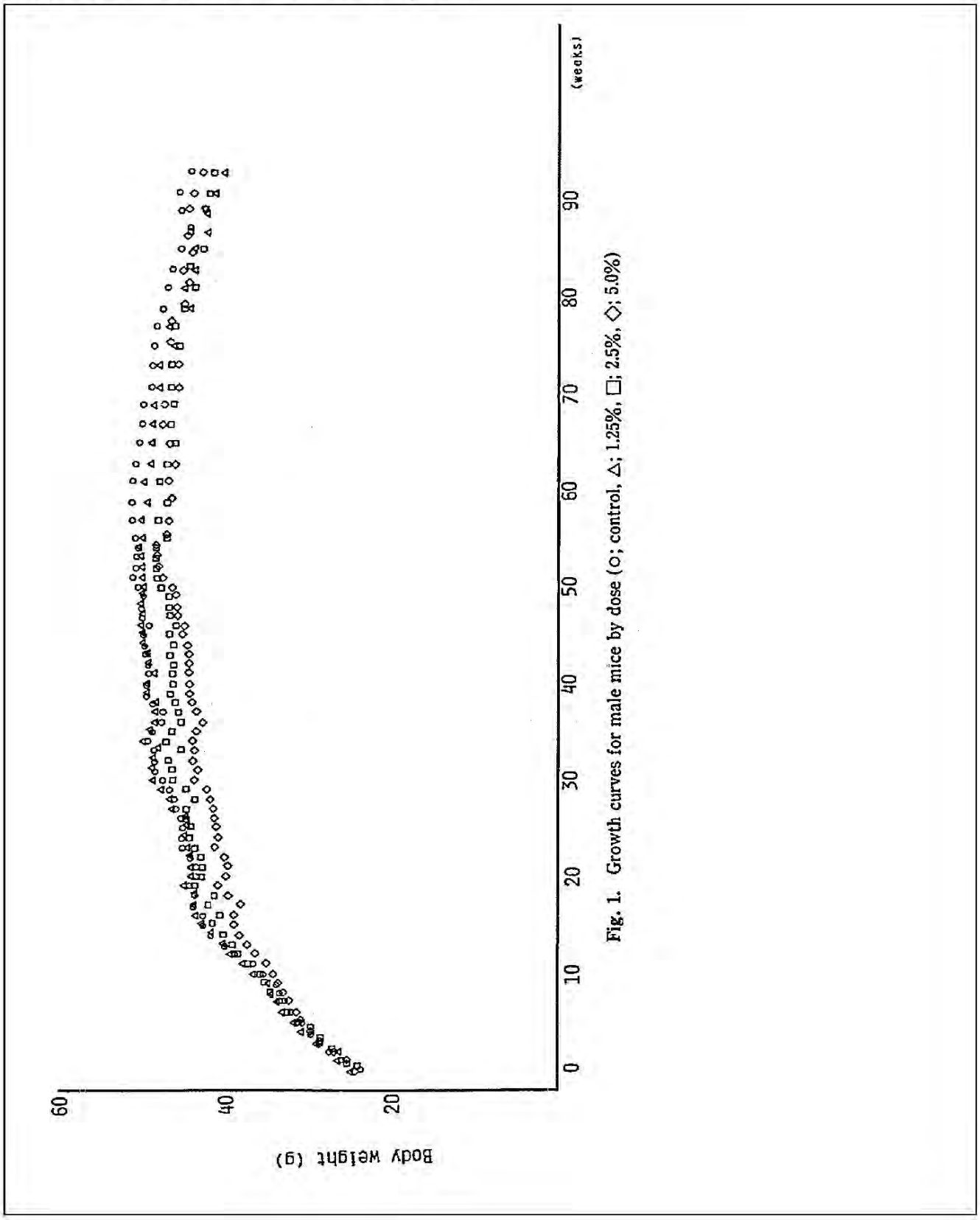

Fig. 1. Growth curves for male mice by dose (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

Fig. 2: Growth Curves for Female Mice by Dose

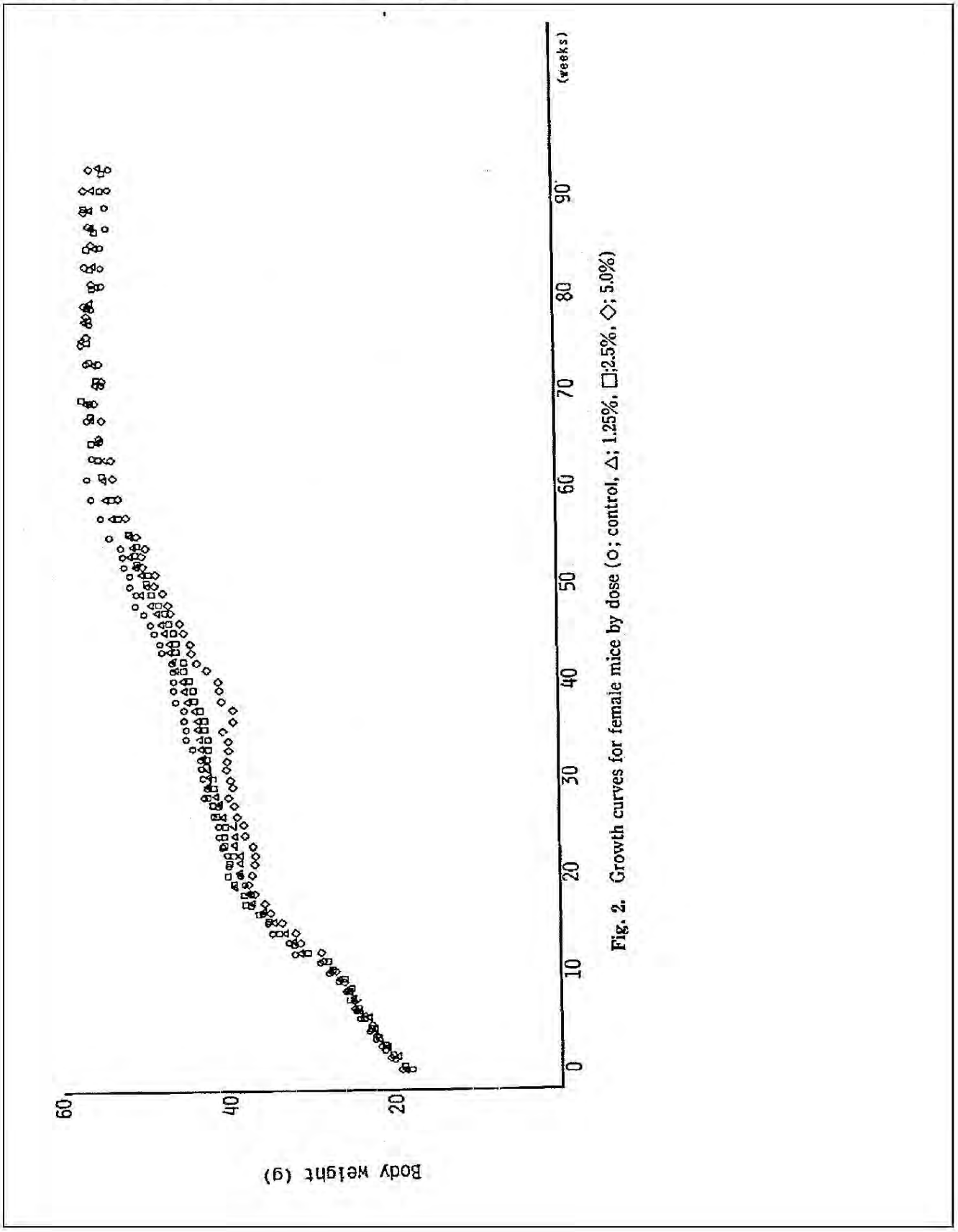

Fig. 2. Growth curves for female mice by dose (○; control, △; 1.25%, □;2.5%, ◇; 5.0%)

Fig. 3 and 4: Survival curves for Male and Female Mice by Dose

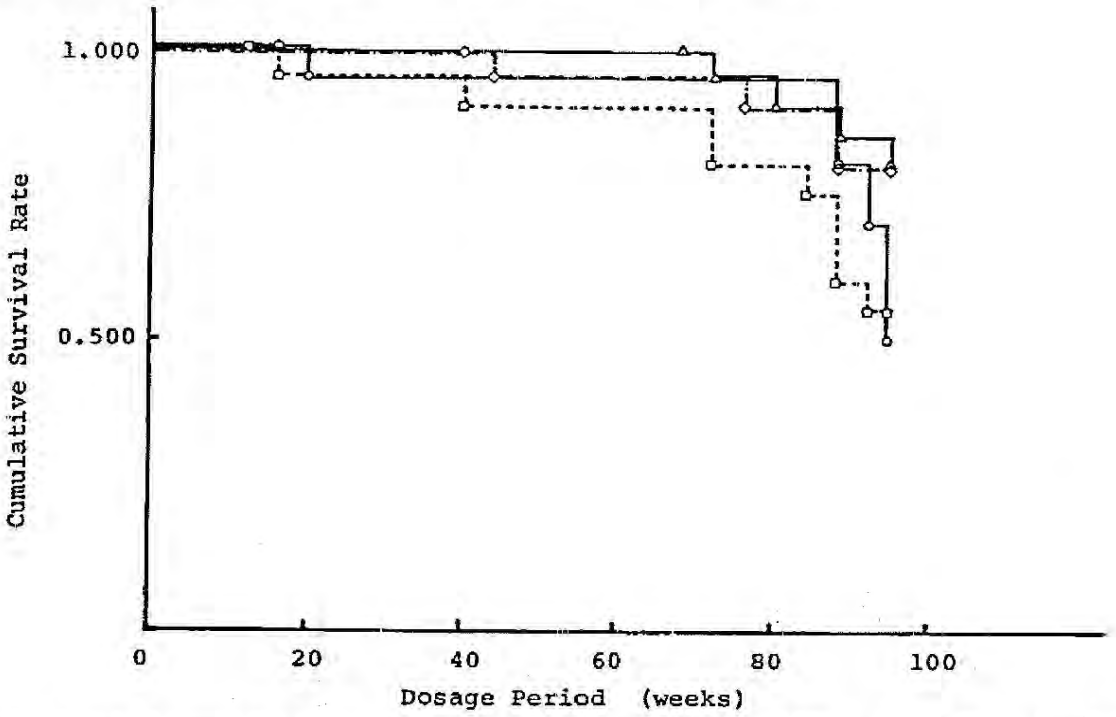

Fig. 3. Survival curves for male mice by dose (—○—; control, —△—; 1.25%, --□--; 2.5%, --◇--; 5.0%)

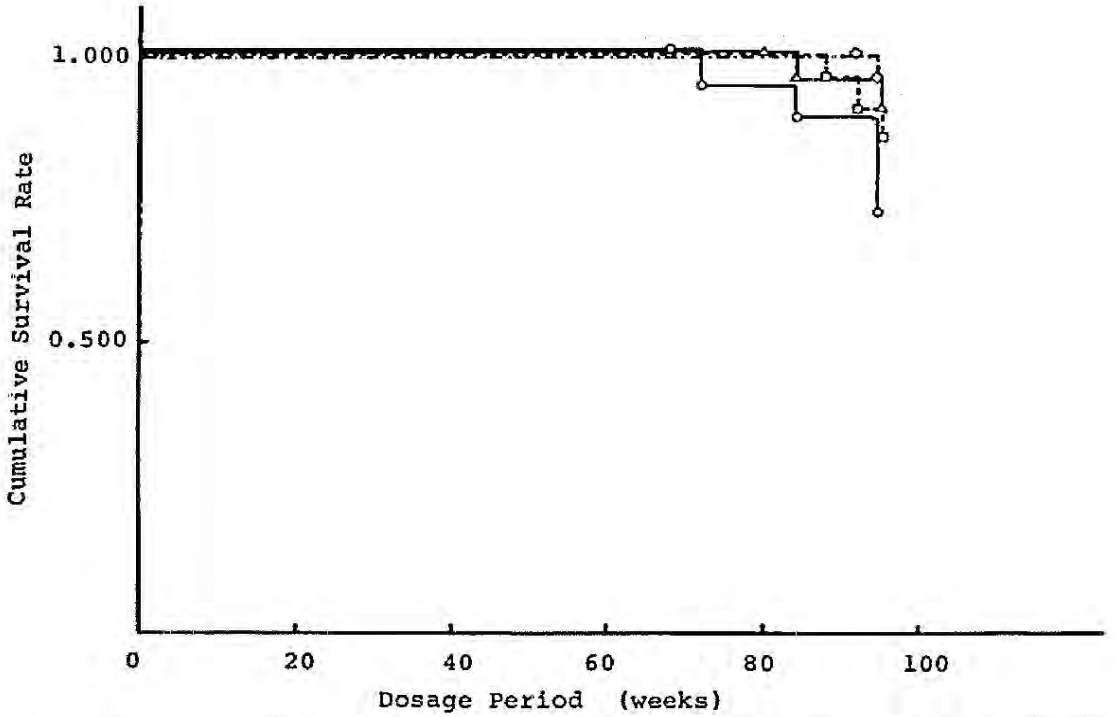

Fig. 4. Survival curves for female mice by dose (—○—; control, —△—; 1.25%, --□--; 2.5%, --◇--; 5.0%)

Table 4-1: Blood Chemistry Observations of Mice by Sex (Male)

Table 4-1. Blood chemistry observations of mice by sex (Male)

