	COMMENTS FROM ...
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_6_4-1. Table for Micronucleus Test In Vivo

Route	Dose (mg/kg)	% of MNPCE or MNRET (after the final treatment)								
		24 h			48 h			72 h		
		Mean	SD	p	Mean	SD	p	Mean	SD	p
ip	0	0.16	0.06		0.18	0.08		0.16	0.15	
	500	0.16	0.18	0.598	0.18	0.05	0.593	0.12	0.08	0.788
	1000	0.28	0.10	0.143	0.20	0.22	0.507	0.18	0.10	0.530
	2000	0.14	0.09	0.696	0.28	0.19	0.202	0.26	0.13	0.192
				(0.411)			(0.132)			(0.090)
ip	0	0.16	0.06		0.18	0.08		0.16	0.15	
	500	0.16	0.05	0.598	0.18	0.05	0.593	0.12	0.08	0.788
	1000	0.28	0.11	0.143	0.20	0.22	0.507	0.18	0.96	0.530
	2000	0.14	0.09	0.696	0.28	0.19	0.202	0.26	0.13	0.192
	5000	0.08	0.10	0.930	0.18	0.10	0.617	0.20	0.08	0.419
				(0.822)			(0.321)			(0.133)
po	0	0.28	0.13		0.18	0.15		0.06	0.06	
	500	0.10	0.07	0.990	0.16	0.11	0.685	0.20	0.10	0.046
	1000	0.24	0.11	0.721	0.16	0.11	0.685	0.14	0.17	0.172
	2000	0.12	0.13	0.979	0.16	0.15	0.685	0.22	0.08	0.029
	5000	0.22	0.16	0.788	0.24	0.11	0.332	0.22	0.11	0.029
				(0.679)			(0.249)			(0.032)

ip: intraperitoneal

po: per oral

MNPCE: Micronucleated polychromatic erythrocytes

MNRET: Micronucleated reticulocytes

P: p value of pairwise comparisons and that of the Cochran-Amitage trend test in parentheses.

Section 6.6.5 **Annex Point IIA, VI, 6.6.5**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian cytogenetic test in cells other than bone marrow	
	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 an *in vivo* mammalian cytogenetic test in cells other than bone marrow is only required when a negative results is obtained in the following test: Annex Point IIA6.6.4 *In vivo* mammalian bone-marrow cytogenetic test (chromosome analysis), or micronucleus test. but a positive result was obtained in one of the following tests: Annex Point IIA6.6.1 *In vitro* gene mutation study in bacteria Annex Point IIA6.6.2 *In vitro* cytogenicity study in mammalian cells Annex Point IIA6.6.3 *In vitro* gene mutation assay in mammalian cells. No *in vitro* genotoxicity studies have demonstrated a positive result for silicon dioxide (please refer to Document IIIA, Sections 6.6.1, 6.6.2 and 6.6.3 of this dossier). Therefore, further testing to determine the genotoxicity of silicon dioxide *in vivo*, including the mammalian cytogenetic test in cells other than bone marrow is not considered necessary. Notwithstanding the above, published data on rats exists that demonstrates that amorphous silicon dioxide is not mutagenic in cells other than bone marrow (please see attached study summary). This confirms findings in the *in vitro* studies that silicon dioxide is not genotoxic.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.6.5 Annex Point IIA, VI, 6.6.5	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies *In vivo* mammalian cytogenetic test in cells other than bone marrow

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

Section 6.6.5

Annex Point IIA6.6.5

Genotoxicity in vivo: Inhalation (1 of 1)

Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

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.11 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)
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.
3.1.2.4	Maximum tolerable dose	No mortality shown at maximum attainable level (477 mgm^{-3})[1]. [redacted]
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	200-250g
3.2.6	Number of animals per group	4

Section 6.6.5

Annex Point IIA6.6.5

Genotoxicity in vivo: Inhalation (1 of 1)

Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

3.2.7	Control animals	Yes.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Number of applications	Continuous: 6hday^{-1}, 5 daysweek^{-1} for 13 weeks
3.3.2	Interval between applications	Not applicable.
3.3.3	Postexposure period	3 and 8 months
		Inhalation
3.3.4	Type	Aerosol; 100% test material.
3.3.5	Concentration	50.4 ± 19.0 mgm^{-3} 6hday^{-1}, 5 daysweek^{-1} for 13 weeks
3.3.6	Vehicle	Not applicable.
3.3.7	Concentration in vehicle	Not applicable.
3.3.8	Total volume applied	Not reported.
3.3.9	Controls	Filtered air.
3.4	**Examinations**	
3.4.1	Clinical signs	Not reported.
3.4.2	Tissue	Lung tissue.
		Number of animals: All animals
		Number of cells: Not applicable.
		Time points: 6.5 and 13 weeks exposure; 12 and 13 weeks post-exposure
		Type of cells Lung tissue.
		Parameters: Number of neutrophils and macrophages.
		Amount of proteinaceous fluid.
		Amount of Alveolar type-II epithelial cell proliferation.
		MIP-2 gene expression.
		TUNEL staining.
3.5	**Further remarks**	Also analysed: lung silica burdens; bronchoalveolar lavage fluid.

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	No effects reported.

Section 6.6.5 — **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 — Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

4.2	**Haematology / Tissue examination**	*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. *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.
4.3	**Genotoxicity**	No.
4.4	**Other**	*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 6_6_5-1). *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 6_6_5-2).

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 $MgSO_4$

Section 6.6.5 **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

(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 counted. Mutation frequencies were calculated as (number of colonies/treatment)/(plating efficiency)/(10^6 cells) = mutants/10^6 cells.
Statistical analysis: 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 $p < 0.05$.

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 Reliability

3

5.3.2 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. Group sizes would have preferably been larger. However, all aspects of relevant lung tissue and gene damage were assessed and a control group used.
It is acknowledged that a full specification of the material tested is not available. However, current information on this material is available

Section 6.6.5 — **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 — Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

from the manufacturer.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.6.5 **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

Table A6_6_5-1. 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_6_5-2. 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; $p \leq 0.05$.

Section 6.6.5 **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

Fig. 1

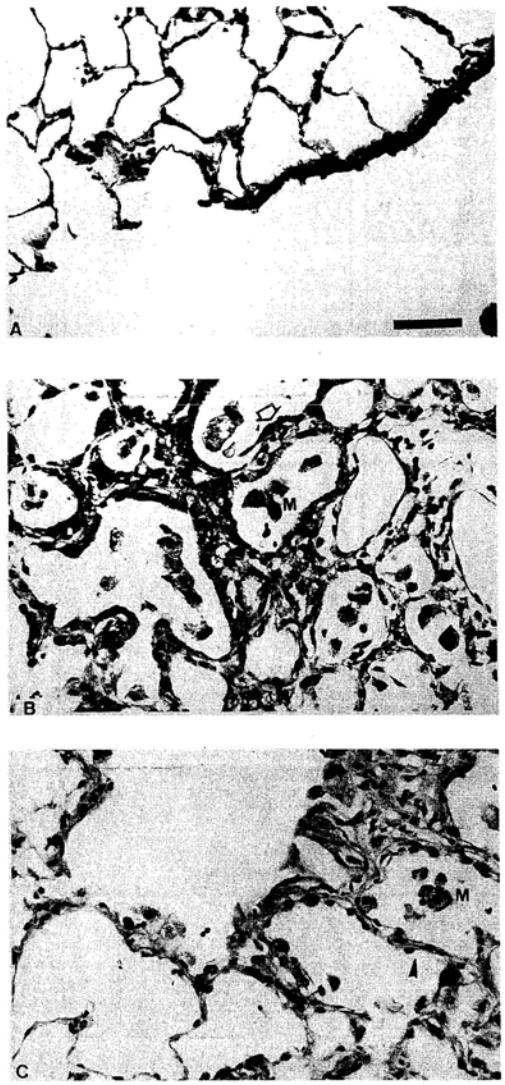


FIG. 1. Histopathology of the lungs from adult rats exposed to room air (A), crystalline silica (B), or amorphous silica (C) for 3 months and examined 8 months after completion of exposure. Note the greatly thickened alveolar septa in B (open arrow) and the normal or slightly thickened septa in C (solid 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

