Fig. 3

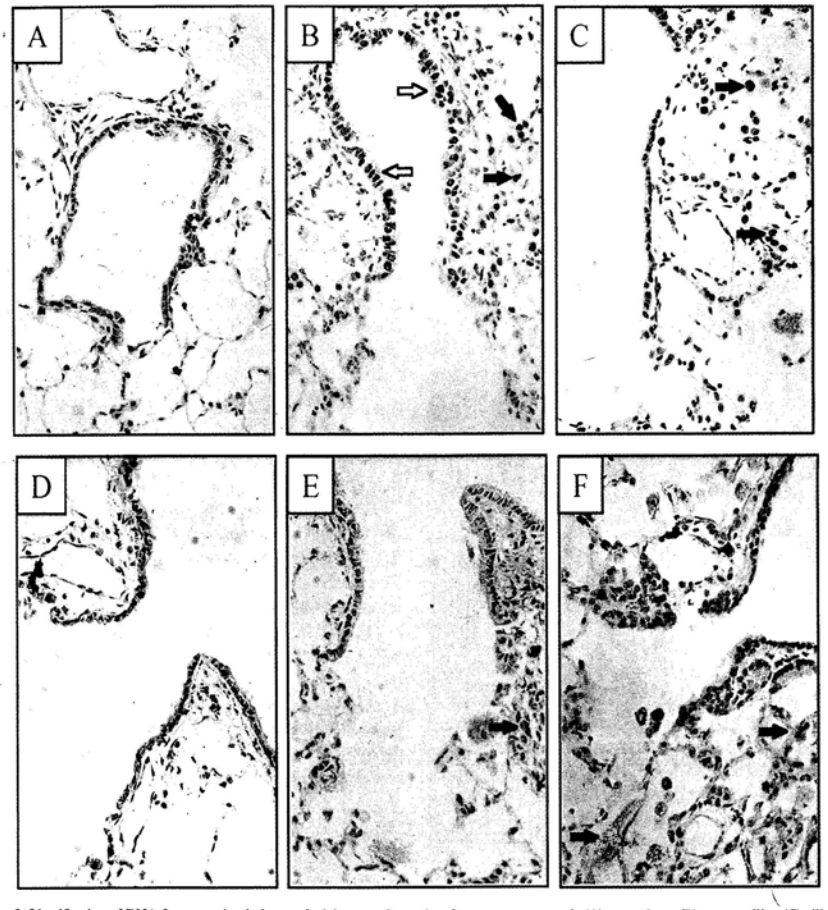


FIG. 3. Identification of DNA fragmentation in lungs of adult rats at 3 months of exposure to room air (A), amorphous (B) or crystalline (C) silica; or at 8 months post-exposure from room air (D), amorphous (E), or crystalline (F) silica. Sections were stained for the presence of free 3′-hydroxyl groups, which was detected as a brown stain. Sections were counterstained with methyl green. Bar in (A) = 100 μm. Filled arrows identify intensely TUNEL-positive cells in the parenchyma and open arrows indicate intensely TUNEL-positive cells in airway.

Fig. 4

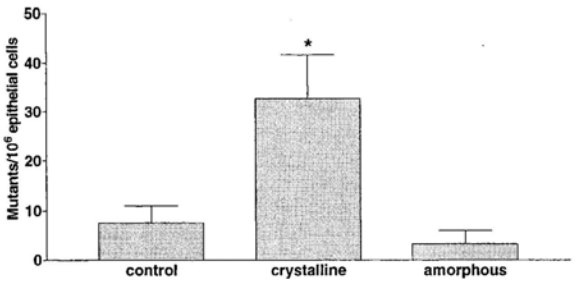


FIG. 4. HPRT mutation frequencies observed for rat alveolar epithelial cells isolated from the lungs of rats exposed subchronically to air or to silica particles. Statistically significant increases in mutation frequencies were detected from epithelial cells obtained immediately after 13 weeks of exposure to 3-mg/m^3 crystalline silica ($p < 0.05$).

Appendix 1

Comparison of silica as submitted in Section 2 and test material

As is shown by the below table, the silica as tested does not deviate significantly from the specification of the silica as stated in Section 2 of Rentokil Initial plc's silicon dioxide dossier as submitted for evaluation under the BPD. Both have high purity, no crystalline content and comparable solubility.

Characteristic	**Aerosil 200[2]** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide	[redacted]	[redacted]
Approved food additive		[redacted]
Amorphous silica	[redacted]	[redacted]
Crystalline content	[redacted]	[redacted]
BET surface area (m^2/g)	[redacted]	
Mean primary particle size	[redacted]	[redacted]
Primary particle shape	[redacted]	
pH value	[redacted]	
Solubility in water	[redacted]	[redacted]
Solubility in organic solvents		[redacted]
Impurities Chloride	[redacted]	[redacted]

[redacted]

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

Section 6.3.1 **Annex Point IIA, VI, 6.3**	**Repeated Dose Toxicity (Oral)** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that: The required route of administration for the short term repeated dose toxicity (28 days) test is the oral route, unless it can be justified that an alternative route is more appropriate. As silicon dioxide is a dust, the most significant route of exposure is via inhalation rather than the oral route. It is on this basis that the 28-day short term repeated dose toxicity study (oral route) is not considered necessary.	
Undertaking of intended data submission [ ]	Not applicable.	

	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 6.3.2 **Annex Point IIA , VI, 6.3**	**Repeated Dose Toxicity (Dermal)** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that a percutaneous study is only required when potential dermal exposure is significant and route-to-route extrapolation is not possible. However, route-to-route extrapolation is possible from oral to dermal in this case. It has been shown that amorphous silicon dioxide does not cause any adverse effects by the oral or inhalation routes (see Document IIIA, Sections A6.4.1 and A6.4.3 for further details). There is no data to suggest that exposure *via* the dermal route is such that it would cause amorphous silicon dioxide to have a more adverse toxicity profile than *via* the oral or inhalation routes. Also, as inhalation is the main route of exposure to amorphous silicon dioxide (as it is a dust), it is best assessed by that route. Given the above, and the fact that Directive 98/8/EC paragraph 19, states that animal testing should be minimised, it is not deemed necessary to generate repeated dose toxicity data *via* the dermal route as it will not provide any useful information for the risk assessment.	
Undertaking of intended data submission [ ]	Not applicable.	

	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 6.3.3 **Annex Point IIA ,VI, 6.3**	**Repeated Dose Toxicity (Inhalation)** Section 6: Toxicological and Metabolic Studies	
	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 **[4]** **Limited exposure** [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that short term repeated dose toxicity (28 days) is intended as a range-finding test and is not required when an adequate subchronic (90 day) toxicity study is available in a rodent. As the subchronic toxicity of silicon dioxide has been considered in Annex Point IIA6.4 Repeated Dose Toxicity, it is therefore not necessary to meet the requirements of the 28 day repeated dose toxicity study.	
Undertaking of intended data submission [ ]	Not applicable.	

	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 6.4.1 **Annex Point IIA, VI, 6.4**	**Subchronic Oral Toxicity Test** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that the required route of administration for the subchronic toxicity test is the oral route, unless it can be justified that an alternative route is more appropriate. As silicon dioxide is a dust, the most significant route of exposure is via inhalation rather than the oral route. It is on this basis that subchronic toxicity test (oral route) is not considered necessary. Notwithstanding the above, the safety profile of amorphous silicon dioxide is well established. The amorphous silicon dioxide as marketed by Rentokil Initial plc for use as an insecticide (PT18) is of food grade and, with regard to long-term ingestion, has an unlimited acceptable daily intake (ADI) set by FAO/WHO[1] alongside its (US) "Generally Regarded as Safe" (GRAS) status[2]. The estimated human oral LD_{50} is >15000 mg/kg. In addition to this, reported for mammalian toxicity (rat LD_{50}) for amorphous silicon dioxide range from >7900, through >10000 and >22500 to >31800mg/kg (see Document IIIA, Section A6.1.1 for further details). Furthermore, data on the subchronic oral exposure of rats to amorphous silicon dioxide is available (see attached study summary) which demonstrates the lack of adverse effects from such exposure. It is noted that the silica differs in its reaction with water compared with the notified silicon dioxide. The test substance in the study is hydrophobic, whereas the notified silicon dioxide is hydrophilic. This surface modification insignificantly affects the toxicological properties of the silica, which is also an approved food additive, and is deemed safe for human consumption. The test substance in the study and the silicon dioxide, which will be marketed by Rentokil Initial as an insecticide (PT18) can therefore be suitably compared, and the data is considered suitable for the risk assessment. **Reference:** [redacted]	
Undertaking of intended data submission [ ]	Not applicable.	

	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 A6.4.1 Subchronic Toxicity: Oral (1 of 1)

Annex Point IIA6.4 **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		
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.26 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**	Aerosil R 972 (Degussa AG, Frankfurt, Germany).
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	Deviating from specification given in section 2 as follows (please see Appendix 1 for justification of test material used):
3.1.2.1	Description	Fluffy powder.
3.1.2.2	Purity (%)	>99.8
3.1.2.3	Impurities (%)	Carbon, bound 1 Al_2O_3 <0.05 Fe_2O_3 <0.01 TiO_2 <0.03 HCl <0.025 Dimethyldichlorosilane <0.1
3.1.2.4	Density	Approx. 2gcm^{-3} (20°C)
3.1.2.5	Particle Size	Approx. 7-16nm
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	Rat.
3.2.2	Strain	Wistar.
3.2.3	Source	Not specified.
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	Male: 120±5g; Female: 130±5g
3.2.6	Number of animals per group	10 male and 10 female.

Section A6.4.1 **Subchronic Toxicity: Oral (1 of 1)**

Annex Point IIA6.4 **Section 6: Toxicological and Metabolic Studies**

3.2.7	Control animals	Yes.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	5 weeks: Low- and mid-dose. 8 weeks: High-dose.
3.3.2	Frequency of exposure	Daily.
3.3.3	Postexposure period	None.
3.3.4	**Oral**	
3.3.4.1	Type	In food.
3.3.4.2	Concentration	In food, 0, 500, 1000, and 2000 mg/kg bw* Drinking water ad libitum. *Because the animals tolerated 2000 mg/kg, the high dose was elevated to 4000 mg/kg after 14 days, to 8000 mg/kg after another 14 days, and finally to 16000 mg/kg.
3.3.4.3	Vehicle	Altromin powder diet (Fa. Altromin GmbH).
3.3.4.4	Concentration in vehicle	Not specified.
3.3.4.5	Total volume applied	Not specified.
3.3.4.6	Controls	Altromin powder diet (Fa. Altromin GmbH).
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. Observed daily.
3.4.1.2	Mortality	Yes. Observed daily.
3.4.2	Body weight	Yes. Measured weekly.
3.4.3	Food consumption	Yes. Observed daily; measured weekly.
3.4.4	Water consumption	No. Water was given *ad libitum*.
3.4.5	Ophthalmoscopic examination	No.
3.4.6	Haematology	Yes. 5 animals from each group. Beginning and end of study. Parameters: Haemoglobin concentration, erythrocyte count, total leukocyte count, blood smear.
3.4.7	Clinical Chemisty	No.
3.4.8	Urinalysis	No.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	No.
3.5.2	Gross and histopathology	Yes. Gross pathology. All dose groups. Liver and kidneys.

Section A6.4.1 **Subchronic Toxicity: Oral (1 of 1)**

Annex Point IIA6.4 **Section 6: Toxicological and Metabolic Studies**

3.5.3	Other examinations	None reported.
3.5.4	Statistics	None reported.
3.6	**Further remarks**	Macroscopic evaluation of treated animals. Microscopic evaluation of liver from highest dose group only.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	16000 mg/kg dose level only: shyness, dirty fur, reduced activity, cachexia and haemorrhage in the mucous membranes of the eyes and nose.
4.1.2	Mortality	16000 mg/kg dose level only: 2 males and 2 females died with severe cachexia in week 8.
4.2	**Body weight gain**	16000 mg/kg dose level only: Weight *loss*, concomitant with decreased food intake (see Figs. 3a and 3b for details). 8000 mg/kg dose level only: Body weight slightly affected (see Figs. 3a and 3b for details).
4.3	**Food consumption and compound intake**	16000 mg/kg dose level only: Food intake reduced with test substance administration level increase.
4.4	**Ophtalmoscopic examination**	None reported.
4.5	**Blood analysis**	
4.5.1	Haematology	1000 mg/kg dose level: Changes in haematology seen sporadically in 2 females (see Table 3 for details). 16000 mg/kg dose level: Condensation of the cytoplasm, loss of the basophilic structure and hyperchromatic and contracted nuclei occurred in the liver cells. Glycogen could not be detected with PAS staining (see Table 3 for details).
4.5.2	Clinical chemistry	None reported.
4.5.3	Urinalysis	None reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	None reported.
4.6.2	Gross and histopathology	16000 mg/kg dose level only: Severe atrophy in the epithelium of the liver.
4.7	**Other**	None reported.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test material was administered in the diet to 3 groups of 10 male and 10 female Wistar rats for 5 weeks in the low- and mid-dose groups and for 8 weeks in the high-dose group. Control groups of 10 males and 10 females were given identical diet without the test material. The animals were housed in a room with a temperature of 23±2°C. The drinking water was offered *ad libitum*. The test substance was mixed

		weekly in the powder diet Altromin taking into account the increasing body weight and the decreasing food intake of the animals. The administered doses were 0, 500, 1000, and 2000 mg/kg. Because the animals tolerated 2000 mg/kg, the high dose was elevated to 4000 mg/kg after 14 days, to 8000 mg/kg after another 14 days, and finally to 16000 mg/kg. Growth, behaviour general condition and food consumption of the rats were observed daily. Body weight and food consumption were measured weekly. At the beginning and end of the experiment, haematological parameters of five animals from each group were determined. The blood was collected from the tail vein under ether. At autopsy, all rats were examined grossly. Kidney and liver samples were fixed in 10% formalin and stained with hematoxylin/eosin after Hotchkiss (PAS staining).
5.2	**Results and discussion**	No treatment-related effects were observed in the low-dose group. Some haematologic changes were seen sporadically in 2 females in the mid-dose group. Noticeable changes only occurred in the high-dose group. These included changes in haematology, atrophy in the epithelium of the liver, cachexia and haemorrhage in the mucous membranes. 2 males and 2 females in the high-dose group died with severe cachexia in week 8)after administration of 16000 mg/kg). No treatment-related effects were found in the kidneys of any animal. It must be noted that the acceptance of treated diet was strongly reduced and that the fraction of the test substance in the diet amounted to approximately 25% by weight (in the high dose group). Malnutrition can also cause changes similar to those found, such as weigh loss, cachexia and decrease of the glycogen content in the liver cells. Therefore it is not clear whether the test substance in a very high dose, the reduced food intake or both caused the described effects. Notwithstanding the above, the study demonstrates that the acute lethal dose range begins at approximately 16000 mg/kg.
5.3	**Conclusion**	
5.3.1	LO(A)EL	1000 mg/kg
5.3.2	NO(A)EL	500 mg/kg
5.3.3	Other	Acute lethal dose range begins at 16000 mg/kg.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. There were deficiencies in the methodology of this study in that the test material was not administered for 90 days in any of the groups (5 weeks: Low- and mid-dose; 8 weeks: High-dose). Also, there was not a post-treatment period. There were also reporting deficiencies as the age of the animals was not reported.