Dosage period (M)	Group (%)	No. of animals	RBC Count (10^4/dl)	RBC HGB (g/dl)	RBC HCT (%)	RBC MCV (fl)	RBC MCH (pg)	RBC MCHC (%)	WBC ($\times10^3$/dl)	Platelet ($\times10^3$/dl)
6	0	6	1005.0± 50.3	15.1±0.59	49.7± 1.87	50.7±0.82	15.4±0.31	30.4±0.15	5.40±2.50	1354.0± 81.5
	1.25	8	989.0± 42.3	15.2±0.62	50.7± 2.16	51.9±1.13	15.1±0.29	30.0±0.28	5.30±1.67	1191.0±118.0
	2.5	1								
	5.0	4	960.0±143.0	14.8±1.89	48.8± 7.62	52.0±1.63	15.9±0.19	30.5±1.09	4.00±2.64	686.0± 35.7
12	0	7	1011.5± 58.2	13.4±3.15	58.6± 4.13	59.6±3.21	15.1±0.72	25.3±0.30	4.20±1.34	1519.0±164.5
	1.25	7	1035.0± 52.3	14.8±0.44	59.1± 1.71	59.7±1.25	14.9±0.29	25.0±0.21	5.60±2.44	1406.0±187.5
	2.5	5	1051.0± 41.7	14.7±0.90	58.1± 3.82	58.2±3.56	14.8±0.89	25.4±0.37	3.60±1.08	1802.0±115.4**
	5.0	7	1052.0± 91.2	15.2±1.06	60.4± 4.31	61.3±3.30	15.4±0.83	25.1±0.47	4.30±0.92	1304.0±289.5
21	0	9	896.3± 44.4	13.2±0.58	49.1± 1.78	54.9±1.64	14.7±0.27	26.8±0.45	7.10±2.93	1430.0±156.0
	1.25	12	1126.0±303.2*	14.9±2.49*	54.8± 7.42*	58.3±4.50*	15.5±1.14*	26.8±0.63	5.30±2.37	1410.0±321.9
	2.5	10	854.9± 49.1	12.7±0.90	50.5± 7.62	56.9±5.07	15.0±0.96	27.0±0.70	7.20±1.80	1559.0±152.9
	5.0	13	762.4±195.3*	12.3±2.94	44.7±11.10	55.5±2.11	16.1±1.85*	27.3±0.61	6.76±2.77	1344.0±234.8

Mean±SD was calculated by the method of rejection limit from Smirnov
*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 4-2: Blood Chemistry Observations of Mice by Sex (Female)

Table 4-2. Blood chemistry observations of mice by sex (Female)

Dosage period (M)	Group (%)	No. of animals	RBC Count (10^4/dl)	RBC HGB (g/dl)	RBC HCT (%)	RBC MCV (fl)	RBC MCH (pg)	RBC MCHC (%)	WBC ($\times10^3$/dl)	Platelet ($\times10^3$/dl)
6	0	1								
	1.25	7	1028.0± 43.6	15.9±0.53	53.2±1.69	53.4±1.27	16.0±0.43	29.9±0.43	4.30±1.10	978.0± 77.6
	2.5	0								
	5.0	4	1004.0± 28.0	15.8±0	51.9±0.75	52.2±0.50	15.9±0.10	30.5±0.44		1075.0±299.5
12	0	9	936.2± 24.4	14.3±0.41	55.5±1.67	59.2±1.20	15.3±0.20	25.8±0.19	3.40±2.30	1213.0±130.9
	1.25	7	964.3± 32.7	14.5±0.40	56.9±1.59	59.0±1.29	15.2±0.36	25.7±0.17	4.30±1.57	1170.0±135.1
	2.5	9	952.2± 53.4	14.6±0.85	56.6±3.23	59.4±1.01	15.3±0.27	25.8±0.37	2.20±0.84	1070.0±148.4
	5.0	8	913.5± 73.5	14.5±0.82	45.9±8.12*	49.9±5.03**	15.9±0.86	32.2±4.84**	2.30±0.69	941.1±164.2
21	0	12	929.5± 97.4	14.2±0.82	52.7±4.89	59.3±2.2	15.6±0.56	26.4±0.37	5.00±2.44	902.1±262.8
	1.25	17	884.0± 91.2	14.1±0.91	54.5±2.33	60.1±1.76	15.7±0.37	26.2±0.22	2.80±0.81*	1035.0±159.3
	2.5	17	869.8±100.0	14.1±0.52	54.3±1.29	59.8±1.17	15.6±0.12	26.2±0.32	3.70±1.89	973.4±268.7
	5.0	14	823.6±181.4	14.1±0.88	54.3±1.80	59.7±1.25	15.7±0.42	26.3±0.33	3.50±1.22	897.2±130.6

Mean±SD was calculated by the method of rejection limit from Smirnov
*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 5-1: Group Mean Organ Weights of Liver, Kidney, Spleen, Heart and Brain for Mice (Male)

Teble 5-1. Group mean organ weights of liver, kidney, spleen, heart, and brain for mice (Male)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	1.54±0.20	0.31±0.04	0.10±0.02	0.19±0.02	0.47±0.02
	1.25	10	1.51±0.13	0.32±0.03	0.10±0.01	0.19±0.02	0.48±0.02
	2.5	10	1.74±0.23	0.36±0.03**	0.11±0.01	0.21±0.02*	0.45±0.03
	5.0	10	1.53±0.19	0.34±0.03	0.08±0.009*	0.19±0.02	0.45±0.03
12	0	10	1.65±0.08	0.35±0.02	0.11±0.02	0.22±0.02	0.44±0.03
	1.25	10	1.81±0.14**	0.35±0.03	0.12±0.02	0.22±0.02	0.48±0.04*
	2.5	10	1.75±0.12	0.32±0.02**	0.10±0.01	0.20±0.02**	0.47±0.02*
	5.0	10	1.66±0.16	0.35±0.02	0.10±0.01	0.21±0.02	0.47±0.04
21	0	10	1.82±0.33	0.32±0.05	0.12±0.03	0.23±0.02	0.49±0.03
	1.25	16	2.07±0.90	0.33±0.06	0.12±0.05	0.21±0.04	0.46±0.03*
	2.5	11	1.86±0.25	0.34±0.07	0.13±0.04	0.21±0.02*	0.45±0.06
	5.0	16	1.81±0.16	0.36±0.06	0.13±0.04	0.22±0.03	0.46±0.03*

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 5-2: Group Mean Organ Weights of Liver, Kidney, Spleen, Heart and Brain for Mice (Female)

Teble 5-2. Group mean Organ weights of liver, kidney, spleen, heart, and brain for mice (Female)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	1.39±0.19	0.27±0.05	0.11±0.03	0.16±0.02	0.50±0.03
	1.25	10	1.28±0.17	0.23±0.04	0.10±0.02	0.15±0.01	0.49±0.02
	2.5	10	1.31±0.12	0.23±0.02*	0.11±0.02	0.13±0.01**	0.49±0.04
	5.0	10	1.17±0.08**	0.21±0.03**	0.09±0.009	0.13±0.01**	0.46±0.02**
12	0	10	0.51±0.22	1.27±0.05	0.11±0.02	0.17±0.02	0.48±0.04
	1.25	10	1.49±0.24	0.23±0.03*	0.11±0.02	0.16±0.03	0.49±0.01
	2.5	10	1.43±0.19	0.21±0.01**	0.09±0.02*	0.15±0.01*	0.52±0.02*
	5.0	10	1.37±0.14	0.24±0.02	0.10±0.01	0.14±0.01*	0.48±0.01
21	0	13	1.66±0.29	0.26±0.01	0.22±0.06	0.16±0.03	0.49±0.02
	1.25	18	1.81±0.36	0.26±0.05	0.18±0.04	0.18±0.04	0.49±0.03
	2.5	17	1.91±0.38	0.34±0.05**	0.24±0.08	0.17±0.02	0.46±0.03**
	5.0	19	1.76±0.29	0.28±0.03*	0.19±0.08	0.17±0.02	0.48±0.03

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 7: Group Mean Body Weights, Food Intake and Intake of SYLOID for Male Rats in Lifespan Study

Table 7. Group mean body wights, food intake, and intake of SYLOID for male rats in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	103
0 (Control)	Body weight (g)	134.6± 7.8	273.5±11.8	376.3±16.4	428.9±20.4	470.0±27.4	455.7±37.0	420.0±55.6
	Food intake (g/day)		15.9± 0.48	15.1± 0.38	15.2± 0.78	15.4± 0.65	14.7± 0.41	15.6± 2.93
1.25	Body weight (g)	134.2± 9.5	280.5±13.0*	396.6±18.9**	458.9±22.4**	498.5±24.6**	467.6±36.9	453.2±65.6
	Food intake (g/day)		16.4± 0.34*	16.1± 0.52**	16.4± 0.70*	16.1± 0.19*	15.2± 0.82	16.3± 1.45
	Daily intake of SYLOID (g/kg/day)		0.71	0.50	0.46	0.40	0.41	0.44
	Cumulative dose of SYLOID (g/rat)		6.71	21.19	42.96	72.58	115.64	143.46
2.5	Body weight (g)	134.3± 8.5	274.0±11.7	386.3±16.4**	438.8±19.2	477.9±19.6	464.4±24.8	429.2±67.1
	Food intake (g/day)		16.0± 0.39	15.9± 0.48**	15.5± 0.88	16.2± 0.78	15.1± 1.06	14.5± 3.39
	Daily intake of SYLOID (g/kg/day)		1.46	1.04	0.89	0.86	0.83	0.85
	Cumulative dose of SYLOID (g/day)		12.95	41.05	81.44	137.68	225.01	179.55
5.0	Body weight (g)	133.4± 6.9	272.9±11.4	377.2±16.0	427.7±17.2	458.7±27.3	464.8±31.6	423.6±70.1
	Food intake (g/day)		16.5± 0.56*	15.9± 0.47**	15.6± 0.43	16.2± 0.33*	16.2± 0.75*	15.4± 2.93
	Daily intake of SYLOID (g/kg/day)		3.0	2.12	1.85	1.76	1.77	1.83
	Cumulative dose of SYLOID (g/rat)		27.24	84.01	166.29	280.37	463.22	581.18

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 8: Group Mean Body Weights, Food Intake and Intake of SYLOID for Female Rats in Lifespan Study

Table 8. Group mean body wights, food intake, and intake of SYLOID for female rats in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	103
0 (Control)	Body weight (g)	112.0± 5.9	176.9± 7.3	225.5±11.2	258.8±12.9	327.0±19.8	371.4±30.0	391.0±21.8
	Food intake (g/day)		10.4± 0.28	10.0± 0.35	10.2± 0.85	11.3± 0.36	12.1± 0.62	15.4± 4.08
1.25	Body weight (g)	110.9± 6.0	174.0± 8.8	222.9±12.9	257.7±17.4	323.3±33.2	383.4±34.0	402.2±70.4
	Food intake (g/day)		10.3± 0.27	9.8± 0.38	10.4± 0.53	11.9± 0.37*	12.4± 0.32	14.9± 2.84
	Daily intake of SYLOID (g/kg/day)		0.75	0.54	0.50	0.46	0.40	0.48
	Cumulative dose of SYLOID (g/rat)		4.70	13.67	27.56	48.40	82.85	107.25
2.5	Body weight (g)	110.0± 6.0	173.0± 8.4*	221.0±10.7	248.6±15.0**	306.1±24.1**	357.2±27.7	360.9±47.4*
	Food intake (g/day)		10.2± 0.45	9.8± 0.27	9.8± 0.42	11.8± 0.76	11.7± 1.43	13.0± 1.70
	Daily intake of SYLOID (g/kg/day)		1.45	1.13	1.00	0.98	0.83	0.88
	Cumulative dose of SYLOID (g/rat)		9.38	27.26	53.60	93.31	159.34	205.02
5.0	Body weight (g)	108.5± 6.0**	174.2± 8.8	223.2±10.8	252.9±10.4	309.7±17.7**	363.6±25.7	358.5±56.4*
	Food intake (g/day)		11.1± 0.42**	10.4± 0.37*	10.2± 0.46	11.9± 0.84*	13.2± 0.83	12.7± 2.67
	Daily intake of SYLOID (g/kg/day)		3.21	2.33	2.02	1.94	1.81	1.78
	Cumulative dose of SYLOID (g/rat)		20.28	58.45	114.18	197.53	335.19	435.33

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Fig. 5: Growth Curves for Male Rats fed Various Diets