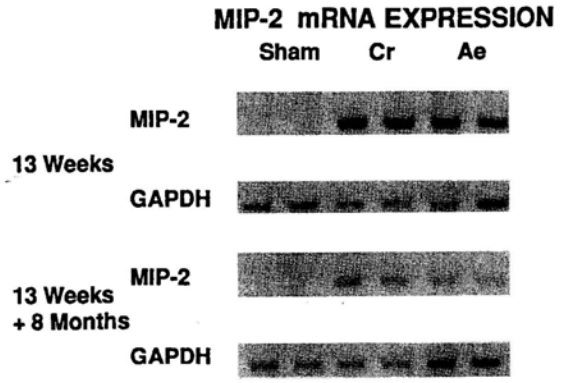


FIG. 2. Effect of subchronic inhalation of SiO_2 particles on expression of mRNA for MIP-2 and GAPDH in rat lung. Shown are negatives of ethidium bromide-stained gel with the Rt-PCR products for MIP-2 and GAPDH amplified for rat lung RNA. Rats were exposed to air or amorphous (Ae) or crystalline (Cr)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

Section 6.6.5 **Genotoxicity in vivo: Inhalation (1 of 1)**

Annex Point IIA6.6.5 Section 6: Toxicological and Metabolic Studies

In vivo mammalian cytogenetic test in cells other than bone marrow

Fig. 3

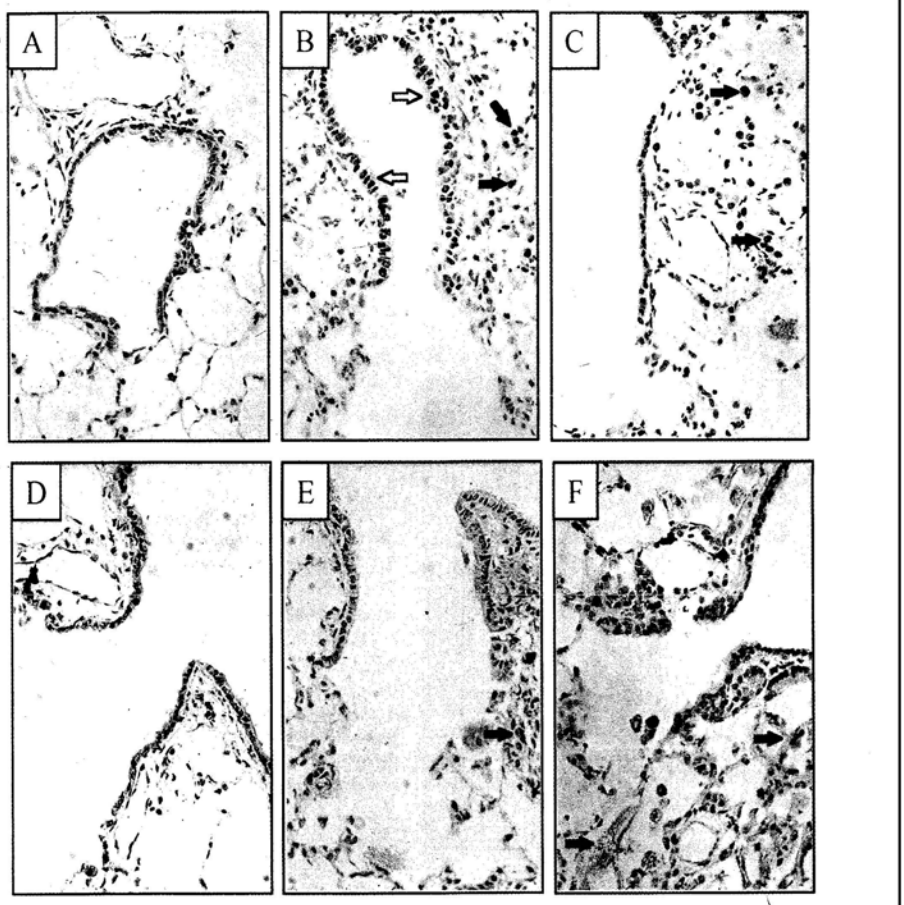


FIG. 3. Identification of DNA fragmentation in lungs of adult rats at 3 months of exposure to room air (A), amorphous (B) or crystalline (C) silica; or at 8 months post-exposure from room air (D), amorphous (E), or crystalline (F) silica. Sections were stained for the presence of free 3′-hydroxyl groups, 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