Section A6.4.1 Subchronic Toxicity: Oral (1 of 1)

Annex Point IIA6.4 **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 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	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	

Fig. 3a Fig. 3b

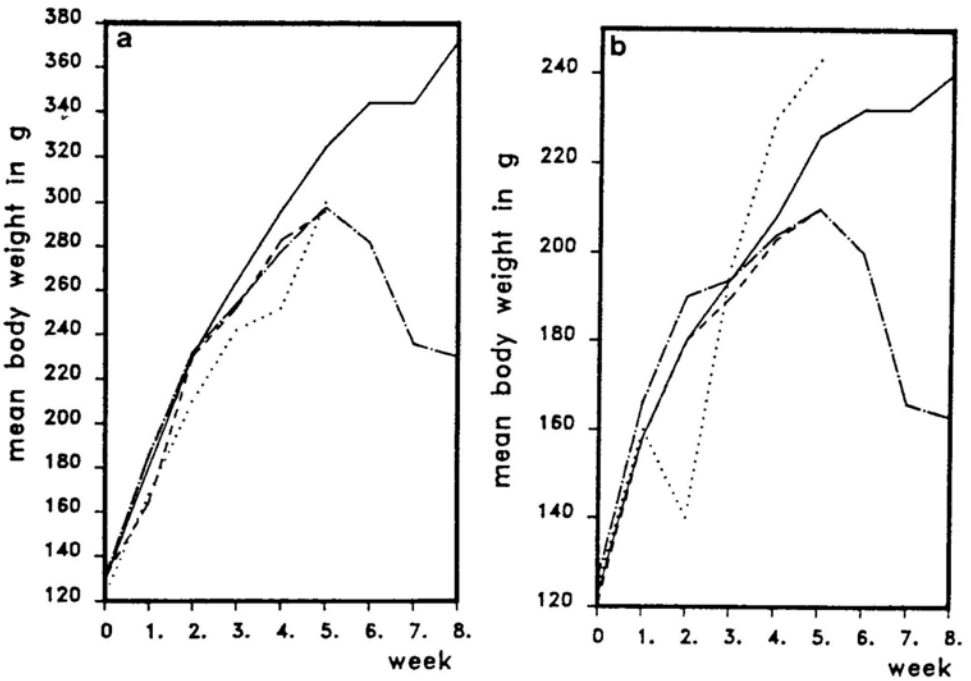


FIG. 3. (a) Body weight of male rats fed FHS for 5 or 8 weeks. (b) Body weight of female rats fed FHS for 5 or 8 weeks (—) 0 mg/kg body wt., (---) 1000 mg/kg body wt., (· · ·) 500 mg/kg body wt., (-·-·-) 2000–16,000 mg/kg body wt.

Table 3. Results of haematology

TABLE 3

HEMATOLOGY DATA FOR RATS FED FHS FOR 5 WEEKS

Dose (mg/kg)	Blood sampling time	Hemoglobin (g/dl)	Erythrocytes (per pl)	Leukocytes (per nl)	Differential leukocyte count (%)[a]					
					Segm neutr	Stab neutr	Baso	Eos	Mono	Ly
Males										
0	Week 0	12.8	6.1	14.8	8	4	0	2	2	84
	Week 8	11.9	6.7	20.7	7	2	0	3	1	87
500	Week 0	12.6	5.2	18.2	10	3	0	1	1	85
	Week 5	12.6	5.8	16.5	6	4	0	0	0	90
1000	Week 0	13.1	5.9	16.0	8	4	0	0	2	86
	Week 5	12.6	5.5	13.3	4	3	0	1	0	92
2000–16000	Week 0	12.9	6.0	14.1	10	2	1	0	3	84
	Week 8	14.3	6.3	18.7	14	5	0	0	1	80
Females										
0	Week 0	12.7	6.8	18.9	8	9	0	1	1	81
	Week 8	12.3	6.4	20.4	11	5	0	1	1	82
500	Week 0	12.9	6.0	16.3	11	2	0	1	2	84
	Week 5	11.1	5.2	14.2	6	3	0	0	0	91
1000	Week 0	13.0	6.1	15.2	6	8	0	2	0	84
	Week 5	11.6	6.0	17.0	6	5	0	1	0	84
2000–16000	Week 0	13.2	6.2	14.2	10	6	0	0	1	83
	Week 8	14.5	6.5	18.5	15	4	0	2	1	78

[a] Abbreviations used: Segm neutr, segmented neutrophils; stab neutr, stab. neutrophils; baso, basophilic leukocytes; eos, eosinophilic leukocytes; mono, monocytes; ly, lymphocytes.

Appendix 1

Comparison of silica as submitted in Section 2 and test material

As is shown by the below table, the silica as tested does not deviate significantly from the specification of the silica as stated in Section 2 of Rentokil Initial plc's silicon dioxide dossier as submitted for evaluation under the BPD. Both are food grade materials with high purity, no crystalline content, comparable solubility and comparable particle size. Although the test material as used, FHS, is surface modified, as stated in the test report, the surface modification insignificantly affects the toxicological properties of the parent silica.

Characteristic	**Fumed Hydrophobic Silica (FHS)** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide	[redacted]	[redacted]
Approved food additive	[redacted]	[redacted]
Amorphous silica	[redacted]	[redacted]
Crystalline content	[redacted]	[redacted]
Particle size	[redacted]	[redacted]
Solubility in water	[redacted]	[redacted]
Solubility in organic solvents	[redacted]	[redacted]
Impurities		
Bound carbon	[redacted]	[redacted]
Al_2O_3 (%)	[redacted]	
Fe_2O_3 (%)	[redacted]	
TiO_2 (%)	[redacted]	
HCl (%)	[redacted]	
Dimethyldichlorosilane (%)	[redacted]	
Surface modification	[redacted]	[redacted]

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

Section 6.4.2 **Annex Point IIA, VI, 6.4**	**Subchronic Dermal Toxicity Test** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [4]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that a percutaneous study is only required when potential dermal exposure is significant and route-to-route extrapolation is not possible. However, route-to-route extrapolation is possible from oral to dermal in this case. It has been shown that amorphous silicon dioxide does not cause any adverse effects by the oral or inhalation routes (see Document IIIA, Sections A6.4.1 and A6.4.3 for further details). There is no data to suggest that exposure *via* the dermal route is such that it would cause amorphous silicon dioxide to have a more adverse toxicity profile than *via* the oral or inhalation routes. Also, as inhalation is the main route of exposure to amorphous silicon dioxide (as it is a dust), it is best assessed by that route. Given the above, and the fact that Directive 98/8/EC paragraph 19, states that animal testing should be minimised, it is not deemed necessary to generate subchronic dermal toxicity data as it will not provide any useful information for the risk assessment.	
Undertaking of intended data submission [ ]	Not applicable.	

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 6.4.3 Annex IIA, VI, 6.4	**Subchronic Inhalation Toxicity Test** Section 6: Toxicological and Metabolic Studies	
	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:

Data is available from public domain studies on the subchronic effects of amorphous silicon dioxide on rats (see attached study summaries). The public domain studies show that any adverse effects to rats from the inhalation of amorphous silicon dioxide for 90 days are reversible.

It is noted that the silicas differ in their reactions with water compared with the notified silicon dioxide. The test substances within the study are both hydrophobic and hydrophilic, whereas the notified silicon dioxide is hydrophilic. However, for this endpoint we shall only be concerned with the available data regarding both tested hydrophilic silicas. All data referring to the hydrophobic silica will be excluded from evaluation.

This data is considered suitable for the risk assessment due to the similarity of the test substances and the silicon dioxide that will be marketed by Rentokil Initial as an insecticide (PT18). A full comparison of the test substances and the silicon dioxide marketed as an insecticide is given in the attached study summaries.

Therefore it is not considered scientifically necessary to carry out a subchronic toxicity study for silicon dioxide.

Even though this is the case for silicon dioxide, and it forms part of the justification for not submitting data on the long-term toxicity of this compound, data on the subchronic toxicity of silicon dioxide is not considered scientifically necessary for the following additional reasons:

The Biocidal Products Directive (98/8/EC, "the Directive") requires long-term testing in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences of chronic exposure (i.e. chronic toxicity and carcinogenicity) to the biocidal active substance. The Directive states in Article 8 (5) that "information which is not necessary owing to the nature of the biocidal product or its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification acceptable to the competent authority must be submitted..." A more detailed waiving concept is given in the TNsG on data requirements. In addition, the TNsG gives the strong recommendation "to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated".

Behind this background, the waiver concept outlined in the TNsG on data requirements is considered applicable for silicon dioxide with regard to the long-term toxicity studies and therefore a scientific justification for waiving these studies are presented below.

- 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 waxed, 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 [17]. 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 chronic toxicity or genotoxicity 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 [15]. 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].

Exposure to increasing concentrations of silicon dioxide: Effects and observations

Below is a summary of the long-term toxicity studies available for silicon dioxide. They are summarised in full under the relevant end points in Document IIIA.

Chronic, oral
Takizawa et al. orally administered 0, 0.125, 2.5 and 5% amorphous silica to $B_6C_3F_1$ mice and Fisher rats 93 weeks and 103 weeks respectively and found that repeated oral administration produced no significant treatment-related effects. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – Study summary 1 of 1).

Chronic, inhalation
Schepers exposed Wistar rats, guinea pigs and rabbits to 126 mg/m^3 amorphous silica by inhalation for a maximum of 24 months. No radiographic signs of lung disease in animals at the end of their maximal period of silicon dioxide inhalation were found. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – Study summary 1 of 2).

Choudat et al studied the health records and chest x-rays of 131 workers (male), 90 of which were the control group and 41 of which were the test group. The 41 men were exposed to 0 – 3.4 mg/m^3 respirable dust over a mean exposure period of 8 years. It was shown that the 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. Exposure to amorphous silica dust may induce a mild small airway disease, only in comparison to a control group. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – study summary 2 of 2).

Repeated dose, inhalation
Reuzel et al. exposed Wistar rats to up to 30 mg/m^3 amorphous silica by inhalation for 90 days. It was found that amorphous silicas did not induce persistent granulomas and the adverse affects in the respiratory tract partly or completely regressed. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.4 – Study summary 1 of 2).

Johnston et al. exposed Fischer-344 rats to 50 mg/m^3 amorphous silica by

inhalation for 90 days. It was found that amorphous silicon dioxide did not cause gene mutation, partly because of its low biopersistence and that the effects of exposure were reversible as demonstrated by the post-exposure results. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.4 – Study summary 2 of 2).

Carcinogenicity
Takizawa et al. orally administered 0, 0.125, 2.5 and 5% amorphous silica to $B_6C_3F_1$ mice and Fisher rats 93 weeks and 103 weeks respectively and found that repeated oral administration produced no significant treatment-related effects. (Referenced and summarised in Document IIIA, Annex point IIA, 6.7 – Study summary 1 of 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 a chronic toxicity 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 which consider subchronic toxicity performed to specific guidelines, 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 6.4 **Annex II, VI, 6.4**	**Subchronic Inhalation Toxicity Test** 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*
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 A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 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	No data protection claimed.
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.29 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**	Aerosil 200 and Sipernat 22S (Degussa AG, Frankfurt, Germany). Note that the results for the quartz used in this study have been ignored as not relevant as it is not amorphous silicon dioxide. The results for Aerosil R974 have also been excluded from the evaluation due to the silica being hydrophobic.
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	Deviating from specification given in section 2 as follows (please see Appendix 1 for justification and further details of test materials used):
3.1.2.1	Description	Fluffy powders.
3.1.2.2	Purity	>99.8% (Aerosil 200); 98% (Sipernat 22S)
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	Rat
3.2.2	Strain	SPF-bred Wistar (Cpb: WU, Wistar random)
3.2.3	Source	TNO Central Institute for the Breeding of Laboratory Animals
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	6 week old
3.2.6	Number of animals per group	70 males and 70 females
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Inhalation

Section A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	5 days per week	
3.3.3	Postexposure period	0, 13, 26, 39 and 52 weeks	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration	Aerosil 200: 1, 6, 30 mg/m³ ; Sipernat 22S: 30 mg/m³
		Analytical concentration	Aerosil 200: 1.3 ± 0.1, 5.9 ± 0.2, 31.0 ± 0.9;; Sipernat 22S: 34.9 ± 0.5
3.3.4.2	Particle size	MMAD not reported.	
		Specification of particles (mean primary particle size):	
		Aerosil 200: 12 nm	
		Sipernat 22S: 18 nm	
		Geometric agglomerate/aggregate size distribution: 1-120 µm	
3.3.4.3	Type or preparation of particles	Aerosols generated using dust generators.	
3.3.4.4	Type of exposure	Whole body	
3.3.4.5	Vehicle	Not applicable.	
3.3.4.6	Concentration in vehicle	Not applicable.	
3.3.4.7	Duration of exposure	6 h	
3.3.4.8	Controls	No exposure.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes. Daily.	
3.4.1.2	Mortality	Not reported.	
3.4.2	Body weight	Yes. Weekly during exposure period; 4 weekly postexposure.	
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: 10 rats/sex/group Time points: 13 week intervals Parameters: Cell count, haemoglobin content, packed cell volume, white-cell count, differential white-cell count, prothrombin time and thrombocytes.	