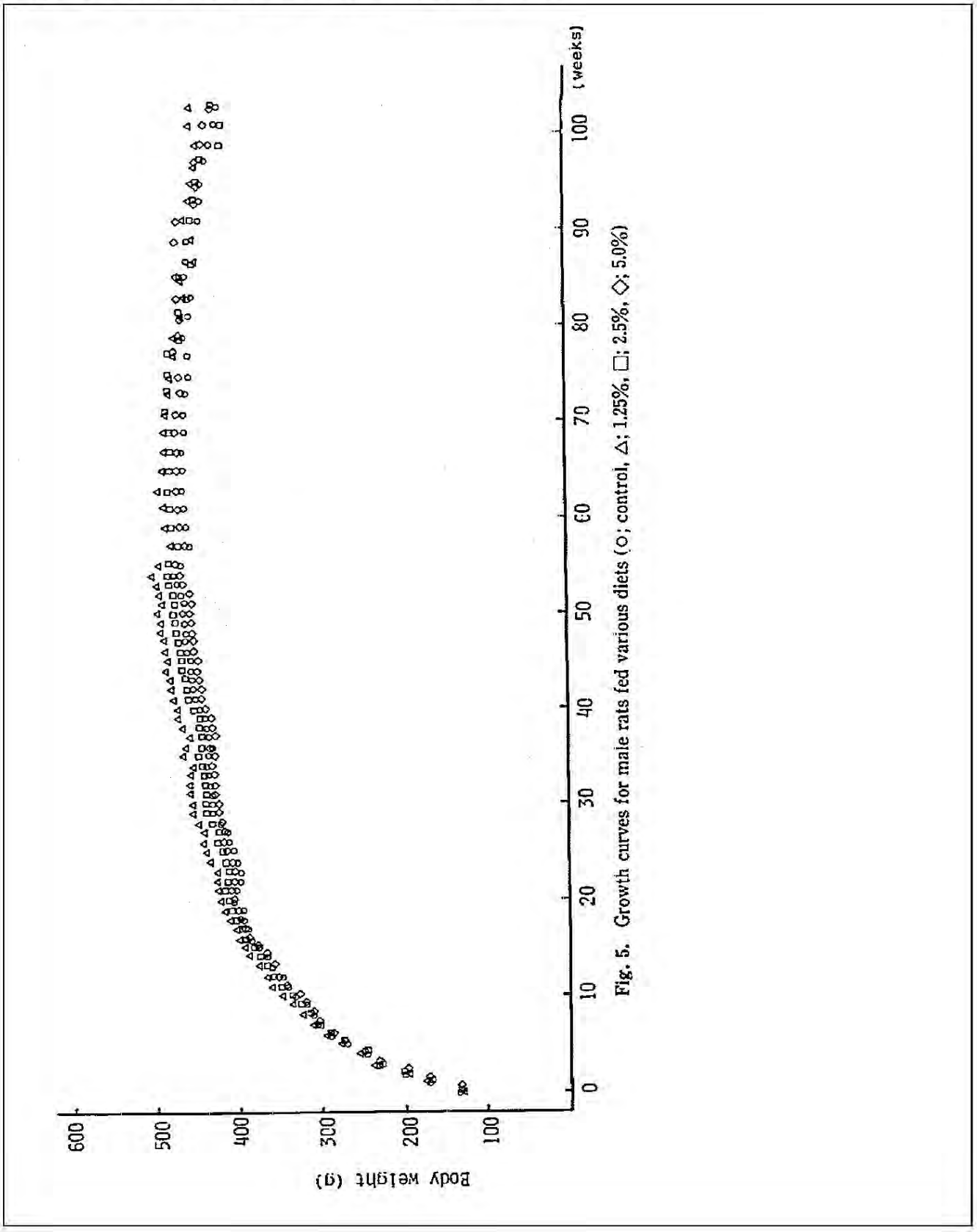

Fig. 5. Growth curves for male rats fed various diets (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

Fig. 6: Growth Curves for Female Rats fed Various Diets

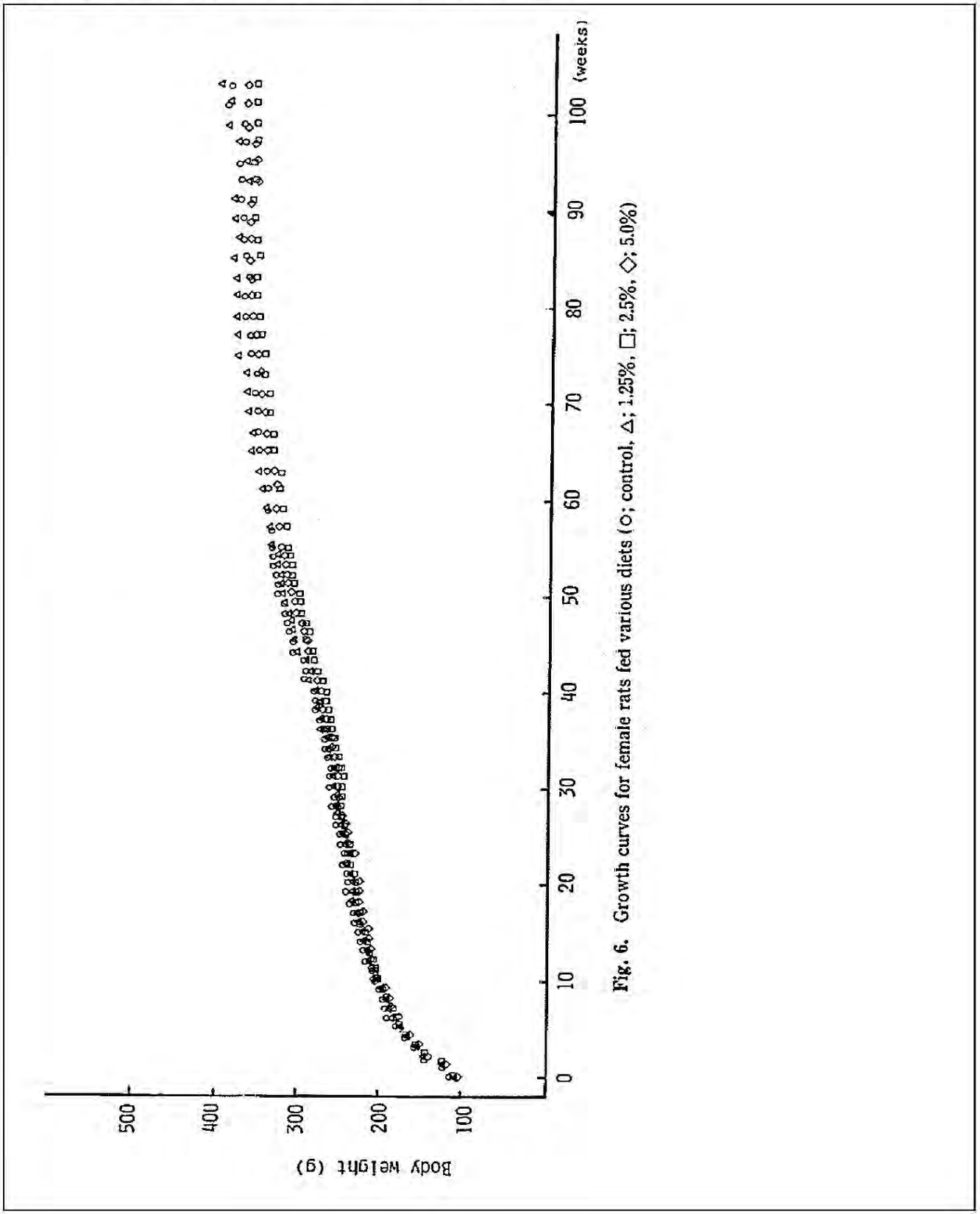

Fig. 6. Growth curves for female rats fed various diets (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

Fig.7 and 8: Survival curves for Male and Female Rats by Dose

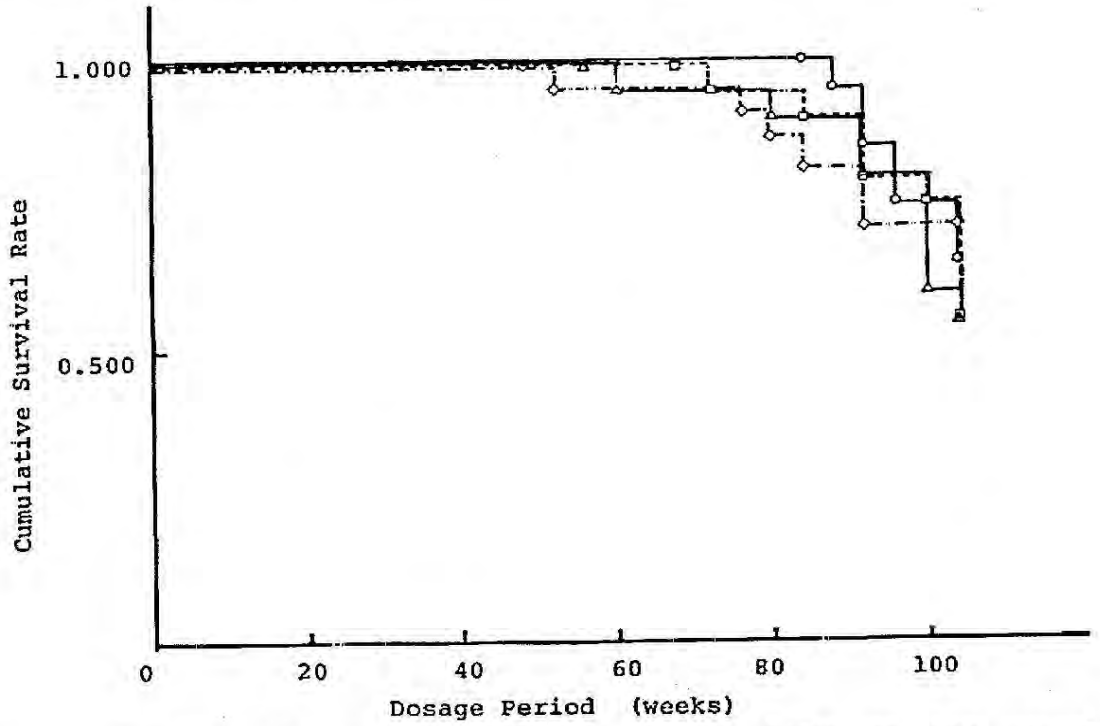

Fig. 7. Survival curves for male rats by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, —◇—; 5.0%)

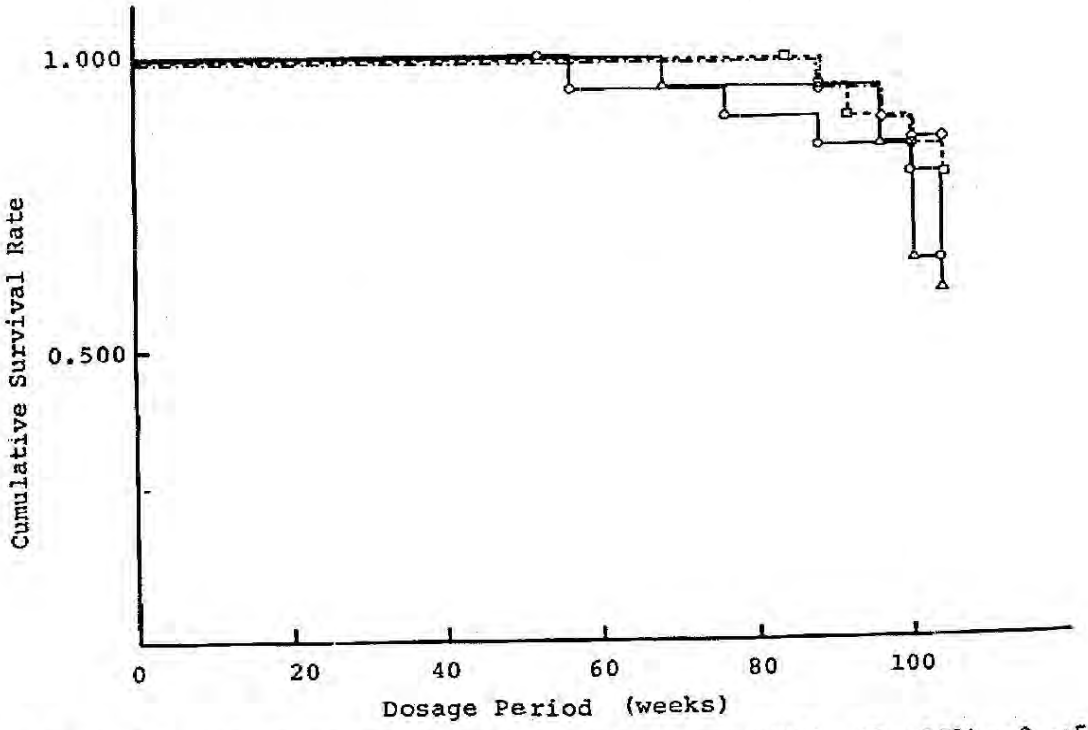

Fig. 8. Survival curves for female rats by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, —◇—; 5.0%)

Table 9-1: Haematological Observations of Rats by Sex (Male)

Table 9-1. Hematological observations of rats by sex (Male)

Dosage period (M)	Group (%)	No. of animals	R B C					WBC ($\times 10^3$/dl)	Platelet ($\times 10^3$/dl)
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (%)		
6	0	8	974.3± 59.0	16.5±0.92	51.4±3.07	17.1±0.08	32.3±0.20	5.30± 2.48	642.9± 59.4
	1.25	10	1003.0± 18.1	16.7±0.35	52.4±1.89	16.7±0.35**	31.8±0.25**	6.20± 1.16	731.6± 44.1**
	2.5	8	965.3± 29.4	16.7±0.33	50.9±1.29	17.2±0.32	32.6±0.64	5.70± 1.87	688.3± 44.9
	5.0	9	993.0± 24.8	16.6±0.79	51.9±1.97	16.9±0.20*	31.9±0.65	5.16± 1.19	686.0± 35.7
12	0	10	1011.0± 41.5	16.1±0.72	64.0±2.86	16.2±0.73	25.1±0.16	3.9± 1.30	627.4± 54.8
	1.25	10	983.6± 9.4	16.2±0.37	64.6±1.03	16.4±0.33	25.0±0.31	4.7± 0.85	631.0± 28.0
	2.5	10	979.3± 16.0	16.2±0.21	64.7±0.91	16.5±0.24	25.1±0.17	5.1± 0.94*	671.4± 25.8
	5.0	9	973.1± 41.3	15.9±0.61	63.1±2.39	16.3±0.21	25.2±0.29	4.5± 0.38	662.9± 26.4
24	0	12	810.4±222.3	11.8±3.51	45.6±14.5	14.6±3.65	25.7±0.56	8.5± 6.26	991.2±374.9
	1.25	9	740.9±142.0	12.2±3.05	48.5±12.2	16.9±0.63	25.2±0.35**	23.3±22.70	716.0±177.4
	2.5	10	775.5±286.2	12.7±5.49	48.6±20.9	16.3±4.28	26.2±1.89	4.5± 0.91	689.9± 71.4*
	5.0	13	723.1±226.6	11.8±4.21	45.8±17.3	15.6±3.25	26.2±2.38	7.2± 4.62	695.1±239.7*