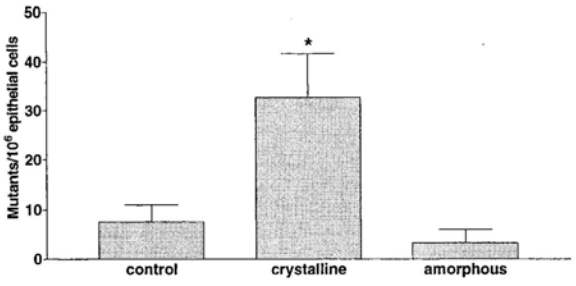


FIG. 4. HPRT mutation frequencies observed for rat alveolar epithelial cells isolated from the lungs of rats exposed subchronically to air or to silica particles. Statistically significant increases in mutation frequencies were detected from epithelial cells obtained immediately after 13 weeks of exposure 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 6.6.6 **Annex Point IIA, VI, 6.6.6**	**Genotoxicity in vivo** Section 6: Toxicological and Metabolic Studies Rodent dominant lethal test or *In vivo* mammalian germ cell cytogenetics	
	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 [ ]	**Technically not feasible** [ ] **Scientifically unjustified [4]**	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	It is not necessary to submit a Rodent dominant lethal test or *in vivo* mammalian germ cell cytogenetics because 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 these tests are only required when a positive result is obtained in the following test: Annex Point IIA6.6.4 *In vivo* mammalian bone-marrow cytogenetic test (chromosome analysis), or micronucleus test. The above test was only required if a positive result was obtained in the following: Annex Point IIA6.6.1 *In vitro* gene mutation study in bacteria Annex Point IIA6.6.2 *In vitro* cytogenicity study in mammalian cells Annex Point IIA6.6.3 *In vitro* gene mutation assay in mammalian cells. Negative results were obtained in all of the above tests, and in addition there is one *in vivo* study available for silicon dioxide which shows it is non-mutagenic in rat alveolar cells. (Refer to Document IIIA, section 6.6.5 for details). In view of this available data, an *in vivo* mammalian bone marrow cytogenetic test for chromosomal damage, or a micronucleus test are not required and thus it follows that a test to assess possible germ cell effects is also not required.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.6.6 Annex Point IIA, VI, 6.6.6	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies Rodent dominant lethal test or *In vivo* mammalian germ cell cytogenetics

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

Section 6.6.7	Metabolites of Concern in Mammals 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** [4]	
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 data on metabolites of concern will not be required if the results are negative from 6.6.1, 6.6.2 and 6.6.3 and no metabolites of concern are formed in mammals. It is not considered scientifically necessary to carry out testing on metabolites of concern (in mammals) for amorphous silicon dioxide as the results from 6.6.1, 6.6.2 and 6.6.3 (see Document IIIA, Sections 6.6.1, 6.6.2 and 6.6.3 for further details) are negative and there is no indication from the ADME data to suggest any metabolites of concern (see Document IIIA, Section 6.2 for further details).	
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 6.7 Annex Point IIA, VI, 6.7	Carcinogenicity 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 carcinogenic effects of amorphous silicon dioxide on rats and mice (see attached study summary). The study shows that after administration of the test substance in the diet for approximately 22 - 24 months respectively, no significant treatment related effects were found. 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). It is not considered necessary to provide data in further species as data is also available from several public domain studies conducted using data from humans. Although not carcinogenicity studies *per se*, pulmonary effects of inhaled silica were investigated, therefore tumours would have been reported. In the first of these studies (study summaries attached), it was found that, after a mean exposure of 8 years to inhaled silica, no tumours were formed. In a further study, it was found that after a mean exposure of 8.6 years to inhaled silica, no tumours were formed. Additionally, in a public domain study on inhalation exposure to humans it was found that after inhalation exposures ranging from 1 to 16 years, no tumours were formed. Finally, data from a further public domain study shows that after occupational inhalation exposure to silica of up to 14 years, no tumours were formed. In fact, in all of these studies, no adverse effects were reported. Additionally, based on other available data, the International Agency for Research on Cancer (IARC) have concluded that amorphous silicon dioxide is not carcinogenic to animals or humans[1]. Therefore it is not considered scientifically necessary to carry out a carcinogenicity 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 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 carcinogenicity 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 [2]. Silicon dioxide and silicates are present in practically all plants and animals and in natural waters [3,4]. 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 [3]. Silicon dioxide is an approved food additive, assigned the E number E551 [5], and is used as an anti-caking agent. Silicon dioxide has been given an acceptable daily intake of "not limited" [6]. In addition, silicon dioxide is approved for use in plastic material coming into contact with food, without hazard to public health [7]. 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 [8]. Estimates indicate that 4,400,000 people are exposed to amorphous silicas in their work environments [8]. 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 [2].