Section A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

3.4.7	Clinical Chemistry	Yes. Number of animals: 10 rats/sex/group Time points: 13 week intervals Parameters: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea, total protein, creatinine, total bilirubin, calcium, potassium, sodium, inorganic phosphate, cholesterol and glucose.
3.4.8	Urinalysis	Yes. Number of animals: 10 rats/sex/group Time points: 13 week intervals Parameters: appearance, volume, density, pH, protein, occult blood, glucose and ketones. Also microscopy of sediment.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. Organs: lungs, adrenals, brain, heart, kidneys, liver, spleen, testes and thymus.
3.5.2	Gross and histopathology	Yes. Number of animals: 50% of rats at each stage. Time points: 13 week intervals. Organs: lungs, mediastinal and hylus lymph nodes, trachea, larynx, adrenals, aorta, axillary lymph nodes, brain (brainstem, cerebrum and cerebellum), caecum, coagulating glands, colon, duodenum, epididymes, eyes, heart, ileum, jejunum, kidneys, liver, mammary glands, mesenteric lymph nodes, nose (nasal cavity), oesophagus, ovaries, pancreas, parathyroids, parotid salivary glands, pharynx, pituitary, prostate, rectum, seminal vesicles, skeletal muscle (thigh), skin/subcutis (flank), spinal cord, spleen, sternum with bone marrow, stomach, sublingual salivary glands, testes, thymus (if identifiable), thyroid, urinary bladder and uterus. Also respiratory tract and regional lymph nodes.
3.5.3	Other examinations	Collagen and silicon content in lungs and associated lymph nodes. Reversibility (clearance from lungs).
3.5.4	Statistics	Body weights were analysed by an analysis of co-variance (Cochran, 1957) followed by the Dunnett's multiple comparison test (Dunnett, 1955). Analysis of variance (Steel and Torrie, 1960) followed by the Dunnett's multiple comparison test were applied to the organ weights and haematological and biochemical data. Incidences of histopathological changes were analysed by the Fisher exact probability test (Siegel, 1956).
3.6	**Further remarks**	2 week concentration finding studies were performed to assess appropriate concentrations for the 90 day study.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	A concentration-related increase in respiration rate which returned quickly to normal postexposure was noted in animals exposed to Aerosil 200.
4.1.2	Mortality	No mortality reported at any dose level.

Section A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

4.2	**Body weight gain**	At the end of the exposure period, body-weight gain was 5-10% lower in males exposed to 30 mg/m^3 Aerosil 200 or 30 mg/m^3 Sipernat 22. After 13 weeks postexposure, weights had returned to normal (see Table A6_3-1).
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophtalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	At the end of the exposure period, neutrophilic leucocyte counts were higher in most groups than in controls, but the elevation was only statistically significant in rats exposed to 30 mg/m^3 Aerosil 200. These counts returned to normal within 13 weeks postexposure (see Table A6_3-2). Red blood cell counts, haemoglobin content and packed cell volumes had slightly increased in males exposed to 30 mg/m^3 Aerosil 200 at the end of the exposure period. The remaining haematological parameters that were examined did not show differences that could be related to treatment.
4.5.2	Clinical chemistry	No effects.
4.5.3	Urinalysis	Urine analyses were essentially negative.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At the end of the exposure period both absolute and relative lung weight had increased in all treated groups in comparison with the controls and the increases were statistically significant in all groups except that exposed to 1 mg/m^3 Aerosil 200. The increase was greater in males than in females, and of the groups exposed was the greatest in the group exposed to 30 mg/m^3 Aerosil 200 (see Fig. 1). During the first 13 weeks after exposure, this difference had decreased significantly or disappeared. Only rats exposed to 30 mg/m^3 Aerosil 200 took up to 26 weeks to return to normal. No other organ weight increases were observed.
4.6.2	Gross and histopathology	Most exposed rats killed at the end of the exposure period had swollen and spotted lungs with a spongy consistency and/or irregular surface and enlarged lung-associated lymph nodes. At 26 weeks postexposure, the gross changes had disappeared in all groups. Microscopic changes were observed mainly in the lungs (see Table A6_3-3). Changes in rats killed at the end of the exposure period comprised slight to severe accumulation of alveolar macrophages, intra-alveolar granular material, cellular debris and polymorphonuclear leucocytes in the alveolar spaces and increased septal cellularity, seen as an increase the number of type II pneumocytes and macrophages within the alveolar walls. The most severe changes were found in rats exposed to Aerosil 200 (see Fig. 2) and the mildest changes in those exposed to Sipernat 22S. Alveolar bronchiolisation, characterised by cuboidal cells lining the alveolar spaces instead of the normal flat cells, occurred mainly in males exposed to 6 or 30 mg/m^3 Aerosil 200_These changes disappeared partly or completely postexposure. In rats exposed to 30

mg/m^3 Aerosil 200, accumulations of alveolar macrophages were still found 52 weeks postexposure. In rats exposed to Sipernat 22S these lesions were found until 39 weeks postexposure.

Accumulation of intra-alveolar granular material, cellular debris and polymorphonuclear leucocytes were occasionally found in the group exposed to 30 mg/m^3 Aerosil 200 (see Fig. 2). Rats exposed to Sipernat 22S recovered completely from the slight increases in septal cellularity that were observed at the end of the exposure period. A lesser degree in recovery was observed in rats exposed to Aerosil 200. Alveolar bronchiolisation persisted in some rats exposed to Aerosil 200.

Focal interstitial fibrosis, seen as amorphous eosinophilic, collagen-containing thickenings of the septa were first observed 13 after exposure in all exposed groups. During the postexposure period, this condition disappeared completely in rats exposed to Sipernat 22S but became more severe in rats exposed to Aerosil 200.

Alveolar cholesterol clefts were seen in some rats exposed to 30 mg/m^3 Aerosil 200 13, 26 and 39 weeks postexposure. The presence of these clefts was associated with the presence of macrophages and polymorphonuclear leucocytes in the alveoli. One male in the 6 mg/m^3 Aerosil 200 group also had cholesterol clefts in the lungs 13 weeks postexposure. This condition had ceased 52 weeks postexposure.

Granulomas, seen as aggregates of macrophage-like cells were scattered throughout the lungs of a few rats exposed to 30 mg/m^3 Aerosil 200 (see Fig. 3). This lesion disappeared completely in rats of the Aerosil 200 group within 13 weeks postexposure.

Microscopic changes in the associated lymph nodes were characterised by a considerable accumulation of macrophages with or without cellular necrosis. They were found in all groups. However, granulomas were not found in the lymph nodes.

Treatment-related changes were found in the nose of all rats at the end of the exposure period only. They comprised focal necrosis and rhinitis and slight degeneration of the olfactory epithelium. The nature of this reversible damage to the nose was similar in all test groups indicating a rather non-specific irritating effect.

The other organs examined, including the liver, did not reveal treatment-related lesions.

4.7 Other

Lung collagen contents

At the end of the exposure period, the lung collagen content of all exposed groups was higher than that of the controls (see Fig. 4) and the increases were statistically significant in all groups except females exposed to 1 mg/m^3 Aerosil 200. The increase was most pronounced in rats exposed to 30 mg/m^3 Aerosil 200. In rats exposed to Aerosil 200, the increase was clearly concentration dependent and generally more pronounced in males than in females. During the postexposure period, lung collagen levels gradually decreased in all exposure groups but after 1 year postexposure only lung collagen content of rats exposed to 1 mg/m^3 Aerosil 200 or 30 mg/m^3 Sipernat 22S had reached values similar to that of controls.

Silicon in the lungs and their associated lymph nodes

Silicon levels in the lungs of males (except those exposed to Aerosil 200) were higher 26 weeks postexposure than 13 weeks postexposure.

This phenomenon, which there was no explanation for, was not observed in females (see Fig. 5). In this respect, it should be emphasised that large differences in silicon levels were also observed between individual animals of the same group at the same stage. Despite the inexplicable results in males, the overall picture is clear. Silicon was present in 1 male 52 weeks postexposure. Silicon was detected in the lungs of 100% and lymph nodes of 50% of the animals exposed to Sipernat 22S and killed at the end of the exposure period. During postexposure, the amounts of silicon in the lungs decreased quickly and could not be detected in the lungs 39 weeks postexposure. Sipernat 22S disappeared more slowly from the lung associated lymph nodes than the lungs. Aerosil 200 was very quickly cleared from the lungs and the associated lymph nodes.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

6-week-old male (n=490) and female (n=490) SPF-bred Wistar rats (Cpb:WU, Wistar random) were housed singly in stainless-steel wire cages in Hazleton H 1000 inhalation chambers throughout the whole 13-week exposure period. The chambers were kept at 21-23°C and 65-75% relative humidity, with an air-flow of approximately 40m^3/h. The rats were provided *ad lib.* With unfluoridated tap-water and fed the Institute's stock diet for rats. During exposure the rats were deprived of food and water. After the exposure period those rats that were retained for the post-treatment periods were transferred from the inhalation chambers to an animal room and housed in wire-mesh, stainless-steel cages, five males and five females to a cage.

Aerosols were generated using the Institute's dust generators, which were composed of a dust feed mechanism and an atomiser operated by compressed air. The concentrations of test material in the test atmospheres were determined by gravimetry. Samples of the test atmospheres were drawn through glass fibre filters and the filters weighed just before and after sampling.

There were six test groups and one control group each containing 70 males and 70 females. The rats were exposed for 6h/day, 5 days/week for 13 weeks to concentrations of test material as described above. After the exposure period and 13, 26, 39 and 52 after exposure 20, 10, 10, 10, and 20 rats/sex/group, respectively were killed. From 50% of the rats killed at each stage, tissues and organs were collected for analysis. From the other 50%, one lung was assessed for collagen content.

5.2 Results and discussion

When inhaled, the test materials adversely affected the respiratory tract. The changes induced by the test materials were generally most apparent by the end of the exposure period, but disappeared more or less quickly within 1 year after the end of exposure.

In all groups, lesions regressed (albeit not completely in Aerosil 200 exposed rats). The formation of collagen fibres and hyalinisation in the granulomatous lesions (which do not regress) were not observed.

All of the dusts were completely cleared from the lungs.

Aerosil 200 induced the most pronounced responses of alveolar macrophages, both at the end of the exposure period and during the post-exposure period, despite the fact that this material was cleared the fastest. Sipernat 22S caused only a very slight response of alveolar macrophages, though it was present for more than 26 weeks after the end of exposure. The fast dissolution of Aerosil 200 will have resulted

Section A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

		in relatively high concentrations of dissolved SiO_2, explaining why it induced more severe lung changes than the other test materials. The increases in neutrophilic leucocyte counts in rats exposed to Aerosil 200 were found to parallel the occurrence of changes in the lungs, and indeed were considered to be a reflection of inflammatory pulmonary reactions. The test materials did not induce persistent granulomas and the adverse affects in the respiratory tract partly or completely regressed.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Aerosil 200: 5.9 mg/m^3 Sipernat 22S: 34.9 mg/m^3
5.3.2	NO(A)EL	Aerosil 200: 1.3 mg/m^3
5.3.3	Other	None.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. It is acknowledged that this study has not been performed or reported in accordance with approved test guidelines. The report does not state a defined LO(A)EL or NO(A)EL. However, the group sizes and test/recovery periods were appropriate for the method. Also, the pathological, histopathological, haematological etc. parameters measured during the testing were relevant for the required investigation and reporting.

Section A6.4.3 Repeated dose toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.4 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	

Table A6_3-1. Body weights of rats exposed to amorphous silica for 13 weeks and then observed for up to 52 weeks.

Weeks after exposure†	Control	Body weights (g) of rats exposed to: Aerosil 200 1 mg/m³	Aerosil 200 6 mg/m³	Aerosil 200 30 mg/m³	Aerosil R 974 (30 mg/m³)	Sipernat 22S (30 mg/m³)
				Males		
−13	109 ± 2 (70)	107 ± 1 (70)	108 ± 1 (70)	106 ± 1 (70)	108 ± 2 (70)	108 ± 1 (70)
0	380 ± 5 (70)	378 ± 6 (70)	369 ± 4 (70)	355 ± 5** (70)	380 ± 4 (70)	363 ± 5* (70)
13	441 ± 7 (50)	443 ± 6 (50)	437 ± 6 (50)	423 ± 6 (50)	446 ± 6 (49)	428 ± 6 (49)
26	492 ± 8 (40)	495 ± 8 (40)	487 ± 8 (40)	480 ± 9 (40)	594 ± 8 (39)	484 ± 8 (39)
39	516 ± 14 (20)	524 ± 11 (20)	511 ± 14 (20)	509 ± 14 (20)	516 ± 12 (20)	494 ± 12 (19)
52	530 ± 17 (20)	534 ± 14 (20)	528 ± 15 (20)	535 ± 14 (20)	536 ± 12 (20)	509 ± 13 (18)
				Females		
−13	107 ± 1 (70)	106 ± 1 (70)	109 ± 1 (70)	107 ± 1 (70)	105 ± 1 (70)	107 ± 1 (70)
0	227 ± 2 (70)	231 ± 2 (70)	233 ± 2 (70)	226 ± 2 (70)	229 ± 2 (70)	223 ± 3 (69)
13	254 ± 3 (50)	258 ± 3 (50)	261 ± 3 (50)	259 ± 3 (50)	254 ± 2 (50)	253 ± 3 (49)
26	274 ± 5 (30)	285 ± 5 (30)	283 ± 6 (30)	288 ± 6 (29)	280 ± 4 (29)	284 ± 6 (29)
39	295 ± 8 (20)	298 ± 7 (20)	306 ± 9 (20)	317 ± 10 (19)	296 ± 7 (18)	311 ± 10 (19)
52	315 ± 8 (19)	312 ± 7 (20)	318 ± 9 (20)	335 ± 11 (19)	301 ± 9 (17)	334 ± 14 (18)

Week 13 = start of exposure

Values are means ± SEM for the numbers of rats indicated in parentheses

Values marked with asterisks differ significantly (covariance and Dunnett's tests, two-sided) from the corresponding control value (*P <0.05; **P <0.01).

Table A6_3-2. Neutrophilic leucocyte count for rats exposed to amorphous silica or quartz for 13 weeks then observed for up to 52 weeks.

Group	Neutrophilic leucocyte count (10^8/litre) in post-exposure wk: 0	13	26	39	52
			Males		
Controls	20 ± 3	14 ± 4	20 ± 3	15 ± 2	31 ± 6
Aerosil 200 (30 mg/m³)	44 ± 6**	21 ± 2	17 ± 2	20 ± 3	22 ± 2
Aerosil R 974 (30 mg/m³)	26 ± 3	19 ± 3	20 ± 2	14 ± 3	20 ± 3
Sipernat 22S (30 mg/m³)	28 ± 2	21 ± 4	24 ± 9	24 ± 3	19 ± 3
Quartz (60 mg/m³)	48 ± 4**	94 ± 12**	73 ± 10**	75 ± 4**	95 ± 14**
			Females		
Controls	12 ± 1	13 ± 2	12 ± 3	13 ± 2	14 ± 2
Aerosil 200 (30 mg/m³)	35 ± 6**	21 ± 2	19 ± 2	9 ± 1	19 ± 3
Aerosil R 974 (30 mg/m³)	24 ± 3	21 ± 2	12 ± 2	17 ± 5	17 ± 3
Sipernat 22S (30 mg/m³)	25 ± 4	18 ± 3	12 ± 2	15 ± 2	22 ± 4
Quartz (60 mg/m³)	31 ± 7*	47 ± 4**	55 ± 8**	54 ± 5**	88 ± 11**

Values are means ± SEM for groups of 10 rats, and those marked with asterisks differ significantly (ANOVA and Dunnett's test, two-sided) from the corresponding control value (*P < 0.05; **P < 0.01).

Table A6_3-3. Summary of incidences of treatment-related microscopic changes in the lungs of rats exposed to amaorphous silica or quartz and then observed for up to 52 weeks.