Mean±SD was calculated by the method of rejection limit form Smirnov
*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 9-2: Haematological Observations of Rats by Sex (Female)

Table 9-2. Hematological observations of rats by sex (Female)

Dosage period (M)	Group (%)	No. of animals	RBC Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (%)	WBC ($\times10^3$/dl)	Platelet ($\times10^3$/dl)
6	0	6	894.8± 36.0	16.6±0.73	51.1± 2.24	18.6±0.25	32.5±0.61	3.38±0.83	696.8± 23.7
	1.25	10	889.4± 24.4	16.4±0.55	52.0± 0.91	18.5±0.28	31.8±0.87	5.21±1.32**	683.9± 64.9
	2.5	9	883.0± 40.4	16.4±0.78	51.1± 2.38	18.6±0.20	32.1±0.80	4.01±1.68	700.0± 41.5
	5.0	9	883.6± 34.4	16.3±0.63	51.2± 2.44	18.4±0.42	31.8±0.85	4.58±1.61	648.3±194.3
12	0	10	883.7± 14.6	15.7±0.25	62.5± 0.99	17.7±0.23	25.1±0.29	3.3 ±0.46	574.9± 30.1
	1.25	9	876.3± 14.0	15.5±0.25	62.0± 1.04	17.7±0.21	25.0±0.23	3.1 ±0.55	598.1± 56.7
	2.5	9	880.0± 35.4	15.6±0.72	62.2± 2.41	17.7±0.26	25.1±0.35	3.4 ±0.29	601.0± 55.4
	5.0	9	890.0± 11.8	15.9±0.22	63.0± 0.73	17.8±0.44	25.2±0.14	3.0 ±0.60	606.9± 27.7*
24	0	13	790.0± 33.8	14.4±0.56	55.7± 2.35	18.3±0.46	25.9±0.35	6.0 ±2.59	662.0±144.6
	1.25	9	813.4± 39.2	14.6±0.68	57.2± 2.46	17.9±0.66	25.6±2.70	5.0 ±1.28	668.4±159.9
	2.5	14	668.0±155.3**	12.1±2.85*	46.5±11.50*	18.4±3.70	26.0±1.56	6.0 ±2.78	732.1±324.2
	5.0	16	785.9± 56.9	14.3±0.86	56.2± 2.52	18.2±0.54	25.8±0.42	4.8 ±1.96	741.9±127.7

Mean±SD was calculated by the method of rejection limit from Smirnov
*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 10-1: Group Mean Organ Weights of Liver, Kidney, Spleen, Heart and Brain for Mice (Male)

Table 10-1. Group mean organ weights of liver, kidney, spleen, heart, and brain for rats (Male)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	9.08±0.39	1.09±0.03	0.69±0.03	1.02±0.13	1.96±0.05
	1.25	10	10.10±0.61**	1.10±0.05	0.77±0.05**	1.05±0.07	2.00±0.04
	2.5	10	9.41±0.39	1.16±0.14	0.75±0.06*	1.01±0.05	1.85±0.08**
	5.0	10	9.43±0.97	1.12±0.07	0.70±0.03	1.06±0.09	2.02±0.07*
12	0	10	9.76±0.32	1.19±0.08	0.81±0.05	1.17±0.06	1.93±0.10
	1.25	10	10.99±0.78**	1.23±0.08	0.90±0.08**	1.22±0.08	2.09±0.04**
	2.5	10	10.26±0.62	1.17±0.08	0.85±0.04	1.12±0.05	1.92±0.07
	5.0	10	10.24±0.51**	1.26±0.06**	0.81±0.05	1.10±0.05**	2.13±0.08**
21	0	13	12.42±2.09	1.32±0.16	1.19±0.36	1.55±0.24	2.06±0.11
	1.25	11	13.20±1.20	1.50±0.21*	2.64±1.66*	1.52±0.21	2.08±0.06
	2.5	11	12.10±1.10	1.35±0.11	1.24±0.32	1.45±0.29	2.03±0.05
	5.0	15	12.45±2.60	1.54±0.59	1.69±1.09	1.52±0.19	2.01±0.11

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 10-2: Group Mean Organ Weights of Liver, Kidney, Spleen, Heart and Brain for Mice (Female)

Table 10-2. Group mean organ weights of liver, kidney, spleen, heart, and brain for rats (Female)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	5.42±0.57	0.76±0.09	0.52±0.03	0.74±0.08	1.86±0.07
	1.25	10	5.68±0.51	0.79±0.08	0.51±0.03	0.75±0.06	1.91±0.04
	2.5	10	5.44±0.33	0.70±0.05	0.49±0.03*	0.67±0.06*	1.08±0.07
	5.0	10	5.30±0.37	0.70±0.07	0.47±0.03**	0.64±0.05**	1.80±0.05**
12	0	10	7.52±0.24	0.84±0.05	0.63±0.04	0.77±0.05	1.86±0.07
	1.25	10	7.18±0.52	0.77±0.07*	0.62±0.04	0.77±0.07	1.89±0.05
	2.5	10	6.96±0.43**	0.79±0.06	0.57±0.04**	0.77±0.04	1.80±0.08
	5.0	10	7.01±0.29**	0.80±0.06	0.58±0.04	0.74±0.06	1.92±0.10
21	0	13	11.23±1.34	1.03±0.12	0.87±0.26	0.91±0.10	1.86±0.07
	1.25	12	11.13±2.33	1.09±0.09	0.84±0.16	0.95±0.07	1.91±0.09
	2.5	16	9.69±1.32**	1.01±0.08	2.12±2.70	0.92±0.09	1.84±0.08
	5.0	18	9.57±1.51**	1.06±0.13	0.80±0.19	0.88±0.09	1.81±0.12

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Section A6.5 Chronic toxicity (Inhalation 3 of 3)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable, published data.
1.2.2	Companies with letter of access	Not applicable, published data.
1.2.3	Criteria for data protection	No data protection claimed.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Not carried out to guideline B.30 in Annex V of Directive 67/548/EC
2.2	**GLP**	No. Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.
		3 MATERIALS AND METHODS
3.1	**Test material**	3 types of amorphous silica: Silica G: Silica gel, Silica P: Precipitated silica and Silica F: Fume silica No other information available on the specification or purity of the test materials.
3.1.1	Lot/Batch number	Not reported.
3.1.2	Specification	Not reported.
3.1.2.1	Description	Not reported.
3.1.2.2	Purity	Not reported.
3.1.2.3	Stability	Not reported. However, silicon dioxide is known to be a stable compound (melting point >1500°C).
3.2	**Test Animals**	
3.2.1	Species	Rats; Guinea pigs; Monkeys
3.2.2	Strain	Sprague-Dawley rats; Hartley guinea pigs; Cynomolgus, *Macaca fasicularis* monkeys
3.2.3	Source	Rats - Laboratory Supply Company, Inc, Indianapolis, Ind Guinea pigs - Sweetwater Farms, Hillsboro, Ohio Monkeys - Primate Imports Corp., Long Island, N.Y.
3.2.4	Sex	Male.
3.2.5	Age/weight at study initiation	Age not reported. Rats - 300-380g

Section A6.5 — Chronic toxicity (Inhalation 3 of 3)

Annex Point IIA, VI, 6.5 — **Section 6: Toxicological and metabolic studies**

		Guinea pigs - 400-800g
		Adult Monkeys - 2300-5400g
3.2.6	Number of animals per group	Rats - 80
		Guinea pigs - 20
		Monkeys - 10
3.2.7	Control animals	Yes, present in the same number as animals under test.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	12 – 18 calendar months.
3.3.2	Frequency of exposure	Exposures were conducted for 5.5 to 6 h per day, 5 days per week for a maximum of 12 – 18 calendar months.
3.3.3	Post exposure period	Not reported.
3.3.4	**Inhalation**	
3.3.4.1	Concentrations	Daily target concentration in the chambers was 15 mg/m^3
3.3.4.2		
3.3.4.3	Particle size	See Table 2 A6_5-2 and A6_5-3.
3.3.4.4	Type or preparation of particles	Dust
3.3.4.5	Type of exposure	Whole body
3.3.4.6	Vehicle	No vehicle used: Test subjects were exposed to 100% silica dust.
3.3.4.7	Concentration in vehicle	Not applicable. No vehicle used.
3.3.4.8	Duration of exposure	5.5-6 h/day
3.3.4.9	Controls	Filtered air 24 h/day
3.4	**Examinations**	
3.4.1	Observations	Not reported.
3.4.1.1	Clinical signs	Not reported.
3.4.1.2	Mortality	Rats sacrificed after 3, 6 and 12 months exposure.
		Guinea pigs after 12 months exposure.
		Monkeys silica F and G after 13 months exposure and silica P after 18 months exposure.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Fed standard laboratory pellet diets. Monkeys given fresh fruit (oranges, bananas or apples) twice a week. Food was available at all times except during the exposure period.
3.4.4	Water consumption	Tap water was available *ad libitum*.

Section A6.5 Chronic toxicity (Inhalation 3 of 3)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Blood was obtained by cardiac puncture at the terminal sacrifice. Parameters: glutamate-oxaloacetate transaminase, lactic dehydrogenase, alkaline phosphatise, total bilirubin, albumin, total protein, cholesterol, uric acid, blood urea nitrogen, glucose, inorganic phosphate and calcium. In addition, white and red blood counts, differential, hematocrit and haemoglobin analyses were performed on 10 animals from each exposure group.
3.4.7	Clinical Chemistry	Prior to exposure and after 3, 6 and 12 – 14 months of exposure pulmonary function tests were performed on the monkeys.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and pathology**	Rats sacrificed after 3, 6 and 12 months exposure. Guinea pigs after 12 months exposure. Monkeys silica F and G after 13 months exposure and silica P after 18 months exposure.
3.5.1	Organ Weights	Not applicable.
3.5.2	Gross and histopathology	At autopsy all organs were examined grossly and selected tissues were removed, fixed in formalin and processed for histopathological examinations. Lungs were visually inspected and perfused at 21 cm of water pressure with buffeted neutral formalin. Haematoxylin-eosin-stained sections were made of the following tissues from each animal and examined microscopically: lungs (all lobes), thyroid, heart, tracheobronical lymph nodes, mesenteric lymph nodes, liver, spleen, kidneys, testis, pancreas, adrenals and skin. In addition sections of the urinary bladder, prostate, stomach and duodenum were made from each monkey. Lung tissue from three monkeys exposed to each of the silicas was processed and examined by scanning and transmission electron microscopy and with an electron microprobe. All monkey lungs were stained with Masson's trichrome stain and a reticulin stain, and examined by light microscopy and polarizing light microscopy. At terminal sacrifice, a portion of the lung from each monkey was excised and analysed for hydroxyproline. Refer to Table A6_5-4 at the end of this study summary for concentrations of hydroxyproline found in lungs of monkeys exposed to synthetic amorphous silica at 15 mg/m^3 for 13 – 18 months. In addition nine rat lungs were analysed for total silicon by plasma emission spectroscopy. Refer to table A6_5-5 at the end of this study summary for silicon concentrations found in lungs of rats exposed by inhalation to synthetic amorphous silicas.
3.5.3	Other examinations	None.
3.5.4	Statistics	Yes: Multivariate one-way analyses of covariance between the control and each exposed group were calculated. The dependent variables (pulmonary functions) were placed into two groups for the analysis. The ventilatory mechanics group included resistance at low frequency (RLLF), compliance at low frequency (CLLF), forced expiratory flow at 25 percent vital capacity (FEF25), forced expiratory flow at 10 percent

		vital capacity (FEF10%), closing volume (CV), nitrogen washout (N_2), and volume of isoflow (VISFL). The lung volume group included forced vital capacity (FVC), inspiratory capacity (IC), residual volume (RV), and total lung capacity (TLC). If the multivariate analysis indicated a significant difference, then each response variable was analysed individually by adjusted univariate analysis.
3.6	**Further remarks**	Not applicable.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Not reported.
4.1.2	Mortality	Rats sacrificed after 3, 6 and 12 months exposure. Guinea pigs after 12 months exposure. Monkeys silica F and G after 13 months exposure and silica P after 18 months exposure.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophtalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	There were no statistically significant differences between the silica exposed groups and the control groups with regard to any of the clinical chemical and haematological parameters measured except for alkaline phosphatase levels in silica F monkeys, which were elevated compared to control group values ($p < 0.05$). This elevation did not correlate with any observed pathology and is probably not the result of exposure to amorphous silica. Refer to Table A6_5-4 at the end of this study summary, which presents the hydroxyproline concentrations in the monkey lungs. No elevations of lung hydroxyproline were evident in any of the exposed groups compared with the control group. Refer to Table A6_5-5 at the end of this study summary, which presents the silicon concentrations in the rat lungs. The results suggest that some amorphous silica was deposited in the rat lungs, but the number of analyses were too few to allow comparisons between exposure groups.
4.5.2	Clinical chemistry	Pulmonary Function Mean body weights and sizes of the monkeys differed significantly between the exposed and control groups after 0, 3 and 6 months of exposure, but not at 12 – 14 months, only the pulmonary function data collected at the latter interval were used to compare exposed with control groups. Refer to Table A6_5-7 at the end of this study summary for the pulmonary function tests.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Not reported.

Section A6.5 Chronic toxicity (Inhalation 3 of 3)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

4.6.2 Gross and histopathology

The most significant alterations related to exposures to amorphous silicas were confined to the lungs of monkeys. Regardless of the type of silica to which they were exposed, the lungs of each monkey contained large numbers of macrophage and mononuclear cell aggregates. Amorphous silica could not be seen by light microscopy, but cytoplasmic alterations indicated its presence. Microprobe studies of these alterations revealed the presence of silica in all those examined. Cell aggregates varied in size from 40 - 600µm in diameter and occurred in the walls of respiratory bronchioles, alveolar ducts, around venules and arterioles and occasionally in alveolar walls distant from the aforementioned structures. Their frequency varied with type of silica.