* 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 [9,10].
  [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[2], 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" [6]. 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[2].

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 carcinogenicity study on silicon dioxide as it will not add any useful information to the risk assessment. It has been shown in the human risk assessment that compared to exposures *via* the diet and the environment, exposure from silicon dioxide as an insecticide is insignificant. The risk assessment for human exposure to silicon dioxide, when applying the representative product RID Insect Powder shows that exposure to silicon dioxide does not exceed agreed, well established maximum exposure limits for safe working conditions with silicon dioxide and nuisance dust. The toxicological profile of silicon dioxide has been well established with a large body of data available in the public domain. The operator exposure limits that have been set for nuisance particles and dusts are also based on a large amount of available data. As shown above, data is available on the effects of exposure to amorphous silicon dioxide and this data shows that there are no lasting adverse effects. Although this data has its limitations and there are no studies available performed to specific guidelines which consider chronic toxicity or carcinogenicity, 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.

Crystalline material
It is well established that carcinogenicity is associated with the occupational inhalation of **crystalline** silicon dioxide. Therefore, it must be stressed that the material to be marketed for use as an insecticide (PT18) as supported by this dossier is **amorphous** silicon dioxide (with no crystalline content). The differences between crystalline and amorphous silica have been reviewed and studies conducted show that there are no

associations between carcinogenicity and amorphous silicon dioxide but many between carcinogenicity and silicon dioxide with crystalline content.

References

[redacted]

[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_7a – Study Summary has been removed.

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	No
1.2.1	Data owner	
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study investigated the pulmonary effects of occupational exposure to amorphous silica. While it did not set out to confirm the carcinogenic potential of silica *per se*, such effects would have been reported in this study. Therefore it considered relevant for this end point.
2.2	**GLP**	No No information available about whether the study was conducted in accordance with GLP.
2.3	**Deviations**	No Standard test guidelines were not followed.

3 MATERIALS AND METHODS

3.1	**Test material**	Amorphous silica, containing 3.8% of respirable dust. No other information available on the specification or purity of the test material.
3.1.1	Lot/Batch number	Not reported.
3.1.2	Specification	Not reported.
3.1.2.1	Description	Not reported.
3.1.2.2	Purity	Not reported.
3.1.2.3	Stability	Not reported.
3.2	**Test Animals**	
3.2.1	Species	Human.
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Male.

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7 Section 6: Toxicological and Metabolic Studies, inhalation, man

3.2.5	Age/weight at study initiation	Average age of test group: 42 Average weight of test group: 79 kg. For more details refer to Table A6_7-1 at the end of this study summary.
3.2.6	Number of animals per group	Number of subjects in test group: 41 For more details refer to Table A6_7-1 at the end of this study summary.
3.2.6.1	at interim sacrifice	Not applicable: No sacrifice group.
3.2.6.2	at terminal sacrifice	Not applicable. No sacrifice group.
3.2.7	Control animals	Yes. For details of test group, refer to Table A6_7-1 at the end of this study summary.
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Duration of treatment	Mean duration of exposure was 8 years (range 1-28 years)
3.3.2	Interim sacrifice(s)	Not applicable: no sacrifice group.
3.3.3	Final sacrifice	Not applicable: no sacrifice group.
3.3.4	Frequency of exposure	Daily
3.3.5	Postexposure period	Not reported.
		Inhalation
3.3.6	Concentrations	Nominal concentration Not reported. Analytical concentration Total dust concentration ranged from 0 to 10.5 mg/m³ Respirable dust concentration ranged from 0 to 3.4 mg/m³.
3.3.7	Particle size	Not reported.
3.3.8	Type or preparation of particles	Not reported.
3.3.9	Type of exposure	Whole body.
3.3.10	Vehicle	No vehicle used: Test subjects were exposed to 100% silica dust.
3.3.11	Concentration in vehicle	No vehicle used.
3.3.12	Duration of exposure/day	8h/day
3.3.13	Controls	Sham exposure