Lesion	Time after exposure (wk)	No. of rats	Occurrence of lesion in: Males in Group†: A	B	C	D	E	F	G	Females in Group†: A	B	C	D	E	F	G
Granuloma-like lesions	0	10	0	0	0	2	10**	0	10**	0	0	0	0	10**	0	9**
	13	5	0	0	1	0	5**	0	5**	0	0	1	0	5**	0	5**
	26	5	0	0	0	0	3	0	5**	0	0	0	0	1	0	5**
	39	5	0	0	0	0	1	0	4*	0	0	0	0	1	0	5**
	52	10	0	0	0	0	0	0	10**	0	0	0	0	0	0	10**
Accumulation of alveolar macrophages	0	10	4	10**	10**	10**	10**	10**	10**	1	10**	10**	10**	10**	10**	10**
	13	5	1	2	5**	5**	5**	5**	5**	0	5**	5**	5**	5**	5**	5**
	26	5	0	1	5**	5**	5**	5**	5**	0	1	4*	5**	3	5**	5**
	39	5	0	0	3	5**	4*	2	5**	1	0	3	4	4	2	5**
	52	10	1	1	1	10**	0	4	10**	0	1	4	8**	1	0	10**
Cellular debris	0	10	0	2	10**	10**	3	2	10**	0	2	6	10**	8**	2	10**
	13	5	0	0	0	2	0	0	5**	0	0	0	0	0	0	5**
	26	5	0	0	0	0	0	0	5**	0	0	0	1	0	0	5**
	39	5	0	0	0	0	0	0	5**	0	0	0	0	0	0	5**
	52	10	0	0	0	1	0	0	10**	0	0	0	1	0	0	10**
IPLI	0	10	1	10**	10**	10**	3	2	10**	0	8**	10**	10**	8**	0	9**
	13	5	0	0	1	1	0	1	5**	0	1	3	5**	5**	0	5**
	26	5	0	0	0	0	0	0	5**	0	0	0	1	0	0	5**
	39	5	0	0	0	0	0	0	5**	0	0	0	0	0	0	5**
	52	10	0	0	0	0	0	0	10**	0	0	0	0	0	0	10**
Increased septal cellularity	0	10	1	10**	10**	10**	3	2	10**	1	9**	9**	10**	10**	6	10**
	13	5	0	0	5**	5**	5**	0	5**	0	1	3	5**	5**	0	5**
	26	5	0	0	5**	3	5**	0	5**	0	1	1	4*	4*	0	5**
	39	5	0	0	0	4*	4*	0	5**	0	0	0	3	4*	0	5**
	52	10	1	1	2	7*	2	4	10**	0	0	3	7*	2	0	10**
Alveolar bronchiolization	0	10	0	0	5	10**	2	0	4	0	0	0	1	0	0	0
	13	5	0	0	1	4*	1	0	1	0	0	0	1	0	0	0
	26	5	0	0	0	4*	1	0	4*	0	0	0	1	0	0	2
	39	5	0	0	0	1	1	0	5**	0	0	0	2	0	0	5**
	52	10	1	2	0	1	0	1	10**	0	1	0	2	0	0	10**
Focal interstitial fibrosis	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13	5	0	0	2	5**	3	1	2	0	1	1	4*	0	0	0
	26	5	0	0	3	4*	1	0	3	0	1	2	5**	0	0	4*
	39	5	0	0	3	5**	0	0	5**	0	0	1	4*	0	0	5**
	52	10	0	0	2	9**	0	0	10**	0	1	1	10**	0	0	10**
Cholesterol clefts	0	10	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	13	5	0	0	1	5**	0	0	0	0	0	0	2	0	0	0
	26	5	0	0	0	4*	0	0	3	0	0	0	0	0	0	2
	39	5	0	0	0	0	0	0	2	0	0	0	1	0	0	5**
	52	10	0	0	0	0	0	0	10**	0	0	0	0	0	0	10**

IPLI = intra-alveolar polymorphonuclear leucocytic infiltration

†Groups were exposed for 13 wk as follows: A—no treatment (controls); B—1 mg Aerosil 200/m³; C—6 mg Aerosil 200/m³; D—30 mg Aerosil 200/m³; E—30 mg Aerosil R 974/m³; F—30 mg Sipernat 22S/m³; G—60 mg quartz/m³.

Values are for the number of rats shown, and those marked with asterisks differ significantly (Fisher's exact probability test) from the corresponding control value (*$P < 0.05$; **$P < 0.01$).

Fig. 1

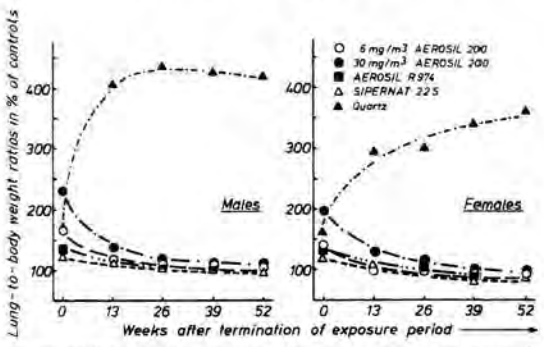


Fig. 1. Relative lung weights expressed as percentage of controls in rats exposed to amorphous silica or quartz for 13 wk and killed at 13-wk intervals after exposure. Exposures were as follows: ○, 6 mg Aerosil 200/m³; ●, 30 mg Aerosil 200/m³; ■, 30 mg Aerosil R 974/m³; △, 30 mg Sipernat 22S/m³; ▲, 60 mg quartz/m³. Values are means for groups of 20 (wk 0 and 52) or 10 (wk 13, 26 and 39) rats.

Fig. 2

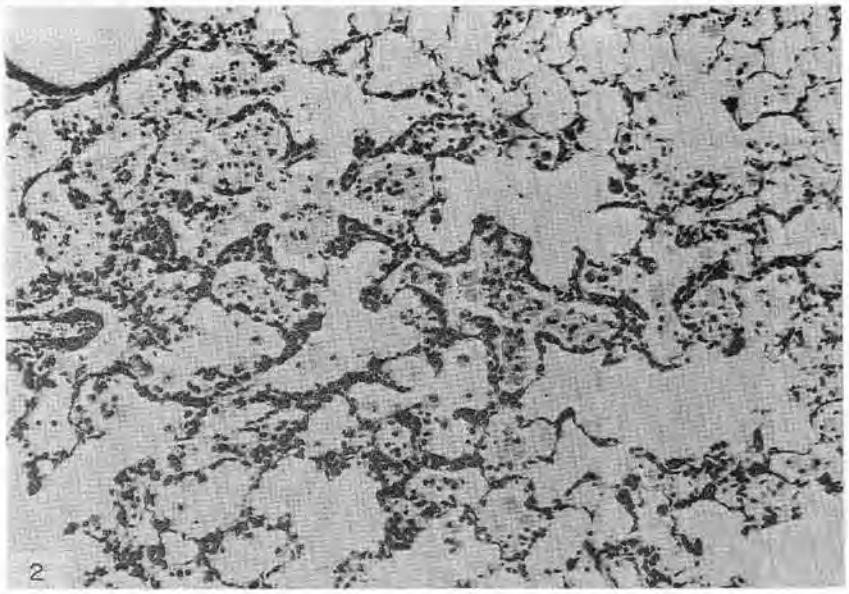


Fig. 3

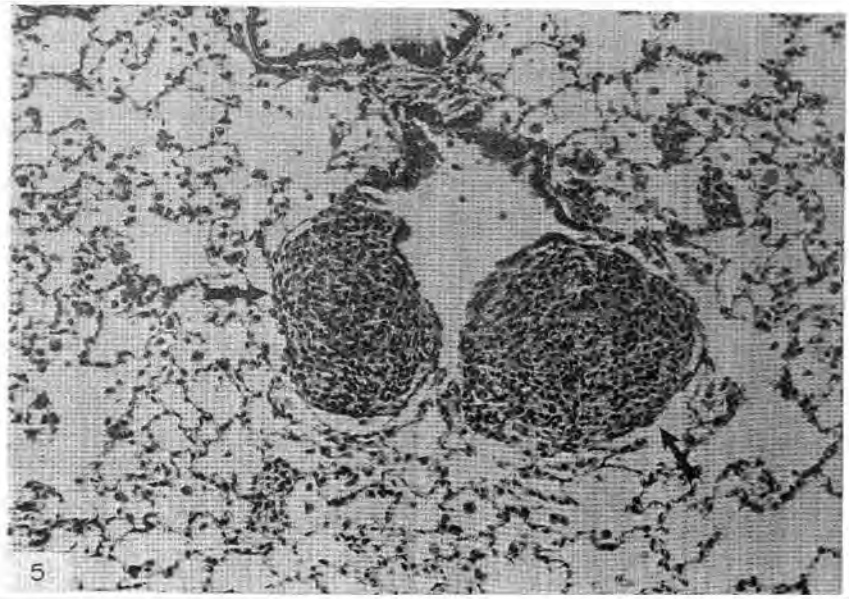


Fig. 4

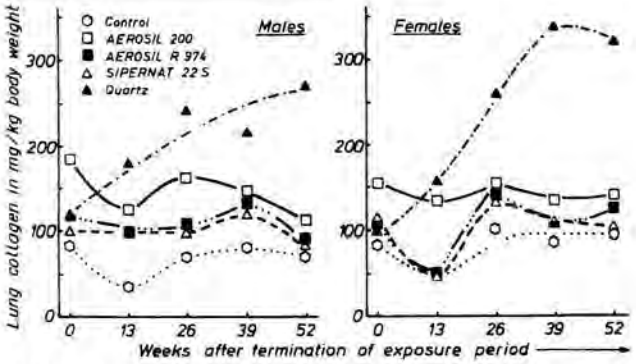


Fig. 2. Lung collagen contents expressed as mg/kg body weight in rats exposed to amorphous silica or quartz for 13 wk and killed at 13-wk intervals after exposure. Exposures were as follows: ◇, controls; □, 30 mg Aerosil 200/m^3; ■, 30 mg Aerosil R 974/m^3 △, 30 mg Sipernat 22S/m^3; ▲, 60 mg quartz/m^3. Values are means for groups of 10 (wk 0 and 52) or 5 (wk 13, 26 and 39) rats.

Fig. 5

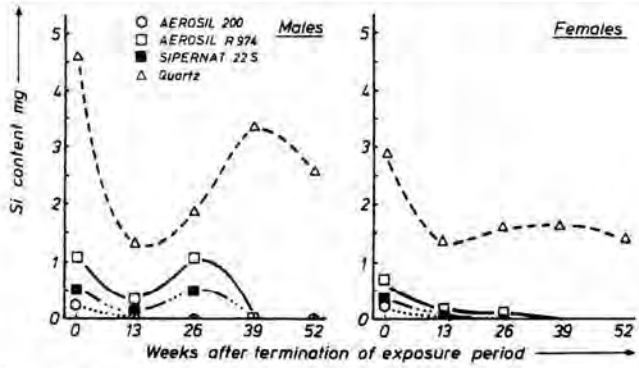


Fig. 3. Total amount of Si in the lungs of rats exposed to amorphous silica or quartz for 13 wk and killed at 13-wk intervals after exposure. Exposures were as follows: ○, 30 mg Aerosil 200/m^3; □, 30 mg Aerosil R 974/m^3; ■, 30 mg Sipernat 22S/m^3; △, 60 mg quartz/m^3. Values are means for groups of 10 (wk 0 and 52) or 5 (wk 13, 26 and 39) rats.

Appendix 1

Comparison of silica as submitted in Section 2 and test material

As is shown by the below table, the silica as tested does not deviate significantly from the specification of the silica as stated in Section 2 of Rentokil Initial plc's silicon dioxide dossier as submitted for evaluation under the BPD. All have high purity, no crystalline content, comparable solubility and comparable particle size.

Aerosil 200

Characteristic	**Aerosil 200** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide		
Approved food additive		
Amorphous silica		
Crystalline content		
BET surface area (m^2/g)		
Mean primary particle size		
Primary particle shape		
pH value		
Solubility in water		
Solubility in organic solvents		
Impurities: Chloride		

Sipernat 22S

Characteristic	**Sipernat 22S** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide		
Approved food additive		
Amorphous silica		
Crystalline content		
BET surface area (m^2/g)		
Mean primary particle size		
Primary particle shape		
Agglomeration size		
pH value		
Solubility in water		
Solubility in organic solvents		
Impurities: Sodium sulphate		

Section A6.4.3 Repeated dose toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.4 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.29 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**	Aerosil 200 (Degussa AG, Frankfurt, Germany). Note: the results from the use of crystalline silica in this study have been ignored as they are not relevant (as it is not amorphous - see Appendix 1).
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	Deviating from specification given in section 2 as follows (please see Appendix 1 for justification of test material used):
3.1.2.1	Description	Powder.
3.1.2.2	Purity	>99.8%
3.1.2.3	Stability	Stability of the test material not reported. However, it is widely reported that amorphous silicon dioxide is a stable compound (melting point >1500°C) which is practically inert and unreactive.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer-344
3.2.3	Source	Not specified in report
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	200-250 g
3.2.6	Number of animals per group	4
3.2.7	Control animals	Yes

Section A6.4.3 Repeated dose toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	6h/day, 5 days/week	
3.3.3	Postexposure period	3 and 8 months	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration	50 mg/m^3
		Analytical concentration	50.4 ± 19.0 mg/m^3
3.3.4.2	Particle size	MMAD: 0.83 µg	
3.3.4.3	Type or preparation of particles	Silica aerosols generated using a screw-feed mechanism (ACCURate, Whitewater, WI).	
3.3.4.4	Type of exposure	Whole body	
3.3.4.5	Vehicle	Not applicable.	
3.3.4.6	Concentration in vehicle	Not applicable.	
3.3.4.7	Duration of exposure	6 h	
3.3.4.8	Controls	Filtered air.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Not reported.	
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	Not reported.	
3.4.7	Clinical Chemistry	Not reported.	
3.4.8	Urinalysis	Not reported.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Not reported.	
3.5.2	Gross and histopathology	Yes. Number of animals: All groups. Time points: 6.5 and 8 weeks of exposure; 3 and 8 months of recovery. Organs: lungs.	