There were up to 40 aggregates / 100 X magnification in the lungs of monkeys exposed to silica P, up to 35 in the lungs exposed to silica F and up to 25 in those exposed to silica G. Frequency and size of the lesions, considering all monkeys in each group, suggested that larger aggregates appeared in the lungs of monkeys exposed to silica P, slightly fewer and smaller ones in those exposed to silica F and considerably fewer and smaller ones in those exposed to silica G.

A difference in type and quantity of extra cellular components was observed. Reticulin fibres were uniformly present in the aggregates in all three groups; collagen was present in significant amounts only in those monkeys exposed to silica F. In six of the nine monkeys exposed to silica F, 5-50% of the aggregates contained collagen in varying amounts. In three of the monkeys, little or no collagen was present.

Collagen fibres wee not seen in any of the aggregates in the lungs of monkeys exposed to silica G and in very few of the lungs of those exposed to silica P.

One uncontrolled variable was observed, some of the monkeys from each group, including the control group, showed varying quantities of birefringent crystals and dark brown or black particles in macrophages in aggregates, primarily around blood vessels and in tracheal lymph nodes. The crystals were identified as mica ($KAlSiO_3$) and kaolin ($AlSiO_3$) by microprobe and electron diffraction analyses. Lungs containing these crystals did not exhibit any more reticulin or collagen than those lungs without it. More collagen occurred in the mica containing aggregates in the lungs of monkeys in the control group. The manner in which the monkeys were exposed to mica and kaolin is unknown.

After 12 months of exposure, 13, 24 and 19 rats were exposed to silicas F, G and P, respectively and 31 in the control group and 15 guinea pigs from each exposure group and control group were autopsied, and tissues examined microscopically. Histopathological examination of the lungs of the rats and guinea pigs revealed far fewer and smaller macrophage aggregates than those seen in monkeys. The concentration of the aggregates was no greater than two or three / 100 X magnification. No aggregates were seen in the control lungs from either rats or guinea pigs. Based on light microscopy, it was estimated that the rats or guinea pig lungs contained <10% of the amount of amorphous silica per unit of weight contained in the monkey lungs.

Interstitial fibrosis associated with dense collections of mast cells appeared in some of the rats in all groups. This seemed to occur more frequently in those exposed to silica F, the presence of lesions in some

of the control group obscured the role that amorphous silica might have played in the development of the lesion.

4.7 Other

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of this study was to compare the pulmonary toxicity of three major types of synthetic amorphous silicas in animals by using the same airborne concentration (15 mg/m^3) as that published by the U.S. Occupational Safety and Health Administration for nuisance dusts.

Test Materials

Three different types of amorphous silicas [silica gel (silica G), precipitated silica (silica P) and fume silica (silica F)] were obtained from commercial manufacturers and stored in their respective shipping containers for the duration of the study. The silicas were analysed for their elemental composition by proton-induced X-ray emission method and for crystalline silica by X-ray diffraction.

The studies were conducted in stainless steel inhalation chambers 60 inches long by 57 inches wide and 160 feet high. Exposures were conducted under dynamic flow conditions with tangential airfeed manifolds maintained at 40 L/min with a pressure of 0.254 cm H_2O. Exposures were conducted for 5.5 – 6 h per day, 5 days per week, for a maximum of 12 – 18 calendar months.

Silica G and P dust aerosols were generated by Wright dust feed mechanisms, which were affixed to each exposure chamber. These dusts were packed into the Wright dust feeder at 2000 lb/ in^2. Silica F dust was generated with a modified fluidised bed. A cyclone was used with the silica F to remove and eliminate the large aggregates, and static eliminators with polonium sources were used with all three dusts to reduce aggregation of the dust aerosols. Filtered and dehumidified compressed air was metered into the dust generators, and the dust aerosols were blown directly into the chamber supply air stream. The supply air was passed through high-efficiency particulate air filters, conditioned to a relative humidity of 50-70 percent and drawn into the chambers.

The daily target concentration in the chambers for all three dusts was 15 mg/m^3 (total dust). Chamber atmospheres were analysed gravimetrically at least three times daily for total dust concentration. A vacuum pump was used to pull chamber air samples through 0.5 or 0.8 µm pore size millipore filters (Millipore Corp., Bedford, Mass.) for silica F 5.0 µm pore size metrical filters (Gelman Instrument Co., Ann Arbor, Mich.) for silica P and silica G for 20 min at a rate of 10 L/min. After each sampling period, adjustments were made to each chamber to maintain a daily concentration times time (C X T) relationship of 90 (15 mg/m^3 X 6h).

Midway through the exposure period, three 8 stage Andersen cascade impactor measurements (Andersen Samplers, Inc., Atlanta, Ga.) were taken from each chamber to determine particle size distributions dynamically on a mass basis. The percent by weight of each silica on each of the impactor stages was determined. Dust in each chamber was also sized by collecting particles on electron microscopy grids, examining and photographing the particles with an electron microscope,

and measuring the diameters of the particles on the photographs.

Since the average daily respirable dust concentration in silica P chamber was much lower (6.9 mg/m^3) than in the other two chambers (Refer to Table A6_5-1 at the end of this study summary), the silica P monkeys were exposed for 18 calendar months (instead of 13) in order to achieve the same total respirable dust dose as that obtained with the other two silicas.

Animals

All animals used in the study were male, caesarean-derived, Sprague-Dawley rats, Hartley guinea pigs and adult Cynomolgus monkeys, *Macaca fasicularis*. Each of the three treatment groups and the control group contained 80 rats (300 – 380g), 20 guinea pigs (400 – 700g) and 10 monkeys (2300-5400g), which were randomly assigned at the start of the study.

The rats and guinea pigs were quarantined for two weeks and the monkeys were quarantined for one month prior to initiation of the inhalation exposures.

Stainless and galvanised steel open wire-mesh cages were used as exposure caging to provide adequate distribution of the dust aerosols within the exposure chambers. All animals were individually marked by toe clipping or tattoo. All three species were individually housed during 6 h exposures, whereas the rats and guinea pigs were housed 2-4 animals per cage at all other times. Control animals were housed in similar cages in separate animal quarters and exposed to filtered air 24 h/day.

Exposed animals were also housed in the animal quarters except during the 6 h inhalation exposure. Rats, guinea pigs and monkeys were fed standard laboratory pellet diets. Monkeys were given fresh fruit (oranges, bananas, or apples) twice a week. Tap water was available *ad libitum*, and food was available to the animals at all times except during the exposure period.

Pulmonary function

Prior to exposure and after 3, 6 and 12 – 14 months of exposure pulmonary function tests were performed on the monkeys. Prior to this testing, the animals were fasted and received no dust exposure for 16-18 h. Animals under test were anesthetised with 25 mg/kg of sodium pentobarbital. Following this an oesophageal balloon was placed in the lower third of the oesophagus and an 18 to 22 F endotracheal tube was inserted into the trachea with the aid of a laryngoscope. The cuff of the endotracheal tube was inflated. The animal was then placed into the chamber, ventral side up, for compliance and resistance testing. For all other tests, the animal was in the vertical position.

Pulmonary mechanics were obtained from simultaneous volume, flow and transpulmonary pressure tracings displayed on a twelve-channel photographic recorder (Electronics for medicine, DR-12). Airflow through the pneumotachograph was measured with a differential transducer and electrically integrated to produce a volume trace.

Dynamic pulmonary compliance (CL_{DYN}) was calculated from simultaneous volume and transpulmonary pressure tracings at points of zero flow. Average flow resistance ($RL_{ave.flow}$) was calculated from the change in transpulmonary pressure (at equal volumes), divided by the

sum of inspiratory and expiratory flow.

All mechanics were measured while the animal was breathing spontaneously through the pneumotachograph only. The animal was inflated for 10 sec initially and periodically throughout the testing to expand atelectatic areas.

The pulmonary function tests requiring breathing manoeuvres [lung volumes, maximum expiratory flow-volume curve (FEF), diffusing capacity (DL_C18_O), single-breath nitrogen washout (ΔN_2), and closing volume (CV)] were performed using a variable pressure plethysmographic chamber. The basic method employed was similar to that used in an external tank respirator; however, a hydraulic control system enabled the operator to bring about inspiration, expiration, breath holding, and breathing rate. Both flow and volume were controlled secondarily by changes in the pressure surrounding the animal. Inspiratory and expiratory airflow could be controlled from very low rates to the maximum within each subject. Volume could be controlled for both maximum inspiration and expiration. Inspiratory capacity (IC) was obtained by rapid depressurisation to 70 cm of water from testing tidal position. Prior to the flow-volume testing, it was determined that plethysmograph pressures of +70 cm of water would be used to produce maximal expirations. Inspection of flow-volume curves at increasing driving pressures showed that flow limitation characteristics had been reached at volumes above 50 % total lung capacity (TLC) when the plethysmograph pressure was greater than +70 cm of water. Therefore FEF at 25% of FVC are values taken at an effort independent zone of the flow-volume curve.

To ensure that sufficient intrathoracic driving pressure was developed oesophageal pressure was recorded during forced expirations. A trans-chestwall pressure gradient was observed. However, intrapleural pressures of 30-35 cm of water were achieved, which are efficient to produce flow maxima. A volume error, as a result of thoracic gas compression, was calculated to be approximately 3 % at 50% TLC with the intrapleural pressure of 30-35 cm of water. This error was considered to be irrelevant because the results were compared in animals tested at the same driving pressures.

Breathing manipulations could be performed in anesthetised animals because of the apnea produced on inflation as a result of the inflation reflex. The inspiratory inhibition had been demonstrated by recording action potentials from the phrenic Inspiratory capacity (IC) and forced vital capacity (FVC) were recorded during a maximum inspiration, followed by a maximum expiration. Flow and volume tracings were recorded which provided the essential data points for calculating forced expiratory flows and volumes ($FEV_{0.5, 1.0}$) and peak expiratory flow (PF). This procedure of maximum inspiration followed by maximum expiration was performed initially and thereafter for all test manoeuvres, which ensured equal volume and flow histories.

5.2 Results and discussion

Table A6_5-1 shows at the 12 month internal the exposure data for rats and guinea pigs in all dust groups were not significantly different from the data for the monkeys at the same 12 month interval.

Powder X-ray diffraction analyses of the three silicas revealed no peaks, which indicate that they did not contain crystalline silica. Elemental analyses by proton-induced X-ray emission revealed a number of trace contaminants. Several of these were present in significant amounts,

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Annex Point IIA, VI, 6.5 **Section 6: Toxicological and metabolic studies**

Refer to Table A6_5-6 at the end of this study summary.

Silica P Pulmonary Function

The multivariate analysis of ventilatory mechanics showed no significant difference between exposed and control groups ($p = 0.33$). The multivariate analysis of the four lung volume parameters indicated a significantly lower overall mean vector response in the exposed group ($p = 0.004$). Of the four parameters, RV and IC showed no significant difference between exposed and control groups when analysed individually. However, both TLC and FVC were each significantly lower in the exposed group as compared with the control group.

Silica F Pulmonary Function

The multivariate analysis of variables associated with ventilatory mechanics indicated a significant difference between the exposed and control groups ($p = 0.004$). All such variables were significant ($p < 0.05$) when analysed individually with the exception of N_2 and VISFL. Multivariate analysis of the four lung volume parameters also showed a significant reduction from control mean levels. All these variables showed a significant ($p < 0.05$) difference from controls when analysed individually with the exception of RV.

Silica G Pulmonary Function

Multivariate analysis of the ventilatory mechanics variables indicated a significant difference between exposed and control groups ($p = 0.014$). Five of the seven variables were significant ($p < 0.05$) in univariate analyses also. The exceptions were CV and VISFL. Univariate analyses of the four variables associated with lung volumes showed no significant differences between the exposed and control groups. Although the multivariate analysis showed a significant ($p = 0.029$) difference, this was misleading, since two variable (RV and IC) showed differences in the opposite direction from what would be expected. There were no significant differences between any of the exposed groups and the control group for DLCO.

The lack of significantly higher concentrations of hydroxyproline in the lungs of monkeys exposed to silica F is interesting. The amount of collagen induced by silica F was less than the variations in concentrations between individual lungs in the control monkeys. Histopathological examination of the lungs is a more sensitive method for detecting early nodular fibrosis than is quantitative hydroxyproline analysis.

The presence of mica and kaolin in the lungs of the monkeys was an undesirable variable. However, it is believed to have not contributed to the induction of macrophage aggregates, reticulin or collagen for three reasons. The greatest amount of collagen was seen in the lungs of the monkeys (silica F) that did not contain birefringent crystals, the concentrations of the crystals in most of the lungs were relatively insignificant and no correlation was seen between the numbers and sizes of the macrophage aggregates, amount of reticulin or collagen, and the concentration of the crystals.