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

3.4	**Examinations**		
3.4.1	Body weight	No	
3.4.2	Food consumption	No	
3.4.3	Water consumption	No	
3.4.4	Clinical signs	Yes: Prevalence of respiratory symptoms such as cough, phlegm, tightness of breath, wheezing, tightness, dyspnoea, asthma	
3.4.5	Macroscopic investigations	Chest radiographs of test subjects were taken which would have found tumours and masses.	
3.4.6	Ophthalmoscopic examination	No	
3.4.7	Haematology	Yes	
		Number of animals:	all test subjects
		Time points:	end of study
		Parameters:	Other: Blood gas concentrations at rest and during exercise. Pulmonary function.
3.4.8	Clinical Chemistry		No
		Number of animals:	Not applicable: not conducted.
		Time points:	Not applicable: not conducted.
		Parameters:	Not applicable: not conducted.
3.4.9	Urinalysis	No	
		Number of animals:	Not applicable: not conducted.
		Time points:	Not applicable: not conducted.
		Parameters:	Not applicable: not conducted.
3.4.10	Pathology	No	
3.4.10.1	Organ Weights	No	
		from:	Not applicable: not conducted.
		Organs:	Not applicable: not conducted.
3.4.11	Histopathology	No	
		from:	Not applicable: not conducted.
		from:	Not applicable: not conducted.
		Organs:	Not applicable: not conducted.
3.4.12	Other examinations	Not reported.	

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7 Section 6: Toxicological and Metabolic Studies, inhalation, man

3.5	**Statistics**	Yes: Contingency tables (2 x 2) with γ^2 tests were used to determine whether relations shown between variables were statistically significant. Quantitative variables were compared by *t* test. Analysis of variance was used to examine the relation of respiratory symptoms, work exposure and smoking to pulmonary function.
3.6	**Further remarks**	None.
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	Not investigated.
4.2	**Food consumption**	Not investigated.
4.3	**Water consumption**	Not investigated.
4.4	**Clinical signs**	Refer to table A6_7-2 at the end of this study summary for details of respiratory symptoms reported, following exposure to amorphous silica.
4.5	**Macroscopic investigations**	No difference was found between the chest radiographs of the control and test group.
4.6	**Ophthalmoscopic examination**	Not investigated.
4.7	**Haematology**	Refer to table A6_7-3 at the end of this study summary for details of blood gas concentrations reported, following exposure to amorphous silica.
4.8	**Clinical Chemistry**	Not investigated.
4.9	**Urinalysis**	Not investigated.
4.10	**Pathology**	Not investigated.
4.11	**Organ Weights**	Not investigated.
4.12	**Histopathology**	Not investigated.
4.13	**Other examinations**	Not reported.
4.14	**Time to tumours**	No tumours reported.
4.15	**Other**	Not reported.

Section A6.7
Annex Point IIA6.7

Carcinogenicity (Inhalation 1 of 3)

Section 6: Toxicological and Metabolic Studies, inhalation, man

5. APPLICANT'S SUMMARY AND CONCLUSION

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

5.1 Materials and methods

The purpose of this study was to determine the pulmonary effects of occupational exposure to amorphous silica compared with a control group. Also, blood gas concentrations, at rest and during exercise, were evaluated as possible indicators of changes in lung function as a result of exposure.

Study population

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

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

Questionnaire

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

Tests of pulmonary function

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

Continued.....

Section A6.7

Annex Point IIA6.7

Carcinogenicity (Inhalation 1 of 3)

Section 6: Toxicological and Metabolic Studies, inhalation, man

5.1 Materials and methods

Continued.....