Section A6.4.3 Repeated dose toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

3.5.3	Other examinations	Lung burden analysis. Bronchoalveolar lavage fluid analysis. RNA isolation/polymerase chain reaction (PCR). Immunohistochemistry. Type-II cell isolation and HPRT assay.
3.5.4	Statistics	Results were evaluated for statistical significance by analysis of variance. Differences from the air control group were determined using Dunnett's test. Statistical significance was considered at $p < 0.05$.
3.6	**Further remarks**	None.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Not reported.
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	Not reported.
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	(See Fig. 1) Elevated numbers of neutrophils and macrophages, first detectable at the first observation point (45 days exposure), decreasing in the post-exposure period. Proliferative response evident at 45 days exposure which decreased precipitously at 8 month exposure point. Fibrosis present in the alveolar septa.
4.7	**Other**	*MIP-2 Gene Expression:* (See Fig. 2) MIP-2 present at end of 13 week exposure period. However, minimal MIP-2 mRNA detected at 8 months post-exposure point. *TUNEL Staining:* (See Fig. 3) Intensely stained TUNEL-positive cells detected throughout the terminal bronchiolar epithelium and throughout the parenchyma. After the 8 month post-exposure period, only faint TUNEL-positive cells were occasionally observed, indistinguishable from the control. *Isolation of alveolar epithelial cells and HPRT mutation frequencies:* (See Fig. 4) No differences between treatment groups and controls.

Lung silica burden: At end of exposure, group-mean lung burden was 882.7 µg/lung. By 3 months after exposure, burdens were significantly decreased relative to the end of exposure and decreased further by 8 months (see Table A6_4_3-1).

BAL fluid: There was significant change in all BAL parameters through exposure, returning to near sham-exposed levels by 3 months post-exposure and values were not significantly different from controls by 8 months post-exposure (see Table A6_4_3-2).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Experimental design: Three groups of male Fischer-344 rats were exposed to filtered air (control) or aerosols of silica (amorphous or crystalline) in compartmentalised 300-litre horizontal laminar flow, whole-body chambers. Silica aerosols were generated using a screw-feed mechanism in combination with a Venturi-type dust feeder. The aerosol was brought to Boltzman equilibrium by passing the airborne particles across a 20-mCi ^{85}K source.
After 6.5 and 13 weeks exposure and 3 and 8 months of recovery, groups of rats were euthanised followed by exsanguination *via* the abdominal aorta, for analysis of lung silica burdens, cellular and biochemical bronchoalveolar lavage fluid markers of lung injury and inflammation, histopathology, inflammatory cytokine gene expression and mutagenesis in alveolar epithelial cells.
Lung burden analysis: A modification of the method of Hemenway *et al.* (1990) was used to determine lung-silica burdens. The lavaged lung was cut into small pieces and placed in the bottom of a platinum crucible, dried overnight in an oven then ashed for 24h using a plasmod asher. Sodium carbonate was added and this then fused for 30min. Crucibles were then cooled to room temp. 10mL of double distilled water was added to the sample, which was then placed in a water bath at 90°C for 15min then sonicated for 20min. Samples were analysed for Si using emission spectroscopy at a wavelength of 251.612.
Bronchoalveolar lavage (BAL) fluid analysis: BAL was performed as described in detail previously (Ferin *et al.*, 1992). Briefly, the lungs and heart were excised, the trachea cannulated and the lungs lavaged 10 times with 5mL of sterile saline each time, at room temp. The BAL fluid was centrifuged and the acellular supernatant was analysed for total protein, lactate dehydrogenase (LDH) and glucuronidase levels or activities. Total protein was determined using the micro-BCA method. LDH was assayed using a Sigma diagnostic kit. The enzyme glucuronidase was measured by the release of p-nitrophenol from the substrate 4-nitrophenylglucuronide, determined at 420nm on a Cary 219 spectrophotometer. BAL fluid cells were quantified by haemocytometric counting, and cell viability was determined by exclusion of trypan blue dye. Cell differentials were performed on cytocentrifuge preparations that were fixed in methanol and stained with Diff-Quik.
Histopathology: After lavage, a lobe of lung was inflated, with 10% neutral buffered formalin (NBF), to the original size. The bronchus was ligated, and the entire lobe immersed in 10% NBF. After a minimum 24h fixation, the lung was grossly slabbed in a radial pattern, with the bronchus as the central point. The lung slab was processed through paraffin, sectioned at 5 microns and stained with Gormor's trichrome. All sections were coded by accession number, which "blinded" the observer from the treatment. The sections were examined for alveolitis: the number of neutrophils and macrophages and the amount of proteinaceous fluid and alveolar type-II epithelial cell proliferation. In addition, the severity of inflammation in bronchioles and bronchi was noted, as was the extent of fibrosis and the relative amount of lung parenchyma affected (diffuseness). All were ranked on a severity score of form 0.0 (no significant lesions) to 4.0 (very severe process) and then summed to yield a toxicity score.

RNA isolation/polymerase chain reaction (PCR): Expression of MIP-

2 mRNA in lungs was assessed, as described elsewhere (Driscoll *et al.*, 1993a,b). Briefly, the left lung lobes from 2 animals/exposure group/time were quick-frozen in liquid nitrogen for later isolation of RNA. RNA was extracted as described by Chomczynski and Sacchi (1987), and mRNA transcript levels were assessed by PCR amplification of the MIP-2 cDNA.

GAPDH mRNA was evaluated concurrently with MIP-2 mRNA as a control. PCR primers were designed from the published sequences for MIP-2 and GAPDH and were as follows:

MIP-2: 5'-GGCACATCAGGTACGATCCAG-3'
5'-ACCCTGCCAAGGGTTGACTTC-3'
GAPDH: 5'-CAGGATGCATTGCTGACAATC-3'
5'-GGTCGGTGTGAACGGATTTG-3'

PCR reactions were overlaid with mineral oil and amplification was carried out through 22-30 cycles of denaturation at 94°C for 1 min, oligo-annealing at 55°C for 1 min and extension at 72°C for 2 min. Reactions were electrophoresed in 1.5% agarose gels containing ethidium bromide in Tris-acetate/EDTA buffer to visualise the MIP-2 and GAPDH PCR products. It was confirmed that the PCR products obtained with the primer sequences were MIP-2 or GAPDH by Southern analysis and using oligonucleotide probes complementary to mRNA sequences internal to the PCR primer sequences used.

Immunohistochemistry: Terminal transferase dUTP nick-end-labelling (TUNEL)-staining on lung sections was performed. Sections were deparaffinised and hydrated before blocking of endogenous hydrogen peroxide with hydrogen peroxide-methanol. TUNEL staining was performed using an ApopTag kit obtained from Oncor, according to the manufacturer's recommendations. Stained sections were photographed using colour-slide film.

Type-II cell isolation and HPRT assay: The rat alveolar type-II cell isolation and the HPRT clonal selection assay were performed as described in detail previously, with the exception that alveolar epithelial cells were harvested from the right lung only in the present study (Driscoll *et al.*, 1995). Briefly, animals were injected with sodium heparin (40 U, ip) before euthanisation. The lungs and trachea were removed and the lungs were perfused, *via* the pulmonary artery, with a buffered salt solution at 9mL/min, using a Harvard infusion pump. The right lung lobe was lavaged 5 times with 5mL sterile BSS and twice with 5mL sterile BSS containing 2.5 mM $CaCl_2$ and 1.2 mM $MgSO_4$ (Ca-Mg BSS). 5 mL of a pronase solution was instilled into the right lung every 5 min for a total of 3 times. The lung tissue was placed in beakers containing 1 mg DNase, 4mL saline and 5mL foetal bovine serum and minced into ~1- to 4-mm pieces. Lung tissue was filtered and the resultant cell suspensions were centrifuged. Cell pellets were resuspended in Ham's F12 medium containing 2% FBS, layered over a Nycodenz gradient and centrifuged for 20 min at 1500 X *g*. The cell layer just beneath the interface was removed, washed twice with saline and resuspended in RluE medium. Staining for alkaline phosphatase activity routinely identified the epithelial cells. Cell counting was performed using a haemocytometer and trypan blue dye exclusion was used to determine cell viability. Freshly isolated alveolar type-II cells were seeded at 2×10^5 epithelial cells/flask into 6 T25 flasks, the cells were allowed to attach overnight and then the culture dishes were washed with RluE cell culture medium to remove non-adherent cells. The cell cultures were fed with medium containing 6TG to select for mutation in the HPRT gene; cultures were re-fed every other day with 6TG-containing medium. After 14-21 days in culture the cells were fixed and immunostained with an antibody to cytokeratins 8, 18 and 19 and 6TG-resistant cytokeratin staining colonies of greater than 50 cells

Section A6.4.3 Repeated dose toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.4 Section 6: Toxicological and Metabolic Studies

		counted. Mutation frequencies were calculated as (number of colonies/treatment)/(plating efficiency)/(10^6 cells) = mutants/10^6 cells.
5.2	**Results and discussion**	After 3 months of increased inflammatory cell levels there was no significant increase in HPRT mutation frequency in rat alveolar epithelial cells. LDH levels in lung lavage were increased most likely due to epithelial cells. This is supported by the increased TUNEL staining in epithelial cells. The increased numbers of cells showing positive TUNEL staining after 90 days of inhalation suggest significant increase of intracellular damage, which may lead to cell death. Cells that were predominantly affected were macrophages and epithelial cells lining the terminal bronchioles. Increased cytotoxicity due to the very high administered dose may have caused necrosis or apoptosis of mutated epithelial cells. The increased cytotoxicity could be a consequence of the very high numbers of alveolar neutrophils and their activation. During recovery there was no persistence of MIP-2 expression or increased level of neutrophils. Any other effects were shown to be reversible after recovery in normal air. The rapid clearance (low biopersistance) of amorphous SiO_2 may be a contributory factor to its lack of cytogeneticity.
5.3	**Conclusion**	Amorphous silicon dioxide does not cause gene mutation, partly because of its low biopersistence. The effects of exposure are reversible as demonstrated by the post-exposure results.
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Not reported.
5.3.3	Other	Not reported.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes. It has not been reported whether or not this study was performed to GLP. Also, as this was not an OECD guideline study, endpoints are not directly comparable. It is acknowledged that a full specification of the material tested is not available. However, current information on this material is available from the manufacturer and has been used. Although this study primarily sets out to investigate the effects of amorphous silicon dioxide with respect to mutagenicity, the analysis of the lungs given (in depth) in this report can be used as a good indicator of the effects that a substance whose primary route of exposure will be inhalation might have. Especially relevant are the endpoints for lung silica-burden, BAL fluid and histopathology which explores the extent of inflammatory processes. This is also helpful in demonstrating the reversibility of the effects from inhaled silica.

Section A6.4.3 Repeated dose toxicity (Inhalation 2 of 2)

Annex Point IIA, VI, 6.4 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	

Table A6_4_3-1. Table for Lung Burdens after Subchronic Exposure of Rats to Amorphous Silica (µg SiO_2/lung)

	Weeks of Exposure		Weeks of Exposure	
Treatment Group	6.5	13	12	32
Control	55.9 ± 40.4	42.5 ± 16.9	28.1 ± 13	39.8 ± 8.7
Amorphous, 50 mg/m^3	755.9 ± 22.9*	882.7 ± 83.1*	156.0 ± 38.6*	92.6 ± 38.6*

Note. Values represent the Mean ± SD; n = 4 rats/treatment

*Significantly different from age-matched control group; $p < 0.05$.

Table A6_4_3-2. Table for Changes in Bronchoalveolar Lavage Fluid Contents in Rats after 6.5 and 13 Weeks of Inhalation Exposure to SiO_2 Particles and 12 or 32 weeks of Recovery (Mean ± SD; n = 4)

	Total cells x 10^7	% AM	% PMN	% Lymph	% Viable	Protein (µg/mL)	LDH (nmol/min/mL)	β Glucuronidase (nmol/min/mL)
Sham Exposure								
Week 6.5	1.09 ± 0.23	99.7 ± 0.36	0.24 ± 0.23	0.77 ± 0.13	93.8 ± 2.74	0.16 ± 0.6	56.2 ± 14.7	0.47 ± 0.11
Week 13	2.89 ± 0.5	98.4 ± 1.9	0.26 ± 0.24	0.64 ± 0.53	94.6 ± 0.63	0.26 ± 0.05	11.7 ± 46.0	0.53 ± 0.01
Recovery								
Week 12	1.76 ± 0.24	98.1 ± 0.47	0.65 ± 0.52	1.22 ± 0.33	92.9 ± 1.1	0.21 ± 0.04	47.9 ± 9.5	0.53 ± 0.07
Week 32	1.20 ± 0.11	98.8 ± 0.43	0.73 ± 0.34	0.48 ± 0.18	94.0 ± 2.4	0.175 ± 0.02	41.6 ± 6.5	0.26 ± 0.06
Amorphous SiO_2								
Week 6.5	16.8 ± 0.54*	42.8 ± 4.9*	55.2 ± 4.8*	2.0 ± 0.8	97.0 ± 0.9	0.94 ± 0.08*	709.4 ± 101.0*	19.3 ± 3.0*
Week 13	16.9 ± 2.2*	42.6 ± 2.9*	55.3 ± 2.2*	2.1 ± 1.2	94.2 ± 1.4	1.59 ± 0.08*	1808.0 ± 631.6*	29.2 ± 2.5*
Recovery								
Week 12	2.7 ± 0.82	88.8 ± 0.9	9.3 ± 0.9*	1.9 ± 0.4	93.8 ± 1.5	0.386 ± 0.06	192.1 ± 57.6*	1.1 ± 0.19
Week 32	2.2 ± 0.08	94.9 ± 1.1	2.6 ± 1.3	2.5 ± 0.5	94.2 ± 2.4	0.339 ± 0.02	152.0 ± 68.8	0.46 ± 0.06

*Significantly different from age-matched control group; $p \leq 0.05$.

Fig. 1

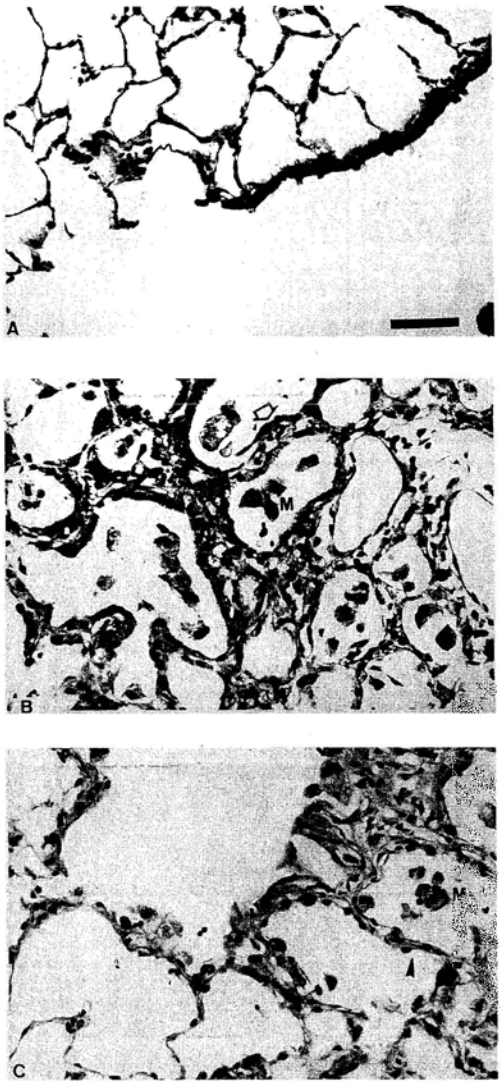


FIG. 1. Histopathology of the lungs from adult rats exposed to room air (A), crystalline silica (B), or amorphous silica (C) for 3 months and examined 8 months after completion of exposure. Note the greatly thickened alveolar septa in B (open arrow) and the normal or slightly thickened septa in C (solid arrow). In both crystalline- and amorphous-exposed animals, there was a persistence of increased numbers of hypertrophic alveolar macrophages (M). All illustrations are of the same magnification; bar = 50 μm.