From the results it is clear that more of the pulmonary function tests were significantly altered in monkeys exposed to silica F. Monkeys exposed to silica F at concentrations considered being the nuisance dust level for a relatively short period of time (13 months) had statistically

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Annex Point IIA, VI, 6.5 **Section 6: Toxicological and metabolic studies**

		significant alterations in most of their pulmonary function tests. Early nodular fibrosis was present in the lungs of several of the monkeys.
5.3	**Conclusion**	The results of the study with monkeys confirm that some synthetic amorphous silicas are capable of inducing pulmonary fibrosis in animals. The results indicate that silica F is more detrimental than silica P or G. The fact that silica F had a smaller particle size than the other test silicas could be the contributing factor. If the concentration and sizes of the macrophage aggregates are good indicators of the amount of silica in the lungs, silica F monkey lungs contained more silica than silica G monkey lungs. This could partially explain the difference in responses between those two groups, but not between the silica F and P groups. The lungs in the latter group contained larger and more macrophage aggregates than the lungs in silica F or G groups. Aluminium and iron compounds have been reported to modify the fibrogenic potency of silica. It is therefore interesting to note that silica P contained 67 times more aluminium and 20 times more iron than silica F. This difference could have contributed to the differences in response.
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Not reported.
5.3.3	Other	None.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that urinalysis parameters were not measured. The study was performed with only a single dose range, so no NOAEL could be established. The test materials used in this study are not identical to that as given in Section 2. The specification of the substances has not been reported. However, this study is deemed appropriate for consideration in the risk assessment as one of the test materials was stated as precipitated amorphous silica, the same as the notified substance.

Table A6_5-1. Summary of inhalation exposure data for monkeys exposed to synthetic amorphous silica dusts at 15 mg/m^3 for 13-18 months.

	Silica		
Parameter	**Fume**	**Precipitated**	**Gel**
Average daily total dust concentration mg/m^3	15	15	15.9
Calendar months of exposure	13	18	13
Exposure, days	262	376	273
Exposure, h	1530	2256	1597
Exposure hours x average daily total dust concentration ÷ 1000	22.95	33.84	25.39
Average daily respirable dust concentration	9.9	6.9	9.4

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mg/m^3			
Exposure hours X average daily respirable dust concentration ÷ 1000	15.15	15.57	15.01

Table A6_5-2. Percent by weight of various size fraction of synthetic amorphous silica dusts[1]

	Silica		
Size Fraction, µm	**Fume**	**Precipitated**	**Gel**
% ≥ 11.0	8	23	16
7.0 – 11.0	10	15	8
4.7 – 7.0	17	16	14
3.3 – 4.7	22	16	21
2.1 – 3.3	17	19	18
1.1 – 2.1	13	9	12
0.7 – 1.1	8	7	7
0.4 – 0.7	4	5	3
0 – 0.4	<1	<1	<1

[1] Size distributions were obtained using an 8-stage Andersen cascade impactor sampled at a rate of 28.3 L/min for 35.3 min. data represents the mean of 2 – 3 samples for each amorphous silica chamber atmosphere. Samples were collected without animals in the chamber.

Table A6_5-3. Particle sizing of amorphous silica chamber atmospheres using electron microscopy[2].

Silica	Geometric mean, µm	% Particles < 5.0 µm	% Particles <1.0 µm
Fume	0.17	99.9	99.5
Precipitated	0.38	98	85
Gel	0.27	99.8	93

[2] The number of particles by count in each size range was determined for each synthetic amorphous silica chamber. Particles were collected on electron microscopy grids, examined and photographed by electron microscopy, with the particle diameters determined by analysis of the photographs taken.

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Table A6_5-4. Concentrations of hydroxyproline found in lungs of monkeys exposed to synthetic amorphous silica at 15 mg/m^3 for 13 – 18 months[3].

	Hydroxyproline		
Exposure groups	**No.**	**µg / mg Lung**	**µg / mg Nitrogen**
Control	10	615 ± 116	746 ± 154
Fume Silica	9	727 ± 268	658 ± 127
Silica Gel	8	537 ± 150	701 ± 171
Precipitated silica	8	346 ± 76	429 ± 72

[3] A portion from each monkey lung was excised and analysed for hydroxyproline following hydrolysis of the tissue protein and reaction with Ehrlich's reagent (dimethylamino benzaldehyde). Total nitrogen was analysed by Kjeldahl. Concentrations expressed are means ± one standard deviation.

Table A6_5-5. Silicon concentrations found in lungs of rats exposed by inhalation to synthetic amorphous silicas[4].

Exposure groups	No.	Mean	Range
Control	3	159	102 – 194
Fume Silica	2	289	252 – 325
Silica Gel	3	453	419 – 471
Precipitated silica	1	946	…..

[4] Lungs were excised, freeze-dried, and analysed for total silicon by plasma emission spectroscopy. Concentrations are expressed as µg silicon / g freeze-dried lung.

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Table A6_5-6. Concentrations of elements in synthetic amorphous silica dust samples[5].

	Amorphous silica, µg/g		
Elements	**Fume**	**Gel**	**Precipitated**
Sulphur (S)	<200	500	3150
Calcium (Ca)	90	360	2000
Aluminium (Al)	26	161	1734
Iron (Fe)	47	104	890
Titanium (Ti)	61	215	445
Zirconium (Zr)	0.9	33	128
Chromium (Cr)	<6	11	36
Manganese (Mn)	<4	9	27
Strontium (Sr)	1.5	1.9	11
Copper (Cu)	1.9	4.0	9.0
Lead (Pb)	1.5	11	4.3
Zinc (Zn)	2.8	3.2	2.7
Iodine (I)	17	<16	<4

[5] Samples of bulk amorphous silicas were analysed for elemental composition using proton-induced X-Ray emission methods. All analysis was formed on digested samples, except analyses for aluminium, zirconium and iodine, which were performed on the powders.

Table A6_5-7. Results of pulmonary function tests in monkeys after 12 to 14 months exposure to synthetic amorphous silica[6,7].

	Groups			
	Control	**Precipitated silica**	**Silica gel**	**Fume silica**
Number of monkeys	10	10	7	9
Mean body weights, g	4470	4335	4686	4411
Pulmonary Function				
Parameter				
RL (average flow), cm H_2O)/L/sec	12.1 ± 4.1	14.9 ± 5.3	19.3 ± 3.1↑	19.0 ± 5.6↑
CL (dynamic), ML/cm H_2O	22.9 ± 10.7	14.3 ± 5.4	11.9 ± 3.5↓	8.8 ± 4.4↓
FEF 75%, mL/sec	648 ± 173	558 ± 192	532 ± 229↓	505 ± 194↓
FEF 90%, mL/sec	262 ± 115	201 ± 86	155 ± 65↓	162 ± 101↓
CV, mL	20.8 ± 9.1	15.2 ± 8.3	23.6 ± 9.7	39.9 ± 34↑
N_2 washout, %N_2/100mL	1.05 ± 0.35	1.63 ± 0.86	0.53 ± 0.26↓	0.80 ± 0.41
Volume of isoflow, mL	28.7 ± 19.6	29.0 ± 14.8	26.4 ± 23	30.9 ± 32
FVC, mL	335 ± 37	282 ± 38↓	329 ± 25	282 ± 33↓
IC, mL	185 ± 20	203 ± 51	196 ± 30	175 ± 19↓

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Section 6: Toxicological and metabolic studies

RV, mL	41 ± 11	48 ± 23	45 ± 19	48 ± 15
TLC, mL	357 ± 39	318 ± 34↓	335 ± 27	304 ± 36↓
DLCO, mL at STPD/min/mm/Hg	1.004 ± 0.325	1.052 ± 0.355	1.231 ± 0.276	1.001 ± 0.183

[6] Data is presented as means ± on stand deviation for each of the parameters.
[7] ↑↓ indicate values significantly different from control (univariate analyses).

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*

Section A6.5 Chronic toxicity (Inhalation 3 of 3)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.6.1 Annex Point IIA, VI, 6.6.1	**Genotoxicity Studies** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	Generation of test data to determine the genotoxicity potential of silicon dioxide is not scientifically necessary. Adequate *in vivo* data exists for this endpoint. No evidence of chromosome damage (micronuclei) was seen in three good-quality studies (one involving oral administration, two involving intraperitoneal injection) where high doses of silicon dioxide were given to mice (Morita et al. 1997). The summary paper does not clarify the exact form used but, if any crystalline material was in the tested chemical, then this would have represented a worst case (since other studies have shown that crystalline silicas are genotoxic whereas amorphous forms are not). This study is fully described in Section 6.6.4. Even though this is the case for silicon dioxide, and it forms part of the justification for not submitting data on the mutagenicity of silicon dioxide, it is not considered scientifically necessary for the following additional reasons: ▪ It is not scientifically necessary on the basis of low exposure to silicon dioxide during its normal use as a biocide. Exposure to amorphous silicon dioxide when used as an insecticide is inconsequential because of the ubiquity of forms of silicon dioxide in the environment. Silicon, in the form of silicon dioxide and silicates (salts of the various silicic acids), occurs abundantly in nature, comprising about 25% of the earth's crust [1]. Silicon dioxide and silicates are present in practically all plants and animals and in natural waters [2,3]. Between 10 and 200 mg silicon dioxide is present in 100g dry weight of normal human tissue. The lungs and lymph nodes of older adults may have levels several times this amount [2]. Silicon dioxide is an approved food additive, assigned the E number E551 [4], and is used as an anti-caking agent. Silicon dioxide has been given an acceptable daily intake of "not limited" [5]. In addition, silicon dioxide is approved for use in plastic material coming into contact with food, without hazard to public health [6]. Synthetic amorphous silicas are widely used in industry (for example as absorbents, dessicants and fillers) and in synthetic fabrics, plastics, lacquers, vinyl coatings, varnish, paper, pharmaceuticals, adhesives, foods, floor waxes, paints, rubber, and inks [7]. Estimates indicate that 4,400,000 people are exposed to amorphous silicas in their work environments [7]. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder, estimates exposure to be 0.0043 mg silicon dioxide/kg/day [*]. To put this exposure into context, and notwithstanding the information given above, the silicon dioxide content of raw potato is reported to be 10.1 mg/kg, and one litre of beer contains 131 mg [1]. * Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide. ▪ In addition to the above, the potential for exposure to silicon dioxide when it is manufactured for use as an insecticide is minimal. Silicon dioxide is manufactured in a completely enclosed system, as is the manufacture of the insecticide product based on silicon dioxide. This means there is no exposure to workers, bystanders or the environment during manufacture. It is estimated that [redacted] of silicon dioxide will be manufactured each year for use as a biocide. This	

amount of silicon dioxide is tiny in comparison to the other non-biocidal uses of silicon dioxide. For example, amorphous silicon dioxide is the main component of glass and in 1995, 12.9 million tonnes of glass was discarded in the US alone [8,9].

[redacted]

- Operator exposure work has been carried out in humans exposed to high concentrations of silicon dioxide [10]. Such data has been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For example, the long term occupational exposure limit for silicon dioxide set in the UK is 2.4 mg/m^3 (respirable dust) (8h time weighted average) [11]. The US threshold limit value (TLV, set by the American Conference of Governmental Industrial Hygienists, ACGIH) for silicon dioxide is 2 mg/m^3 (respirable dust) [12]. In Australia, the long-term occupational exposure limit for silicon dioxide is also 2 mg/m^3 (respirable dust) [13]. The risk assessment for human exposure to silicon dioxide, when applying the representative product, RID Insect Powder shows that exposure to silicon dioxide does not exceed these agreed maximum exposure limits for safe working conditions*. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for an approximation of a theoretical safe value.

  *The risk assessment for human exposure to silicon dioxide shows exposure to RID Insect Powder, under normal working conditions did not exceed the recommended UK maximum exposure limit to amorphous silicon dioxide (set at 2.4 mg/m^3 for respirable dust)***.

  *** Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- There is a substantial volume of information available for amorphous silicon dioxide. The data available are in general agreement, all showing that amorphous silicon dioxide *per se* is intrinsically biologically inert.

There is a substantial volume of information available for silicon dioxide, and while there are no studies available performed to specific guidelines, which consider mutagenicity specifically, it does cover all the major biological considerations. Given the large volume of data available for silicon dioxide, only the typical findings have been summarised below with regards to the chronic toxicity and carcinogenic potential of silicon dioxide. A number of reviews have been conducted by different regulatory bodies including the EPA [14], and the FDA[1], who considered the health aspects of silicon dioxide as a food additive. EPA concluded that silicon dioxide's acute toxicity profile is characterised as moderate to low, and consequently silicon dioxide has been exempted from the requirement of a tolerance limit when applied to growing crops or agricultural commodities. FDA has classified silicon dioxide as Generally Recognised as Safe (GRAS) and has approved its use as a dietary food additive at levels of up to 2% by weight in food. The joint FAO/WHO Expert Committee evaluated a number of food additives. The anti-caking agent silicon dioxide was given an acceptable daily intake of "not limited" [5]. There are two FDA direct food ingredient regulations for silicon dioxide, plus a clearance by the US Department of Agriculture for its use in curing mixes and in animal feed premixes. In agreement with the review by the EPA[14], the FDA concluded that silicon dioxide appears to be biologically inert and there was no evidence available that suggests silicon dioxide is hazardous to humans [1].

Conclusion

It has been demonstrated that the low level of exposure to silicon dioxide during its use as an insecticide (PT18) indicates that it is not scientifically necessary to conduct an *in vitro* bacterial mutagenicity study on silicon dioxide as it will not add any useful information to the risk assessment. It has been shown in the human risk assessment that compared to exposures *via* the diet and the environment, exposure from silicon dioxide as an insecticide is insignificant. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder shows that exposure to silicon dioxide does not exceed agreed, well established maximum exposure limits for safe working conditions with silicon dioxide and nuisance dust. The toxicological profile of silicon dioxide has been well established with a large body of data available in the public domain. The operator exposure limits that have been set for nuisance particles and dusts are also based on a large amount of available data. As shown above, data is available on the effects of exposure to amorphous silicon dioxide and this data shows that there are no lasting adverse effects. Although this data has its limitations and there are no studies available performed to specific guidelines which consider chronic toxicity or mutagenicity specifically, it is considered sufficient to address the toxicity of silicon dioxide particularly given the levels of exposure expected to silicon dioxide through other, non-biocidal uses of silicon dioxide including its use in food.

References

[redacted]

	[redacted]
Undertaking of intended data submission [ ]	Not applicable.