Blood gas concentrations at rest and during exercise

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

Chest Radiographs

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

Assessment of Environmental Exposure

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

5.2 Results and discussion

Smoking habits and characteristics of the population did not differ significantly between the exposed and control groups, except for the percentage of shift workers (61% vs 87% in the exposed group. This was statistically significant with a p value of <0.01). Table A6_7-1 at the end of this study summary gives details of the characteristics of the control group and exposed subjects.

Table A6_7-2 at the end of this study summary shows the prevalence of respiratory symptoms.

Concentrations of blood gas at rest and during exercise were not significantly different between the exposed group and the control group (Table A6_7-2 at the end of this study summary), and no difference was found between the chest radiographs of the two groups.

Continued.....

Section A6.7

Annex Point IIA6.7

Carcinogenicity (Inhalation 1 of 3)

Section 6: Toxicological and Metabolic Studies, inhalation, man

5.2 Results and discussion

Continued.....

All airflow values were lower in the exposed group than in the control (Table A6_7-4 at the end of this study summary) with significant differences for FEV_1 / FVC, FEF_{25-75}, FEF_{50} and FEF_{75}. There was no significant correlation, however between the exposure index and pulmonary function.

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

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

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

In conclusion, exposure to amorphous silica dust may induce a mild small airway disease, only shown by comparison to a control group. This obstruction to air flow is increased by cigarette smoking. Only flow volumes curves were different between groups. Arterial blood gases at rest and during standardised exercise, and chest radiographs were similar between groups.

5.3 Conclusion

5.3.1 Reliability 3

Section A6.7

Annex Point IIA6.7

Carcinogenicity (Inhalation 1 of 3)

Section 6: Toxicological and Metabolic Studies, inhalation, man

5.3.2	Deficiencies	Yes. Study investigated the pulmonary effects of occupational exposure to amorphous silica. While it did not set out to confirm the carcinogenic potential of silica *per se,* such effects would have been reported in this study. Therefore it considered relevant for this end point. There are deficiencies with the study in that body weight, food and water consumption, standard clinical signs, ophthalmoscopic examinations, standard haematology, clinical chemistry, urinalysis, pathology, and histopathology were not conducted. However, with other measured parameters showing no significant effects, this does not appear to adversely affect the study because it would have indicated potential toxicity. Average exposure period was 8 years, as opposed to the recommended (OECD guideline 453) 24 months. However, as this length of time is in excess of the standard timescale for carcinogenicity studies, this is not considered significant. The study was performed with only a single dose range, so no NOAEL could be established. There are deficiencies in the reporting, including full reporting of method and results. The test material used in this study is not identical to that as given in Section 2. However, it is considered sufficiently similar and adds to weight of evidence with regards to the inert nature of silicon dioxide.

Section A6.7 Carcinogenicity (Inhalation 1 of 3)

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7 Carcinogenicity

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

Table A6_7-1. Characteristics of control workers and workers exposed to amorphous silica

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

Table A6_7-2. Prevalence of respiratory symptoms in controls, and workers exposed to amorphous silica

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

Section A6.7 Carcinogenicity

Annex Point IIA6.7

Section 6: Toxicological and Metabolic Studies, inhalation, man

Table A6_7-3. Blood gas concentrations at rest and during exercise in control workers and workers exposed to amorphous silica

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

Table A6_7-4. Pulmonary function in controls and workers exposed to amorphous silica

	Group C (control) Mean (SD)	Group E (exposed group) Mean (SD)	*p* Value
FVC (l)	4.8 (0.8)	4.8 (0.8)	NS
FEV_1 (l/s)	3.9 (0.6)	3.8 (0.6)	NS
FEF_{25-75} (l/s)	4.2 (1.2)	3.6 (1.2)	<0.01
PF (1/s)	10.7 (1.6)	10.3 (1.5)	NS
FEF_{25} (l/s)	8.7 (1.8)	8.2 (1.6)	NS
FEF_{50} (l/s)	5.1 (1.5)	4.5 (1.4)	<0.03
FEF_{75} (l/s)	1.8 (0.7)	1.4 (0.5)	<0.008
FEV_1 / FVC (%)	82.1 (5.6)	79.2 (5.7)	<0.007