Fig. 2

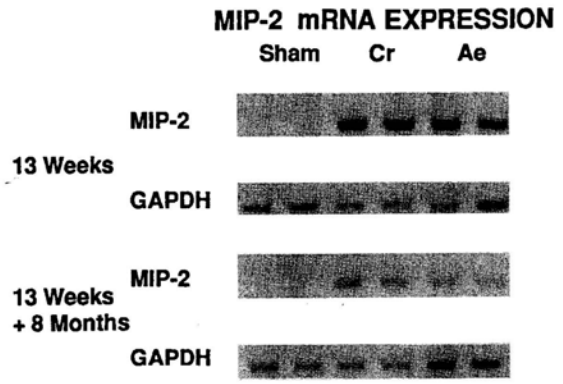


FIG. 2. Effect of subchronic inhalation of SiO_2 particles on expression of mRNA for MIP-2 and GAPDH in rat lung. Shown are negatives of ethidium bromide-stained gel with the Rt-PCR products for MIP-2 and GAPDH amplified for rat lung RNA. Rats were exposed to air or amorphous (Ae) or crystalline (Cr) silica at concentrations of 50 mg/m^3 and 3 mg/m^3, respectively, for 13 weeks, followed by an 8-month recovery period.

Fig. 3

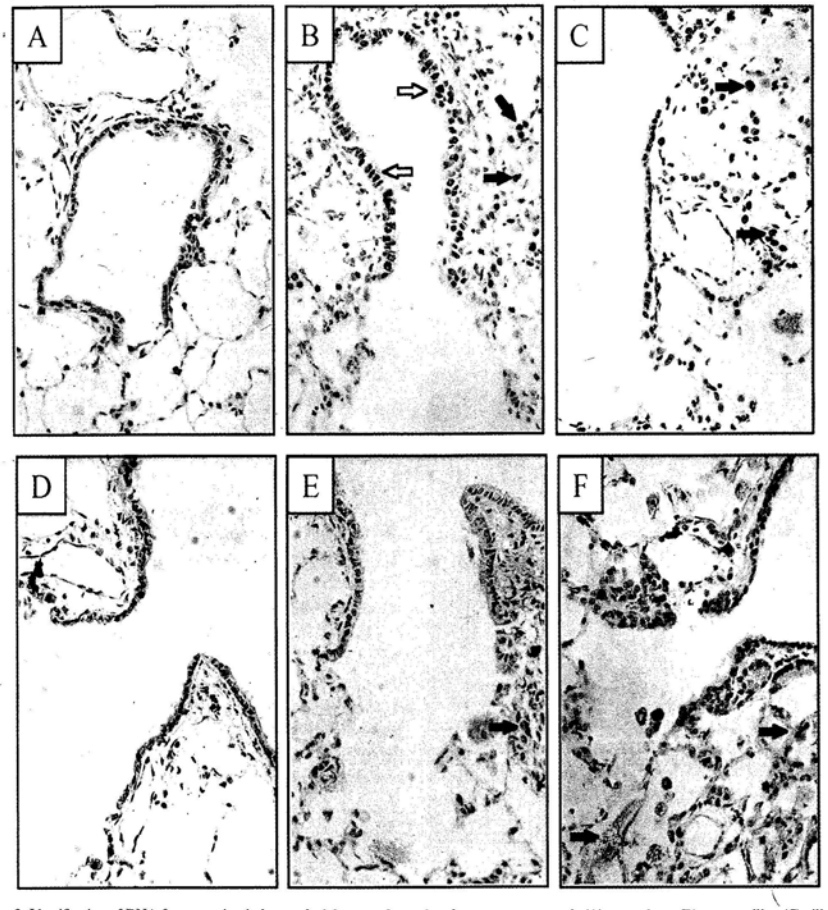


FIG. 3. Identification of DNA fragmentation in lungs of adult rats at 3 months of exposure to room air (A), amorphous (B) or crystalline (C) silica; or at 8 months post-exposure from room air (D), amorphous (E), or crystalline (F) silica. Sections were stained for the presence of free 3′-hydroxyl groups, which was detected as a brown stain. Sections were counterstained with methyl green. Bar in (A) = 100 μm. Filled arrows identify intensely TUNEL-positive cells in the parenchyma and open arrows indicate intensely TUNEL-positive cells in airway.

Fig. 4

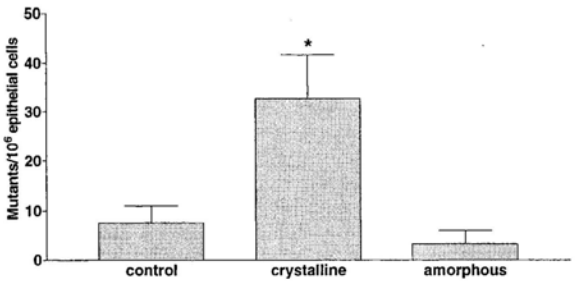


FIG. 4. HPRT mutation frequencies observed for rat alveolar epithelial cells isolated from the lungs of rats exposed subchronically to air or to silica particles. Statistically significant increases in mutation frequencies were detected from epithelial cells obtained immediately after 13 weeks of exposure to 3-mg/m^3 crystalline silica ($p < 0.05$).

Appendix 1

Comparison of silica as submitted in Section 2 and test material

As is shown by the below table, the silica as tested does not deviate significantly from the specification of the silica as stated in Section 2 of Rentokil Initial plc's silicon dioxide dossier as submitted for evaluation under the BPD. Both have high purity, no crystalline content and comparable solubility.

Characteristic	**Aerosil 200[2]** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide	[redacted]	[redacted]
Approved food additive		[redacted]
Amorphous silica	[redacted]	[redacted]
Crystalline content	[redacted]	[redacted]
BET surface area (m^2/g)	[redacted]	
Mean primary particle size	[redacted]	[redacted]
Primary particle shape	[redacted]	
pH value	[redacted]	
Solubility in water	[redacted]	[redacted]
Solubility in organic solvents		[redacted]
Impurities Chloride	[redacted]	[redacted]

[redacted]

Section 6.5 Annex Point IIA, VI, 6.5	Chronic Toxicity Section 6: Toxicological and Metabolic Studies	
	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:

Data is available from one public domain study on the chronic effects of synthetic amorphous silica on rats and mice which shows that no dose-related effects are present (see attached study summary). Also, all effects observed in the subchronic 90-day toxicity test were found to be reversible.

It is not considered necessary to perform testing in a second species as data exists on humans working with amorphous silicon dioxide shows that inhalation (for up to 14 years) produces no adverse effects (see attached study summary for details). In a public domain study monitoring the respiratory health of humans after occupational inhalation exposure, no effects were shown after a mean exposure of 8 years (see attached study summary for details). Also, in a 24 month inhalation study on rats, guinea pigs and rabbits, no signs of lung disease were discovered (see attached study summary for details). Even though this is the case for silicon dioxide, and it forms part of the justification for not submitting data on the long-term toxicity of this compound, data on the chronic toxicity of silicon dioxide is not considered scientifically necessary for the following additional reasons:

The Biocidal Products Directive (98/8/EC, "the Directive") requires long-term testing in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences of chronic exposure (i.e. chronic toxicity and carcinogenicity) to the biocidal active substance. The Directive states in Article 8 (5) that "information which is not necessary owing to the nature of the biocidal product or its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification acceptable to the competent authority must be submitted..." A more detailed waiving concept is given in the TNsG on data requirements. In addition, the TNsG gives the strong recommendation "to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated".

Behind this background, the waiver concept outlined in the TNsG on data requirements is considered applicable for silicon dioxide with regard to the long-term toxicity studies and therefore a scientific justification for waiving these studies are presented below.

- **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 waxed, 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 [17]. 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 chronic toxicity or carcinogenicity 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 [20]. 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].

Exposure to increasing concentrations of silicon dioxide: Effects and observations

Below is a summary of the long-term toxicity studies available for silicon dioxide. They are summarised in full under the relevant end points in Document IIIA.

Chronic, oral
Takizawa et al. orally administered 0, 0.125, 2.5 and 5% amorphous silica to $B_6C_3F_1$ mice and Fisher rats 93 weeks and 103 weeks respectively and found that repeated oral administration produced no significant treatment-related effects. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – Study summary 1 of 1).

Chronic, inhalation
Schepers exposed Wistar rats, guinea pigs and rabbits to 126 mg/m^3 amorphous silica by inhalation for a maximum of 24 months. No radiographic signs of lung disease in animals at the end of their maximal period of silicon dioxide inhalation were found. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – Study summary 1 of 2).

Choudat et al studied the health records and chest x-rays of 131 workers (male), 90 of which were the control group and 41 of which were the test group. The 41 men were exposed to 0 – 3.4 mg/m^3 respirable dust over a mean exposure period of 8 years. It was shown that the 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. Exposure to amorphous silica dust may induce a mild small airway disease, only in comparison to a control group. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.5 – study summary 2 of 2).

Repeated dose, inhalation
Reuzel et al. exposed Wistar rats to up to 30 mg/m^3 amorphous silica by inhalation for 90 days. It was found that amorphous silicas did not induce persistent granulomas and the adverse affects in the respiratory tract partly

or completely regressed. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.4 – Study summary 1 of 2).

Johnston et al. exposed Fischer-344 rats to 50 mg/m^3 amorphous silica by inhalation for 90 days. It was found that amorphous silicon dioxide did not cause gene mutation, partly because of its low biopersistence and that the effects of exposure were reversible as demonstrated by the post-exposure results. (Referenced and summarised in Document IIIA, Annex point IIA, VI, 6.4 – Study summary 2 of 2).

Carcinogenicity
Takizawa et al. orally administered 0, 0.125, 2.5 and 5% amorphous silica to $B_6C_3F_1$ mice and Fisher rats 93 weeks and 103 weeks respectively and found that repeated oral administration produced no significant treatment-related effects. (Referenced and summarised in Document IIIA, Annex point IIA, 6.7 – Study summary 1 of 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 a chronic toxicity 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 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]

9. [redacted]

Undertaking of intended data submission [ ]	Not applicable.

	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_5a – Study summary removed May 2008

A6_5b – Study summary has been removed.

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

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**	HI-SIL 233 (PPG Industries).
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	Please note the following: This study has been performed using a substance that is similar to that in Section 2 of the current dossier. However, impurities are too high for this study to be considered in the risk assessment. This is, however, a useful study as it is indicative of the reversibility of the effects of amorphous silicas and therefore is suitable as a supporting document. Deviating from specification given in section 2 as follows:
3.1.2.1	Description	Completely amorphous silica.
3.1.2.2	Purity (%)	[redacted]
3.1.2.3	Impurities (%)	[redacted]
3.1.2.4	Density	Specific gravity: Particles: 2.30; Bulk: 0.15
3.1.2.5	Particle Size	225-250 Å
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	Rats; Guinea pigs; Rabbits
3.2.2	Strain	Rats: Wistar; Guinea pigs: Saranac colony; Rabbits; Not reported
3.2.3	Source	Rats: Sprague-Dawley colony; Guinea pigs: Not reported; Rabbits; Not

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

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

		reported
3.2.4	Sex	Not reported.
3.2.5	Age/weight at study initiation	Not reported.
3.2.6	Number of animals per group	Rats: 84; Guinea pigs: 167; Rabbits: 19
3.2.7	Control animals	Yes: Rats; Guinea pigs; Rabbits
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Duration of treatment	Maximum 24 months
3.3.2	Frequency of exposure	7 days/week
3.3.3	Postexposure period	Maximum 12 months
3.3.4	**Inhalation**	
3.3.4.1	Concentrations	Nominal concentration: 126 mg/m^3 Analytical concentration: Not reported
3.3.4.2	Particle size	2 to 5 µm
3.3.4.3	Type or preparation of particles	Dust created in a box through mechanical agitation by a rotating paddle aided by a stream of compressed air at the throat of a Venturi tube.
3.3.4.4	Type of exposure	Whole body
3.3.4.5	Vehicle	Not applicable
3.3.4.6	Concentration in vehicle	Not applicable
3.3.4.7	Duration of exposure	8 h
3.3.4.8	Controls	Normal air.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes.
3.4.1.2	Mortality	Yes.
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. Rabbits.

		Erythrocyte count, haemoglobin levels, packed cell volume.
3.4.7	Clinical Chemistry	Not reported.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. All animals. Lung.
3.5.2	Gross and histopathology	Yes. All animals. Lung, lymph nodes.
3.5.3	Other examinations	Lung silica content and retention of silica in lungs. Interactions with tuberculosis infection.
3.5.4	Statistics	Not reported.
3.6	**Further remarks**	None.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Cardiac ventricular pressures were measured in rabbits. The control left-ventricular pressure increased linearly and slightly with age by about 12-mm mercury, an increase of 28%. At the same time the control right-ventricular pressures increased linearly with age from an average starting value of 6-cm H_2O pressure to an average of 14 cm after 24 months, an increase of 130%.

During the exposure phase the left-ventricular pressures rose to an average of 47.7 mm mercury, an increase of 24% above the control values. On cessation of the dust exposure the left-ventricular pressures initially continued to climb above the control values, but within 6 months a decisive change had set in and the differences between the control and test values decreased progressively from 25 to 1% at the end of the 12 months of dust inhalation, a 41% difference between the test and control animals. When the exposure was discontinued, the right-ventricular pressure decreased to 23% lower than the control pressures. The two cross over after about 8 months of elimination of the silica.

Dyspnea on exertion and head elevation occurred to slight degrees in animals exposed for more than 9 months. The sporadic occurrence of these symptoms here suggest that not all of the rabbits became symptomatic as a result of prolonged inhalation, since the majority recovered completely on cessation of exposure. It is likely that the animals that did develop symptoms were the ones that had experienced cardiac punctures and intercurrent viral infections. However, the possibility that the dust inhalation might have played a role cannot be negated.

Neoplasms were found in the lungs of 5 of the control rats, a 10% incidence. Two tumours were found in the lungs of the rats that inhaled HI-SIL 233. Since there were 84 test rats the tumour incidence is 2.4%, which suggest that the HI-SIL exposure definitely did not place these

animals at hazard with respect to neoplasiogenesis.