Section 6.6.1 Annex Point IIA, VI, 6.6.1	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

A6_6_1a – study summary has been removed.

A6_6_1b – study summary has been removed.

Section 6.6.2 Annex Point IIA, VI, 6.6.2	**Genotoxicity Studies** Section 6: Toxicological and Metabolic Studies *In vitro* cytogenicity study in mammalian cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data **[4]**	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure **[4]**	**Other justification** [ ]	
Detailed justification:	A comprehensive search of the published literature did not identify any *in vitro* cytogenicity study (i.e. a test for chromosome damage) in mammalian cells. However, there are several convincing reasons why it is not considered scientifically necessary to submit a new study of this type. ▪ It is not scientifically necessary because adequate *in vivo* data exists for this endpoint. No evidence of chromosome damage (micronuclei) was seen in three good-quality studies (one involving oral administration, two involving intraperitoneal injection) where high doses of silicon dioxide were given to mice (Morita et al. 1997). The summary paper does not clarify the exact form used but, if any crystalline material was in the tested chemical, then this would have represented a worst case (since other studies have shown that crystalline silicas are genotoxic whereas amorphous forms are not). This study is fully described in Section 6.6.4. ▪ It is not scientifically necessary because the silicon dioxide that is the subject of this submission is food grade. That is, its specification matches or betters that of the permitted food additive silicon dioxide. Before being approved or recommended as food additives, compounds such as silicon dioxide are rigorously assessed and periodically reassessed by national and/or international experts. A consideration of genotoxicity is a critical feature of this assessment, and chemicals considered to be genotoxic are not permitted for use in food. Since silicon dioxide of an appropriate specification (matching the Rentokil material) is an approved food additive in the EU (89/107/EEC as amended), it is clear that silicon dioxide grades that meet the food grade specification do not have genotoxic potential. In assessing the acceptability of using amorphous silicon dioxide in food in the EU, the SCF approved its use, and allocated an ADI "not specified" status (SCF, 1991[1]). This is the most favourable opinion that could be reached and is used for substances of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not represent a hazard to health. For that reason, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. ▪ It is not scientifically justifiable because adequate studies have shown that amorphous silicon dioxide is not a chemical carcinogen (see Sections 6.6.1-3). The major role of genotoxicity studies is to screen for possible carcinogenic activity, and genotoxins would be expected to induce tumours in cancer bioassays. In the case of amorphous silicon dioxide, the available carcinogenicity studies showed no evidence of activity, clearly indicating that amorphous silicon dioxide is not genotoxic. The other existing genotoxicity data are consistent with the view that amorphous silicon dioxide is not genotoxic [2]. ▪ It is not scientifically necessary on the basis of low exposure to silicon dioxide during its normal use as a biocide, compared with exposures from other sources.	

Exposure to amorphous silicon dioxide when used as an insecticide is inconsequential because of the ubiquity of forms of silicon dioxide in the environment. Silicon, in the form of silicon dioxide and silicates (salts of the various silicic acids), occurs abundantly in nature, comprising about 25% of the earth's crust[3]. Silicon dioxide and silicates are present in practically all plants and animals and in natural waters[4,5]. Between 10 and 200 mg silicon dioxide is present in 100g dry weight of normal human tissue. The lungs and lymph nodes of older adults may have levels several times this amount[4]. Silicon dioxide is an approved food additive, assigned the E number E551[6], and is used as an anti-caking agent. Silicon dioxide has been given an acceptable daily intake of "not limited" [7]. In addition, silicon dioxide is approved for use in plastic material coming into contact with food, without hazard to public health[8]. Synthetic amorphous silicas are widely used in industry (for example as absorbents, dessicants and fillers) and in synthetic fabrics, plastics, lacquers, vinyl coatings, varnish, paper, pharmaceuticals, adhesives, foods, floor waxes, paints, rubber, and inks[9]. Estimates indicate that 4,400,000 people are exposed to amorphous silicas in their work environments[9]. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder, estimates exposure to be 0.0043 mg silicon dioxide/kg/day *. To put this exposure into context, and notwithstanding the information given above, the silicon dioxide content of raw potato is reported to be 10.1 mg/kg, and one litre of beer contains 131 mg [3].

* Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- In addition to the above, the potential for exposure to silicon dioxide when it is manufactured for use as an insecticide is minimal. Silicon dioxide is manufactured in a completely enclosed system, as is the manufacture of the insecticide product based on silicon dioxide. This means there is no exposure to workers, bystanders or the environment during manufacture. It is estimated that [redacted] of silicon dioxide will be manufactured each year for use as a biocide. This amount of silicon dioxide is tiny in comparison to the other non-biocidal uses of silicon dioxide. For example, amorphous silicon dioxide is the main component of glass and in 1995, 12.9 million tonnes of glass was discarded in the US alone[10,11].
  [redacted]

- Operator exposure work has been carried out in humans exposed to high concentrations of silicon dioxide. Such data has been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For example, the long term workplace exposure limit (WEL) for silicon dioxide set in the UK is 2.4 mg/m^3 (respirable dust) (8h time weighted average)[12]. In an 8-hr work shift, a worker exposed at the UK WEL might inhale 19.2-24 mg silicon dioxide. The US threshold limit value (TLV, set by the American Conference of Governmental Industrial Hygienists, ACGIH) for silicon dioxide is 2 mg/m^3 (respirable dust)[13]. In Australia, the long-term occupational exposure limit for silicon dioxide is also 2 mg/m^3 (respirable dust)[14]. The risk assessment for human exposure to silicon dioxide, when applying the representative product, RID Insect Powder shows that exposure to silicon dioxide does not exceed these agreed maximum exposure limits for safe working conditions*. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for an approximation of a theoretical safe value.

*The risk assessment for human exposure to silicon dioxide shows exposure to RID Insect Powder, under normal working conditions did not exceed the recommended UK maximum exposure limit to amorphous silicon dioxide (set at 2.4 mg/m^3 for respirable dust)***.
** Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- There is a substantial volume of information available for amorphous silicon dioxide. The data available are in general agreement, all showing that amorphous silicon dioxide *per se* is intrinsically biologically inert.

There is a substantial volume of information available for amorphous silicon dioxide covering all the major biological considerations. Given the large volume of data available for silicon dioxide, only the typical findings have been summarised below with regards to the chronic toxicity and carcinogenic potential of silicon dioxide. A number of reviews have been conducted by different regulatory bodies including the EPA[15], and the FDA[3], who considered the health aspects of silicon dioxide as a food additive. EPA concluded that silicon dioxide's acute toxicity profile is characterised as moderate to low, and consequently silicon dioxide has been exempted from the requirement of a tolerance limit when applied to growing crops or agricultural commodities. FDA has classified silicon dioxide as Generally Recognised as Safe (GRAS) and has approved its use as a dietary food additive at levels of up to 2% by weight in food. The joint FAO/WHO Expert Committee evaluated a number of food additives. The anti-caking agent silicon dioxide was given an acceptable daily intake of "not limited" [7], which is the most favourable verdict that can be reached. There are two FDA direct food ingredient regulations for silicon dioxide, plus a clearance by the US Department of Agriculture for its use in curing mixes and in animal feed premixes. In agreement with the review by the EPA[15], the FDA concluded that silicon dioxide appears to be biologically inert and there was no evidence available that suggests silicon dioxide is hazardous to humans[3].

Conclusion

The availability of several good-quality studies showing that silicon dioxide does not cause chromosome damage in mice treated orally or by intraperitoneal injection means that it is not scientifically necessary to conduct an *in vitro* mammalian cytogenicity study on amorphous silicon dioxide as it will not add any useful information to the risk assessment. This is supported by the fact that use as an insecticide (PT18) will result in only a very low level of exposure to silicon dioxide. It has been shown in the human risk assessment that compared to exposures *via* the diet and the environment, exposure from silicon dioxide as an insecticide is insignificant. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder shows that exposure to silicon dioxide does not exceed agreed, well established maximum exposure limits for safe working conditions with silicon dioxide and nuisance dust. The toxicological profile of silicon dioxide has been well established with a large body of data available in the public domain. The operator exposure limits that have been set for nuisance particles and dusts are also based on a large amount of available data. As shown above, data is available on the effects of exposure to amorphous silicon dioxide and this data shows that there are no lasting adverse effects. Although this data has its limitations, it is considered sufficient to address the toxicity of silicon dioxide particularly given the levels of exposure expected to silicon dioxide through other, non-biocidal uses of silicon dioxide including its use in food.

References

[redacted]

	[redacted]
Undertaking of intended data submission [ ]	Not applicable.

Section 6.6.2 Annex Point IIA, VI, 6.6.2	**Genotoxicity Studies** Section 6: Toxicological and Metabolic Studies *In vitro* cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.6.3 Annex Point IIA, VI, 6.6.3	**Genotoxicity Studies** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in mammalian cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [4]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [4]	**Other justification** [ ]	

Detailed justification:

It is not considered necessary to undertake an *in vitro* gene mutation study in mammalian cells as data exists on *in vivo* testing of amorphous silicon dioxide. This public domain study data shows that amorphous silicon dioxide is non-mutagenic in rat alveolar cells *in vivo* (see Document IIIA, Section 6.6.5 for further details).

Even though this is the case for silicon dioxide, testing of the mutagenicity in mammalian cells of silicon dioxide is not considered scientifically necessary for the following additional reasons:

- It is not scientifically necessary on the basis of low exposure to silicon dioxide during its normal use as a biocide.

Exposure to amorphous silicon dioxide when used as an insecticide is inconsequential because of the ubiquity of forms of silicon dioxide in the environment. Silicon, in the form of silicon dioxide and silicates (salts of the various silicic acids), occurs abundantly in nature, comprising about 25% of the earth's crust [1]. Silicon dioxide and silicates are present in practically all plants and animals and in natural waters [2,3]. Between 10 and 200 mg silicon dioxide is present in 100g dry weight of normal human tissue. The lungs and lymph nodes of older adults may have levels several times this amount [2]. Silicon dioxide is an approved food additive, assigned the E number E551 [4], and is used as an anti-caking agent. Silicon dioxide has been given an acceptable daily intake of "not limited" [5]. In addition, silicon dioxide is approved for use in plastic material coming into contact with food, without hazard to public health [6]. Synthetic amorphous silicas are widely used in industry (for example as absorbents, dessicants and fillers) and in synthetic fabrics, plastics, lacquers, vinyl coatings, varnish, paper, pharmaceuticals, adhesives, foods, floor waxes, paints, rubber, and inks [7]. Estimates indicate that 4,400,000 people are exposed to amorphous silicas in their work environments [7]. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder, estimates exposure to be 0.0043 mg silicon dioxide/kg/day [*]. To put this exposure into context, and notwithstanding the information given above, the silicon dioxide content of raw potato is reported to be 10.1 mg/kg, and one litre of beer contains 131 mg [1].

[*] Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- In addition to the above, the potential for exposure to silicon dioxide when it is manufactured for use as an insecticide is minimal. Silicon dioxide is manufactured in a completely enclosed system, as is the manufacture of the insecticide product based on silicon dioxide. This means there is no exposure to workers, bystanders or the environment during manufacture. It is estimated that [redacted] of silicon dioxide will be manufactured each year for use as a biocide. This amount of silicon dioxide is tiny in comparison to the other non-biocidal uses of silicon dioxide. For example, amorphous silicon dioxide is the main component of glass and in 1995, 12.9 million tonnes of glass was discarded in the US alone [8,9].

[redacted]

- Operator exposure work has been carried out in humans exposed to high concentrations of silicon dioxide. Such data has been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For example, the long term occupational exposure limit for silicon dioxide set in the UK is 2.4 mg/m^3 (respirable dust) (8h time weighted average) [10]. The US threshold limit value (TLV, set by the American Conference of Governmental Industrial Hygienists, ACGIH) for silicon dioxide is 2 mg/m^3 (respirable dust) [11]. In Australia, the long-term occupational exposure limit for silicon dioxide is also 2 mg/m^3 (respirable dust) [12]. The risk assessment for human exposure to silicon dioxide, when applying the representative product, RID Insect Powder shows that exposure to silicon dioxide does not exceed these agreed maximum exposure limits for safe working conditions*. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for an approximation of a theoretical safe value.

  *The risk assessment for human exposure to silicon dioxide shows exposure to RID Insect Powder, under normal working conditions did not exceed the recommended UK maximum exposure limit to amorphous silicon dioxide (set at 2.4 mg/m^3 for respirable dust)***.

  *** Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- There is a substantial volume of information available for amorphous silicon dioxide. The data available are in general agreement, all showing that amorphous silicon dioxide *per se* is intrinsically biologically inert.

There is a substantial volume of information available for silicon dioxide, and while there are no studies available performed to specific guidelines, which consider mutagenicity specifically, it does cover all the major biological considerations. Given the large volume of data available for silicon dioxide, only the typical findings have been summarised below with regards to the chronic toxicity and carcinogenic potential of silicon dioxide. A number of reviews have been conducted by different regulatory bodies including the EPA [13], and the FDA[1], who considered the health aspects of silicon dioxide as a food additive. EPA concluded that silicon dioxide's acute toxicity profile is characterised as moderate to low, and consequently silicon dioxide has been exempted from the requirement of a tolerance limit when applied to growing crops or agricultural commodities. FDA has classified silicon dioxide as Generally Recognised as Safe (GRAS) and has approved its use as a dietary food additive at levels of up to 2% by weight in food. The joint FAO/WHO Expert Committee evaluated a number of food additives. The anti-caking agent silicon dioxide was given an acceptable daily intake of "not limited" [5]. There are two FDA direct food ingredient regulations for silicon dioxide, plus a clearance by the US Department of Agriculture for its use in curing mixes and in animal feed premixes. In agreement with the review by the EPA[13], the FDA concluded that silicon dioxide appears to be biologically inert and there was no evidence available that suggests silicon dioxide is hazardous to humans [1].