In the 167 guinea pigs that inhaled HI-SIL 233 one lung tumour was found. This occurred in an animal subjected to silica inhalation and infection with RiRv mycobacteria. The overall tumour incidence is 0.6%. There were no tumours in the control guinea pigs, which derived from the Saranac colony with a known tumour incidence of approximately 1%.

No tumours were observed in the 19 rabbits exposed to HI-SIL 233, and none in the 50 control rabbits.

In one rat, about 8-months-old, the tumour was a papillary adenoma of the kind frequently found in laboratory rats. The adenoma originated in the subpleural zone of the lung and, by expanding peripherally, compressed the surrounding lung parenchyma. No invasion of lung tissue was detectable and there were no metastases to distant organs.

There were identical tumours in rats that had inhaled HI-SIL 233 for 6 months and then were transferred to normal air for 3 months. One animal was 12-months-old. The tumour was, again, a typical rat papillary adenoma which could not be distinguished from the pulmonary tumours found in the control rats.

The tumour which was found in a guinea pig that had been infected with RiRv mycobacteria and four months later commenced inhaling HI-SIL 233 dust for 8 months also was a papillary adenoma of the kind occasionally found in guinea pigs that had not breathed any dust. The tumour was highly cellular with extremely little stroma. It invaded the pulmonary parenchyma along its free edges, but there was no distant dissemination to other parts of the lung or to other organs. There were no dust-induced lesions in the region of the lung where the tumour originated, nor any focal tuberculous process, which suggests that the tumour had nothing to do with either the inhalation of HI-SIL 233 or the infection with the mycobacteria.

A similar papillary adenoma with virtually no stroma was found in a guinea pig that served as an undusted control for a concurrently conducted study. There was no metastastic dissemination of this tumour to other parts of the lungs or to distant organs.

The conclusion is that HI-SIL 233 played a role in the genesis of these tumours, which are of the natural type and prevalence of the rat and guinea pig colonies that participated in this study.

4.1.2 Mortality

See Table A6_5-1. For rats, analysis of the distribution of deaths over the period of the experiment demonstrates that the deaths occurred at a more or less even rate in both the dusted and control animals. The causes of death were approximately the same in variety and prevalence. The majority of the rats died from enzootic pulmonary infections, often culminating in pneumonia or even pulmonary suppuration. A few had severe emphysema, sometimes with associated corpulmonale. Exceptionally, neoplastic disease killed a few animals of both the control and test groups.

The mortality experience for guinea pigs was appreciably lower than that for the rats. No guinea pigs that had been exposed to HI-SIL 233 for 12 months and then returned to normal air died, in sharp contrast to the fate of the rats. Of the 80 guinea pigs continuously exposed to HI-SIL 233 for up to 24 months, 8 died (9.7%). Among the 100 controls, which resided only in normal air over a period extending to 36 months, only 8

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.5

Section 6: Toxicological and metabolic studies

		died. This was typical of this colony of guinea pigs, which had remained infection free for several decades. The slight preponderance of deaths among the exposed group is of minimal significance if the zero death rate among the elimination group is considered, since the combined mortality is only 6.3%. Of the 50 control rabbits, 23 died. Five of these deaths were due to cardiac tamponade resulting from the experimental cardiac punctures. Of the 19 exposed rabbits, 7 died during the study. In three of these cases cardiac tamponade from cardiac punctures was again the cause of death. The overall mortality for the test group was 46% as compared with the 30% mortality among the controls. If the artificial deaths are discounted for both the control and the test group, the mortality rates are respectively 20 and 36%. This suggests that the period of residence of the rabbits in the dust room where they breathed HI-SIL 233 dust was less hazardous than residence in normal air.
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	Rabbit: During the dust-exposure phase, the average erythrocyte counts increased to 6.70 million red cells as compared with the non-exposure count average of 5.51 for rabbits that had lived in normal air only. The exposure average count was within the range of variation for the control series, but higher average values do suggest that there was a minor grade of erythremia in the rabbits by the end of 12 months of inhalation of HI-SIL 233. When the dust exposure was discontinued for 12 months, the average red cell count still was 6.44. Although individual values fell within the range of variation, the high average suggests persistence of mild erythremia with only about 4% decrease of the cell count. There were comparable elevations of the haemoglobin values. The haemoglobin content of erythrocytes in control rabbits was 9.4 g. All the test rabbits registered haemoglobin values above this level at the end of the 12 months of dust exposure. Thus the average measurement was 13.2 g, which was about 40% higher than that of the controls. When the animals returned once more to normal air the haemoglobin continued to build up, attaining an average value of 14.0 g by the end of the 12 months of residence in normal air. This represents a difference of 49% between the test and control animals. Packed cell volumes increased by about 12% during exposure and remained about 5% above normal average values after a year of dust elimination. Significant changes were not observed with respect to mean corpuscular volumes, mean corpuscular haemoglobin or leucocyte counts. The extreme range of variation in the latter reflects the epizootic in the control rabbits.
4.5.2	Clinical chemistry	Not reported.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and**	

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

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

pathology

4.6.1	Organ weights	Changes in the lung weights of test animals are summarised graphically in Fig. 1. The standard weight values for control animals are compared with the weights observed in rats and guinea pigs which were exposed to HI-SIL 233 aerosol by inhalation for periods of 15 and 24 months respectively, and also in rats and guinea pigs which were transferred to normal air, respectively after 6 and 12 months of aerosol exposure. In both animal species the lung weights increased to approximately double the weights of the control animals towards the end of the continuous exposure period. When dust exposure was discontinued, the lung weights rapidly and steadily reverted to normal. In the case of the rats, the average lung weights were the same as those registered for the controls by the end of 9 months in normal air. Reversion to normal was not as complete as in the case of the guinea pigs, but lung weights did ultimately approximate normal levels. The residual slightly elevated lung weights may be due entirely to the slow departure of dust-filled cells, especially from the lungs, because of the tendency of the species to generate large conglomerate multinucleated dust-storing macrophages (koniophores) that are slow in reconverting to individual, smaller macrophage components which would be able to escape from the lungs. Figures 2 and 3 summarise the data obtained through the serial analysis of the lungs of rats and guinea pigs during the exposure phase and after they were returned to normal air following 12-months continuous inhalation of HI-SIL 233 aerosol. In guinea pigs a plateau was established at about the fourth month of dust exposure, which suggests either that, at this stage, elimination of the silica occurred as rapidly as its accumulation or that, after 4 months of dust exposure, the guinea pig lung had attained a tolerance threshold and was no longer able to absorb and retain any additional silica.
4.6.2	Gross and histopathology	The basic biological action in all three animal species was tissue response limited to phagocytosis and focal storage of phagocytes, which resulted in dust cell nidi. At late stages some of these were faintly visible to the naked eye, but no radiographic signs of lung change could be found. There was no necrosis of any of the phagocytes. They tended to form large multinucleated cell masses or koniophores. These cells remained throughout the inhalation phase and retained their ability to undergo mitosis and to phagocytise dust and other materials. On cessation of dust exposure, the koniophores broke up once more into component phagocytes which, with sufficient elapsed time, cleared completely out of the lungs and tracheobronchial lymph nodes, leaving no tissue reminders of their prior occupancy. The accumulation of phagocytes in the lungs of the test animals coincided with progressive storage of HI-SIL 233 particles in the lungs, almost exclusively in the phagocytes. Many phagocytes and retained silica were constantly being removed from the lungs during dust inhalation. Some phagocytes found their way into the hilar lymph nodes, which became enlarged and indurated. Phagocytes filled with particulates were found in the respiratory passages. There was no discernible entry of particulates into the blood stream and no phagocytes or amorphous silica could be demonstrated in remote organs such as liver, kidney, brain and muscle. The phagocytes in the lung had little discernible effect on the lungs except to occupy air space not needed for respiration. Some phagocytes accumulated in lymphatic plexuses around small pulmonary blood

vessels and occasionally around the terminal respiratory passages. When the animals were infected with tubercle bacilli, or as a result of naturally occurring epizootics, the numbers of phagocytes outside the regular storage nidi increased. After the infection terminated, the prevalence of phagocytes decreased.

In occasional animals there were some eosinophils in some of the dust nidi or in surrounding alveoli; occasionally there were plasma cells. No fibrocytes, fibroblasts or mast cells were noted.

There was minimal stimulation of epithelial cell prevalence in some animals. It seems likely that epizootics influenced this trend more than dust inhalation. The silica dust may have played a part, however, since there was decreasing incidence of this feature in animals after removal from the dust. Epithelisation of peribronchiolar alveolar surfaces was observed in some stages. The silica may have stimulated this change when there was concurrent infection. Lesions were particularly impressive in animals that had inhaled HI-SIL 233 continuously after infection with the tubercle bacilli. Epithelisation also occurred in the undusted controls, particularly in animals with tuberculosis infection. The epithelisation was not progressive after dust inhalation was discontinued.

The only consistent stromal change in proximity to the dust cell nidi was a mild grade of reticulum deposition in the alveolar walls, which could also be demonstrated in control animals. However, there appeared to be some focal increase of the response, which decreased after dust exposure was discontinued. No collagen formed in uninfected animals at any stage. Only in guinea pigs infected with tuberculosis was any collagen demonstrated, being increased in the large lesions which developed in animals that were infected and then continuously exposed to the silica dust. There was no more collagen in these lesions in animals that inhaled HI-SIL 233 for up to 24 months than in animals at the 4- and 6-months stages of the study, which suggests that the tuberculosis process was more influential than the inhaled dust in provoking the deposition of collagen.

Elastic tissue did not increase or decrease as a result of dust exposure. Minimal distortion and stenosis of bronchioles and some hypertrophy of the muscle cells in the terminal respiratory passages were noted. This suggests that there may have been bronchospasm and minimal functional bronchiolar obstruction. On cessation of the dust exposure these changes were reversed, which suggests that they were directly due to the inhalation of the silica.

It seems likely that ageing and recurrent epizootic pneumonitis played a significant role in the genesis of the emphysema, since this change also occurred in controls and since dust cessation did not halt the progression of the process. Other varieties of submicron silicas have proven capable of inducing progressive and severe pulmonary emphysema. If the HI-SIL 233 dust played a role, this was minimal and sporadic and dependent on other factor. Other siliceous particles induce significant emphysema by virtue of their destructive effects on the mucosa of the terminal bronchioles and disruption of the continuity of alveolar walls. Neither of these processes was noted in the animals that breathed the HI-SIL 233. The type of emphysema that predominated during the exposure phase was diffuse hypertrophic vesicular distension. After dust exposure was discontinued, destructive vesicular confluence was more prevalent, which suggests that dust inhalation may have played a part in

determining the type of response even though infection may have been more influential.

In rabbits, particularly after dust exposure was terminated, the dominant residual manifestation was alveolar duct distension and focal emphysema. It is likely that some of the regional distension of alveoli was compensatory to partial atelectasis of alveoli, since recovery from this focal emphysema occurred when dust cells were completely evacuated. The presence of hypertrophied bronchiolar muscle cells suggests that bronchiolar spasm was a factor in the peripheral alveolar distension. However, since the emphysema progressed in the animals after discontinuance of dust exposure while other dust-related lesions regressed, it is difficult to explain the emphysema, except in terms of the epizootic pneumonitis.

No damage was inflicted on the main respiratory passages. The epithelium of the trachea and bronchi remained completely normal throughout exposure periods as long as 24 months. In the smaller airways there was some hypertrophy and hyperplasia of epithelium in some animals. The cells remained morphologically normal but more numerous and individually larger. No increase in mitosis of the cells was noted. There may have been some stimulation of cell proliferation, as suggested by the occasional epithelisation of alveolar spaces. An alternative explanation is that these epithelised alveoli may have resulted through delayed desquamation of normal lining cells.

There were no changes in the pleura, nor were there substantial numbers of subpleural nidi of dust-filled cells. This is considerably in contrast to the tendency of other inert dust particles to congregate here in great quantity.

In the tracheobronchial lymph nodes there was some storage of dust-filled phagocytes. Clearance of these cells occurred faster and more completely in the rats and rabbits than in guinea pigs. No structural tissue response accompanied these phagocytes. No collagen could be demonstrated here.

Reversal of lung lesions was relatively speedy and complete.

4.7 Other

Lung silica content and retention of silica in lungs

Less than 10 mg of silica was retained in guinea pigs that had inhaled HI-SIL 233 at a 126 mg/cm^3 concentration. The silica content of the lung ash of undusted controls reached about 0.6 mg % in the same 12-monrth period, and is silica derived from dietary constituents, their air supply having been filtered to exclude extraneous silica.

After 12 months of dust exposure the relative silica content of the lung ash in guinea pigs continued to increase slowly. This is explained by the lack of increase in nonsiliceous mineral content of the lung ash associated with the progressive deposition of fibrous tissue in the lungs. In animals exposed to HI-SIL 233, no such factors operated. In control animals the silica content also continued to increase slowly, since additional silica was derived from the food and water.

On completion of 6 months of elimination, the silica content of the lungs of guinea pigs that had inhaled HI-SIL 233 for 12 months approximated the silica content of the undusted control guinea pigs that had never been exposed to any type of silica. This affirms the virtual complete reversibility of tissue retention of silica in animals that had inhaled silica dust. This near total silica elimination matched the almost

complete regression of the thesaurotic reactions induced by the HI-SIL233 during continuous inhalation.

Interactions with tuberculosis infection

Guinea pigs were given continuous exposure to HI-SIL 233 after they were infected with RiRv mycobacteria. Except for one guinea pig, all the animals survived in spite of the double stress.

The concurrent dust exposure and infection resulted in an initially augmented tuberculous process. In contrast with the control animals, there were conspicuous, relatively large tubercles in the lung parenchyma, some of them thus becoming confluent. The absence of a pleural reaction is noteworthy. The tracheobronchial lymph nodes were enlarged, but not as prominently as in the other animals, and one node contained a large calcific focus. Minute tubercles were discernible in some of the lymph nodes.

Augmented tubercles continued to develop and caseate during the greater part of the first year after infection. Resolution, fibrosis, and calcification were delayed for from 8 to 10 months. Proliferation and hyperplasia of the parenchymal lymph follicles continued to increase during the first 8 months. Exuberant development of the tubercles was seen at the two-month stage, each tubercle being appreciably larger than those of controls. A little later the cellular and capsular components of the tubercles increased to the extent that adjacent tubercles became confluent.

Along the edges of these large tubercles there were koniophores in alveoli which were being incorporated into the tubercle by interstitial extension of the infectious activity. The tubercle bacilli did not affect the vitality of the koniophores.

The marginal extension of the tuberculous process, or perhaps focal pneumonic consolidation of the lung by the tuberculous process, led to the development of large composite lesions. A minimal degree of aggravation of the pneumoconiotic process was seen, the result of the concurrent or prior tuberculous infection.

In some of the affected areas peribronchial inflammatory and infiltrative activity by the end of the first year did not progress any further. Some of this led to marked epithelisation of the alveoli isolated by the inflammatory processes. These epithelised alveoli usually were devoid of phagocytic cells and trapped dust. Aggravation of the pneumoconiotic process occurred within the tuberculous foci. There also was some separate intensification of the dust response at the later stages.

Very little change resulted in the main respiratory passages. The trachea remained intact despite 24 months of dust inhalation. Occasionally tubercles formed in the adventiae of medium-sized bronchi, leading to secondary stenosis, distortion, and distal atelectasis. In the tracheobronchial lymph nodes the tubercle formation was active for several months and then slowly decreased; healing and resolution followed. The infection of the nodes apparently delayed or prevented the accumulation of silica in them and very few koniophores could ever be detected. In the hepatic portal lymph nodes the histological responses were similar to those in the tracheobronchial nodes, and tubercles were more prevalent and considerably enlarged. Resolution of these lesions progressed as in the controls.

The response in the undusted control series of animals was limited to brief activity during the first 4 months, followed by a stage in which the infection remained quiescent, although tubercle bacilli could still be cultured from the lung 24 months after infection.

In a second phase of this study, 26 guinea pigs were infected with RiRv mycobacteria by inhalation. Ten were placed in the HI-SIL 233 room two months after infection; eight 4 months after infection; and eight 6 months after infection. The results are summarised in Table A6_5-2. Although the tuberculous process was reactivated in all the animals, this occurred only transiently and to a slight degree. Decisive stimulation of the infection occurred in the group of animals placed in the dust environment after 2 months following infection. In these guinea pigs there was moderate local extension and caseation of individual tubercles. These lesions persisted for a relatively long period. In the group exposed to dust 4 months after infection, there was less caseation. In the animals whose exposure commenced 6 months after infection no caseation of tubercles was seen. The tubercles remained relatively small but were slow to undergo resolution, and there was a greater tendency to calcification than in the preceding groups.

The response of the lymph nodes was mild. Tubercles were very scarce and small and mainly represented by focal fibrosis. There was mild hyperplasia of the lymphoid tissue, but there was no indication of dust deposition or reaction to dust. When dust exposure was delayed 6 months after infection, virtually no persistent damage of the respiratory passages or of the lymph nodes could be discerned.

No true enhancement of the pneumoconiotic process occurred during the reactivation phase, when the infection preceded dust exposure by more than 2 months. In the group that commenced dust exposure 2 months after infection, there were some areas of peritubercular enhanced cellular reaction and epithelisation of peribronchiolar alveoli which could be ascribed to the dust exposure rather than the tuberculous process. In the animals exposed 4 and 6 months after infection, the pneumoconiosis was decidedly limited, and in many of the animals no evidence of dust storage or reaction was discernible for many months. These findings are not typical of dusts possessing a tuberculosis-promoting capability.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Altogether 496 animals were used in the study, of which 270 were exposed to the dust and 226 served as undusted controls. There were 134 rats, 293 guinea pigs and 69 rabbits.

The dust for inhalation was created in a box through mechanical agitation by a rotating paddle, aided by a jet of compressed air. The resultant aerosol was sucked into a stream of compressed air at the throat of a Venturi tube. This was then expelled into the dusting room. Although the HI-SIL 233 was still fairly lumpy as it left the dusting box, the passage through the Venturi funnel broke up the clusters, and the stream of compressed air further dispersed the particles in a relatively even manner. These particles easily traversed the coarse metal mesh walls of the animal cages. Dust-level measurements on air samples from within the cages did not differ much from those of the general room air. Additionally, the animal cages were regularly rotated within the room to further ensure even and equal dust exposure.

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

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

		For about 8 h each day of the week, the HI-SIL aerosol was dispersed throughout the room. Precautions were taken to keep the dust level as constant as possible. The dust concentration was determined by means of serial electroprecipitator and Millipore filter sampling at weekly intervals. A uniform rate of dispersion of the aerosol was achieved throughout the period of experimentation.
5.2	**Results and discussion**	No radiographic signs of lung disease were observed in the animals at the end of their maximal period of dust inhalation. Minor terminal respiratory passage lesions developed and myohypertrophy of the bronchial walls was seen in some animals. Tuberculouus processes were more efflorescent in exposed animals but readily reversed on cessation of the exposure.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	50 mg/m^3
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. Also urinalysis and clinical chemistry parameters were 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, namely the lack of detail regarding experimental set up. This study has been performed using a substance that is similar to that in Section 2 of the current dossier. However, impurities are too high for this study to be considered in the risk assessment. This is, however, a useful study as it is indicative of the reversibility of the effects of amorphous silicas and therefore is suitable as a supporting document.

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

Annex Point IIA, VI, 6.5

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*

Section A6.5 Chronic toxicity (Inhalation 1 of 2)

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	

Table A6_5-1. Summary of component experiments

Dose (mg/kg)	Blood sampling time	Hemoglobin (g/dl)	Erythrocytes (per pl)	Leukocytes (per nl)	Differential leukocyte count (%)[a]					
					Segm neutr	Stab neutr	Baso	Eos	Mono	Ly
Males										
0	Month 0	13.1	6.6	18.1	6	4	0	1	2	87
	Month 3	13.2	4.2	15.2	12	2	0	2	4	80
	Month 6	13.6	6.2	16.7	15	3	0	2	1	79
	Recovery period	14.2	6.0	16.7	8	5	0	3	2	82
500	Month 0	12.6	6.2	18.2	10	4	0	3	1	82
	Month 3	14.2	6.2	17.1	10	2	0	4	2	82
	Month 6	13.8	6.6	14.5	13	3	0	6	2	76
	Recovery period	14.3	6.6	11.7	4	2	0	4	1	89
Females										
0	Month 0	13.8	6.2	16.2	11	6	0	2	0	81
	Month 3	13.4	6.4	17.2	14	1	0	0	0	85
	Month 6	13.0	5.3	12.2	17	5	0	3	1	74
	Recovery period	13.8	6.4	10.8	12	5	0	1	2	80
500	Month 0	12.9	6.2	14.1	10	6	0	2	1	81
	Month 3	13.6	6.3	12.1	9	2	0	2	3	84
	Month 6	13.9	6.1	11.5	14	5	0	5	1	75
	Recovery period	13.6	6.6	14.1	10	2	0	1	2	85

[a] Abbreviations used: Segm neutr, segmented neutrophils; stab neutr, stab neutrophils; baso, basophilic leukocytes; eos, eosinophilic leukocytes; mono, monocytes; ly, lymphocytes.

Table A6_5-2. Inhalation experiment with guinea pigs

Pulmonary Lesions								
Months		Tubercles					Lymph Follicle Hyper-plasia	Bronchitis and Peri-bronchitis
After Infection	In Dust	Resolving	Spreading	Caseation	Fibrosis	Calcifi-cation		
							Animals placed in dust environment	
4	2	++	...	++	...	...	+	...
6	4	+	...	+	×	...	×	...
10	8	−	...	×	+	...	×	...
14	12	+	×	×	+	...	×	×
							Animals placed in dust environment	
8	4	+	+	×	×	×	...	×
12	8	+	...	×	×	×	×	−
16	12	×	×	...	×	×	×	+
							Animals placed in dust environment	
10	4	+	...	...	+	+	×	++
14	8	+	...	...	+	+	×	+
18	12	+	...	...	+	+	×	+

[a] Each guinea pig was infected with RiRy tubercle bacilli, then groups were placed in the dust environment after 2, 4, and 6 months. All animals (26) were killed for study.
[b] Symbols: + Slight reaction, + + Moderate reaction, + + + Marked reaction, × Slight but inconstant reaction.

Fig. 1 Increase of lung weights of control rats, and progressive increases of the lung weights of guinea pigs

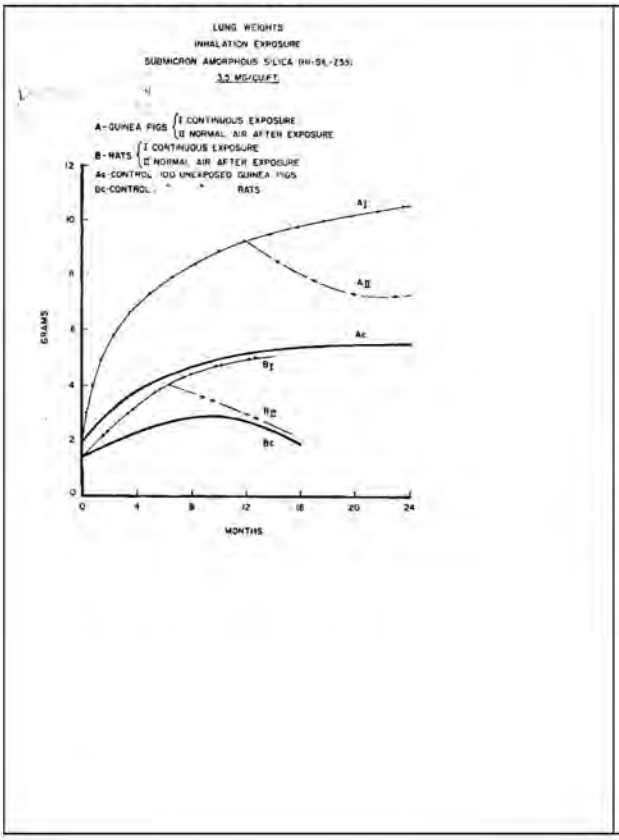


Fig. 2 Gravimetric changes in the lungs of rats after continuous and interrupted inhalation of HI-SIL 233

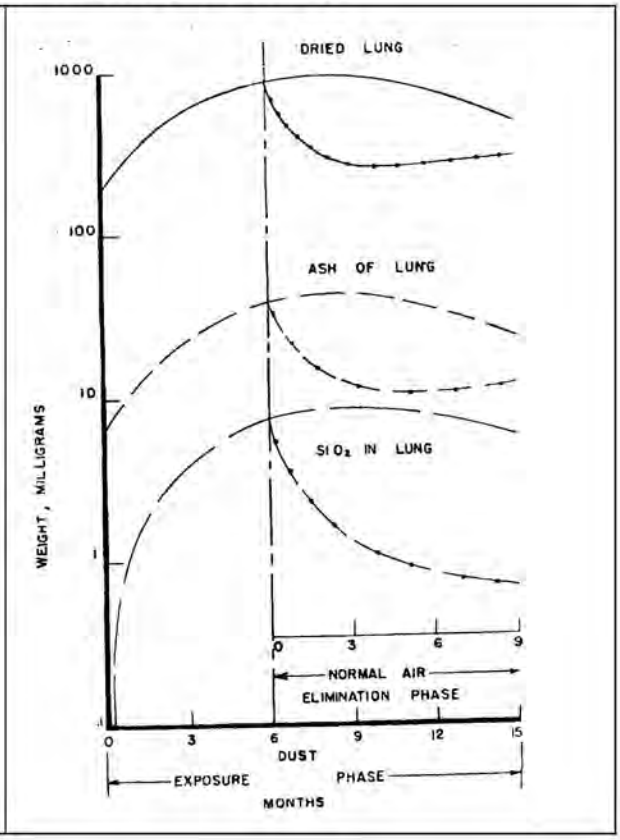
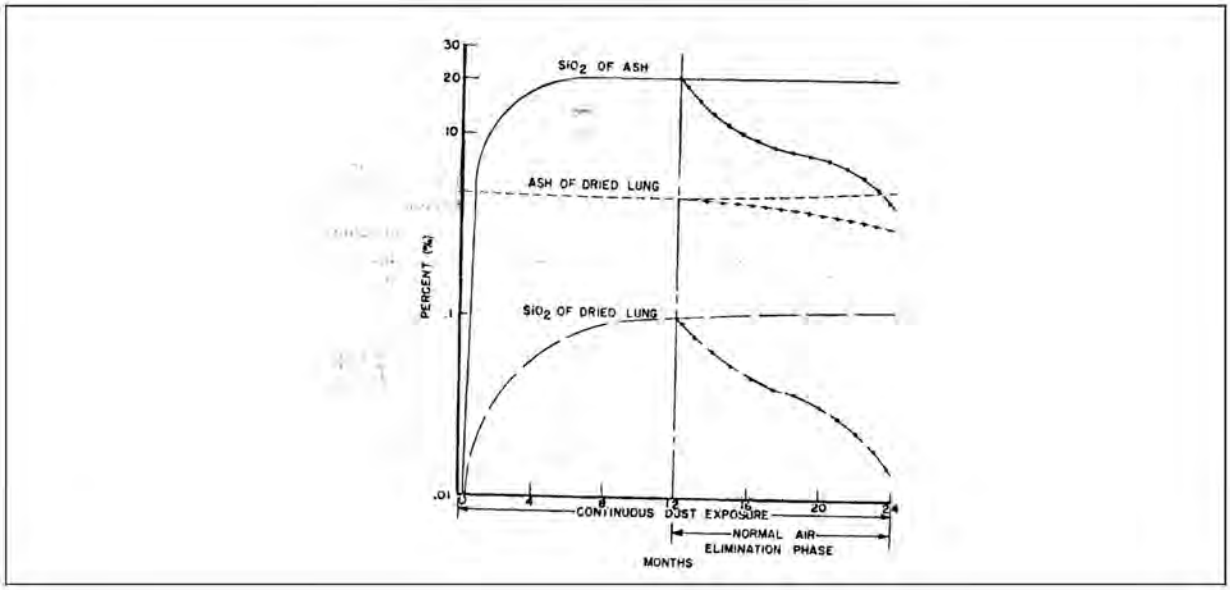


Fig. 3 Silica content of the lungs of guinea pigs as percentile ratios of the weights of the dried lung and lung ash.



Appendix 1

Comparison of silica as submitted in Section 2 and test material

As is shown by the below table, the silica as tested deviates from the specification of the silica as stated in Section 2 of Rentokil Initial plc's silicon dioxide dossier as submitted for evaluation under the BPD in that it has a high level of impurities. Other than that, both have no crystalline content, comparable solubility and comparable particle size.

Although the impurities are too high for this study to be considered in the risk assessment, this is a useful study as it is indicative of the reversibility of the effects of amorphous silicas and therefore is suitable as a supporting document.

Characteristic	**HI-SIL 233** (Silicon dioxide tested)	**Wet Process Silica** (Silicon dioxide marketed by Rentokil)
Purity of silicon dioxide	[redacted]	[redacted]
Approved food additive	[redacted]	[redacted]
Amorphous silica	[redacted]	[redacted]
Crystalline content	[redacted]	[redacted]
Particle size	[redacted]	[redacted]
Solubility in water	[redacted]	[redacted]
Solubility in organic solvents	[redacted]	[redacted]
Impurities		
Fe_2O_3 (%)	[redacted]	[redacted]
TiO_2 (%)	[redacted]	
Al_2O_3 (%)	[redacted]	
CaO	[redacted]	
MgO	[redacted]	
NaCl	[redacted]	
Surface modification	[redacted]	[redacted]