Conclusion

It has been demonstrated that the low level of exposure to silicon dioxide during its use as an insecticide (PT18) indicates that it is not scientifically necessary to conduct an *in vitro* bacterial mutagenicity study on silicon dioxide as it will not add any useful information to the risk assessment.

In addition, there is one *in vivo* study available for silicon dioxide which shows it is non-mutagenic in rat alveolar cells (refer to Document IIIA, Section 6.6.5 for details). It has also been shown in the human risk assessment that compared to exposures *via* the diet and the environment, exposure from silicon dioxide as an insecticide is insignificant. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder shows that exposure to silicon dioxide does not exceed agreed, well established maximum exposure limits for safe working conditions with silicon dioxide and nuisance dust. The toxicological profile of silicon dioxide has been well established with a large body of data available in the public domain. The operator exposure limits that have been set for nuisance particles and dusts are also based on a large amount of available data. As shown above, data is available on the effects of exposure to amorphous silicon dioxide and this data shows that there are no lasting adverse effects. Although this data has its limitations and there are no studies available performed to specific guidelines which consider chronic toxicity or mutagenicity specifically, it is considered sufficient to address the toxicity of silicon dioxide particularly given the levels of exposure expected to silicon dioxide through other, non-biocidal uses of silicon dioxide including its use in food.

References

	[redacted]
Undertaking of intended data submission [ ]	Not applicable.

Section 6.6.3 Annex Point IIA, VI, 6.6.3	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.6.4 **Annex Point IIA,VI, 6.6.4**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* micronucleus test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [3] **Limited exposure [3]**	**Technically not feasible [ ]** **Scientifically unjustified [4]** **Other justification [ ]**	
Detailed justification:	It is not deemed scientifically necessary because adequate *in vivo* data exists for this endpoint. No evidence of chromosome damage (micronuclei) was seen in three good-quality studies (one involving oral administration, two involving intraperitoneal injection) where high doses of silicon dioxide were given to mice (Morita et al. 1997). The summary paper does not clarify the exact form used but, if any crystalline material was in the tested chemical, then this would have represented a worst case (since other studies have shown that crystalline silicas are genotoxic whereas amorphous forms are not). Please see attached study summary for details. It is also not deemed necessary for the following, additional reasons: ▪ It is not scientifically necessary because the silicon dioxide that is the subject of this submission is food grade. That is, its specification matches or betters that of the permitted food additive silicon dioxide. Before being approved or recommended as food additives, compounds such as silicon dioxide are rigorously assessed and periodically reassessed by national and/or international experts. A consideration of genotoxicity is a critical feature of this assessment, and chemicals considered to be genotoxic are not permitted for use in food. Since silicon dioxide of an appropriate specification (matching the Rentokil material) is an approved food additive in the EU (89/107/EEC as amended), it is clear that silicon dioxide grades that meet the food grade specification do not have genotoxic potential. In assessing the acceptability of using amorphous silicon dioxide in food in the EU, the SCF approved its use, and allocated an ADI "not specified" status (SCF, 1991[1]). This is the most favourable opinion that could be reached and is used for substances of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not represent a hazard to health. For that reason, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. ▪ It is not scientifically justifiable because adequate studies have shown that amorphous silicon dioxide is not a chemical carcinogen (see Sections 6.6.1-3). The major role of genotoxicity studies is to screen for possible carcinogenic activity, and genotoxins would be expected to induce tumours in cancer bioassays. In the case of amorphous silicon dioxide, the available carcinogenicity studies showed no evidence of activity, clearly indicating that amorphous silicon dioxide is not genotoxic. The other existing genotoxicity data are consistent	

with the view that amorphous silicon dioxide is not genotoxic [2].

- It is not scientifically necessary on the basis of low exposure to silicon dioxide during its normal use as a biocide, compared with exposures from other sources.

Exposure to amorphous silicon dioxide when used as an insecticide is inconsequential because of the ubiquity of forms of silicon dioxide in the environment. Silicon, in the form of silicon dioxide and silicates (salts of the various silicic acids), occurs abundantly in nature, comprising about 25% of the earth's crust [3]. Silicon dioxide and silicates are present in practically all plants and animals and in natural waters [4,5]. Between 10 and 200 mg silicon dioxide is present in 100g dry weight of normal human tissue. The lungs and lymph nodes of older adults may have levels several times this amount [4]. Silicon dioxide is an approved food additive, assigned the E number E551 [6], and is used as an anti-caking agent. Silicon dioxide has been given an acceptable daily intake of "not limited" [7]. In addition, silicon dioxide is approved for use in plastic material coming into contact with food, without hazard to public health [8]. Synthetic amorphous silicas are widely used in industry (for example as absorbents, dessicants and fillers) and in synthetic fabrics, plastics, lacquers, vinyl coatings, varnish, paper, pharmaceuticals, adhesives, foods, floor waxes, paints, rubber, and inks [9]. Estimates indicate that 4,400,000 people are exposed to amorphous silicas in their work environments [9]. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder, estimates exposure to be 0.0043 mg silicon dioxide/kg/day *. To put this exposure into context, and notwithstanding the information given above, the silicon dioxide content of raw potato is reported to be 10.1 mg/kg, and one litre of beer contains 131 mg [3].

* Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- In addition to the above, the potential for exposure to silicon dioxide when it is manufactured for use as an insecticide is minimal. Silicon dioxide is manufactured in a completely enclosed system, as is the manufacture of the insecticide product based on silicon dioxide. This means there is no exposure to workers, bystanders or the environment during manufacture. It is estimated that [redacted] of silicon dioxide will be manufactured each year for use as a biocide. This amount of silicon dioxide is tiny in comparison to the other non-biocidal uses of silicon dioxide. For example, amorphous silicon dioxide is the main component of glass and in 1995, 12.9 million tonnes of glass was discarded in the US alone [10,11].

  [redacted]

- Operator exposure work has been carried out in humans exposed to high concentrations of silicon dioxide. Such data has been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For example, the long term workplace exposure limit (WEL) for silicon dioxide set in the UK is 2.4 mg/m^3 (respirable dust) (8h time weighted average) [12]. In an 8-hr work shift, a worker exposed at the UK WEL might inhale 19.2-24 mg silicon dioxide. The US threshold limit value (TLV, set by the American Conference of Governmental Industrial Hygienists, ACGIH) for silicon dioxide is 2 mg/m^3 (respirable dust) [13]. In Australia, the long-term occupational exposure limit for silicon dioxide is also 2 mg/m^3 (respirable dust) [14]. The risk assessment for human exposure to silicon dioxide, when applying the representative product, RID Insect Powder shows that exposure to silicon dioxide does not exceed these agreed maximum

exposure limits for safe working conditions*. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for an approximation of a theoretical safe value.

*The risk assessment for human exposure to silicon dioxide shows exposure to RID Insect Powder, under normal working conditions did not exceed the recommended UK maximum exposure limit to amorphous silicon dioxide (set at 2.4 mg/m^3 for respirable dust)**.

** Refer to Document IIIA, section 2.10 for details of human risk assessment for silicon dioxide.

- There is a substantial volume of information available for amorphous silicon dioxide. The data available are in general agreement, all showing that amorphous silicon dioxide *per se* is intrinsically biologically inert.

There is a substantial volume of information available for amorphous silicon dioxide covering all the major biological considerations. Given the large volume of data available for silicon dioxide, only the typical findings have been summarised below with regards to the chronic toxicity and carcinogenic potential of silicon dioxide. A number of reviews have been conducted by different regulatory bodies including the EPA [15], and the FDA[3], who considered the health aspects of silicon dioxide as a food additive. EPA concluded that silicon dioxide's acute toxicity profile is characterised as moderate to low, and consequently silicon dioxide has been exempted from the requirement of a tolerance limit when applied to growing crops or agricultural commodities. FDA has classified silicon dioxide as Generally Recognised as Safe (GRAS) and has approved its use as a dietary food additive at levels of up to 2% by weight in food. The joint FAO/WHO Expert Committee evaluated a number of food additives. The anti-caking agent silicon dioxide was given an acceptable daily intake of "not limited" [7], which is the most favourable verdict that can be reached. There are two FDA direct food ingredient regulations for silicon dioxide, plus a clearance by the US Department of Agriculture for its use in curing mixes and in animal feed premixes. In agreement with the review by the EPA[15], the FDA concluded that silicon dioxide appears to be biologically inert and there was no evidence available that suggests silicon dioxide is hazardous to humans[3].

Conclusion

The availability of several good-quality studies showing that silicon dioxide does not cause chromosome damage in mice treated orally or by intraperitoneal injection means that it is not scientifically necessary to conduct an *in vitro* mammalian cytogenicity study on amorphous silicon dioxide as it will not add any useful information to the risk assessment. This is supported by the fact that use as an insecticide (PT18) will result in only a very low level of exposure to silicon dioxide. It has been shown in the human risk assessment that compared to exposures *via* the diet and the environment, exposure from silicon dioxide as an insecticide is insignificant. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder shows that exposure to silicon dioxide does not exceed agreed, well established maximum exposure limits for safe working conditions with silicon dioxide and nuisance dust. The toxicological profile of silicon dioxide has been well established with a large body of data available in the public domain. The operator exposure limits that have been set for nuisance particles and dusts are also based on a large amount of available data. As shown above, data is available on the effects of exposure to amorphous silicon dioxide and this data shows that there are no lasting adverse effects. Although this data has its limitations, it is considered sufficient to address the toxicity of silicon dioxide particularly given the levels of exposure expected to silicon

dioxide through other, non-biocidal uses of silicon dioxide including its use in food.

References

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.6.4 Annex Point IIA,VI, 6.6.4	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian bone marrow cytogenetic test / micronucleus test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.4 **Annex Point IIA6.6.4**		**Genotoxicity in vivo** **Section 6: Toxicological and Metabolic Studies** Micronucleus test	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable.	
1.2.2			
1.2.3	Criteria for data protection	No data protection claimed.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Not stated	
2.3	**Deviations**	Not applicable – not a guideline study	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Silica, crystalline, CAS: 14808-60-7; Silica silicis, anhydride, CAS: 7631-86-9 Wako Pure Chemical Industries Ltd (Osaka, Japan)	
3.1.1	Lot/Batch number	LKR3258	
3.1.2	Specification	Deviating from specification given in section 2 as follows:	
3.1.2.1	Description	Not specified in report.	
3.1.2.2	Purity	Not specified in report.	
3.1.2.3	Stability	Not specified in report.	
3.1.2.4	Maximum tolerable dose	>5000 mg/kg	
3.2	**Test Animals**	Non-entry field	
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1 (ICR)	
3.2.3	Source	Not stated in report.	
3.2.4	Sex	M	
3.2.5	Age/weight at study initiation	8-10 weeks-old	
3.2.6	Number of animals per group	5m	

3.2.7	Control animals	Yes		
3.3	**Administration/ Exposure**	Oral and intraperitoneal		
3.3.1	Number of applications	2		
3.3.2	Interval between applications	24 h		
3.3.3	Postexposure period	72h		
		Oral		
3.3.4	Type	Gavage		
3.3.5	Concentration	0, 500, 1000, 2000, 5000 mg/kg bw		
3.3.6	Vehicle	Carboxymethyl cellulose		
3.3.7	Concentration in vehicle	As above		
3.3.8	Total volume applied	Not recorded		
3.3.9	Controls	Vehicle		
		Intraperitoneal injection		
3.3.10	Vehicle	Not stated		
3.3.11	Concentration in vehicle	Not stated		
3.3.12	Total volume applied	Not recorded		
3.3.13	dose applied	0, 500, 1000, 2000, 5000 mg/kg bw		
3.3.14	Substance used as Positive Control	Mitomycin C, 0.5 mg/kg		
3.3.15	Controls	Pre-administration sample		
3.4	**Examinations**			
3.4.1	Clinical signs	No		
3.4.2	Tissue	Peripheral blood		
		Number of animals:	all animals	
		Number of cells:	1000	
		Time points:	0, 24, 48, 72 h after treatment	
		Type of cells	Polychromatic erythrocytes / reticulocytes in peripheral blood	
		Parameters:	Numbers of structural aberrations	
3.5	**Further remarks**	None.		
		4 RESULTS AND DISCUSSION		
4.1	**Clinical signs**	Not recorded.		

4.2	**Haematology / Tissue examination**	No effects.	
4.3	**Genotoxicity**	No – See Table 6_4_4-1	
4.4	**Other**	None.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	5 male mice aged 8- to 10-weeks old were acclimatized (fed on pellets and given water *ad libitum*) before being treated twice orally (*via* a vehicle) or by intraperitoneal injection with test substance. Time between administrations was 24 h. At least 1000 polychromatic erythrocytes or reticulocytes (from peripheral blood) were observed for frequency of micronucleation.	
5.2	**Results and discussion**	Negative response in two intraperitoneal tests (up to 5000 mg/kg) were confirmed with an oral test (up to 5000 mg/kg)	
5.3	**Conclusion**	It is concluded that silica is not carcinogenic *in vivo*.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes. It is not reported whether the testing was carried out to GLP, however, sound protocols were followed and it is believed that this does not interfere with the results. A specification for the test substances was not provided, however, as there appears to have been crystalline content in at least one of the materials, this can be taken as a worst case and shows that wholly amorphous silica would not be carcinogenic.	
		Evaluation by Competent Authorities	
		Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		*Give date of action*	
Materials and Methods		*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*	
Results and discussion		*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*	
Conclusion		Other conclusions: *(Adopt applicant's version or include revised version)*	
Reliability		*Based on the assessment of materials and methods include appropriate reliability indicator*	
Acceptability		acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*	
Remarks			