Section A6.7 — **Carcinogenicity (Inhalation 2 of 3)**

Annex Point IIA6.7 — **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.32 in Annex V of Directive 67/548/EC.
2.2	**GLP**	Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.
		3 MATERIALS AND METHODS
3.1	**Test material**	Precipitated amorphous silica.
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	The specification of the substance has not been reported. However, this study is deemed appropriate for consideration in the risk assessment as the test substance was stated as precipitated amorphous silica, the same as the notified substance.
3.1.2.1	Description	Dust.
3.1.2.2	Purity (%)	Not reported.
3.1.2.3	Impurities (%)	Not reported.
3.1.2.4	Density	Not reported.
3.1.2.5	Particle Size	Not reported.
3.1.2.6	Stability	Not reported. However, silicon dioxide is known to be a stable compound (melting point >1500°C).
3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Not specified in report.
3.2.5	Age/weight at study initiation	Range, not reported.

Section A6.7 — Carcinogenicity (Inhalation 2 of 3)

Annex Point IIA6.7 — **Section 6: Toxicological and Metabolic Studies**

3.2.6	Number of animals per group	165	
3.2.6.1	at interim sacrifice	Not applicable. No interim sacrifice carried out.	
3.2.6.2	at terminal sacrifice	Not applicable. No terminal sacrifice carried out.	
3.2.7	Control animals	No.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	Mean 8.6 years.	
3.3.2	Interim sacrifice(s)	Not applicable. No interim sacrifice carried out.	
3.3.3	Final sacrifice	Not applicable. No interim final carried out.	
3.3.4	Frequency of exposure	Not specified.	
3.3.5	Postexposure period	Not applicable.	
		Inhalation	
3.3.6	Type	In air	
3.3.7	Concentration	In air	<1.0 mg/m^3 to >10 mg/m^3
3.3.8	Vehicle	Not applicable.	
3.3.9	Concentration in vehicle	Not applicable.	
3.3.10	Total volume applied	Not specified.	
3.3.11	Controls	Not applicable.	
3.4	**Examinations**		
3.4.1	Body weight	No.	
3.4.2	Food consumption	Not reported.	
3.4.3	Water consumption	Not reported.	
3.4.4	Clinical signs	Yes	
3.4.5	Makroskopic investigations	No.	
3.4.6	Ophthalmoscopic examination	No.	
3.4.7	Haematology	No.	
		Number of animals:	Not applicable.
		Time points:	Not applicable.
		Parameters:	Not applicable.
3.4.8	Clinical Chemistry	No.	
3.4.9	Urinalysis	No.	

3.4.10	Pathology	No.	
3.4.10.1	Organ Weights	No.	
3.4.11	Histopathology	No.	
		from:	Not applicable.
		from:	Not applicable.
		Organs:	Not applicable.
3.4.12	Other examinations	Annual spirometry, chest roentgenogram and respiratory questionnaires.	
3.5	**Statistics**	Student t-test, Fisher's exact test, linear and multiple regression and analysis of variance.	
3.6	**Further remarks**	None.	

4 RESULTS AND DISCUSSION

4.1	**Body weight**	Not reported.
4.2	**Food consumption**	Not reported.
4.3	**Water consumption**	Not reported.
4.4	**Clinical signs**	No effects.
4.5	**Macroscopic investigations**	Not reported.
4.6	**Ophthalmoscopic examination**	Not reported.
4.7	**Haematology**	Not reported.
4.8	**Clinical Chemistry**	Not reported.
4.9	**Urinalysis**	Not reported.
4.10	**Pathology**	Not reported.
4.11	**Organ Weights**	Not reported.
4.12	**Histopathology**	Not reported.
4.13	**Other examinations**	No effects.
4.14	**Time to tumours**	No evidence of tumours.
4.15	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of this study was to determine the pulmonary effects of occupational exposure to amorphous silica.

A test group of 165 amorphous silica workers with at least one years exposure to precipitated amorphous silica (PAS) from two industrial premises were reviewed in this study. It involved analysis of medical records including annual spirometry, chest roentgenogram and most recent respiratory questionnaires. From this, forced vital capacity (FVC), forced expiratory volume (FEV_1) in one second, FEV_1/FVC

		ratio and maximum mid-expiratory flow (FEF_{25-75}) were obtained. Respiratory symptoms and relationship to PAS exposure and smoking history were examined. Chest radiographs of 143 workers were examined to identify radiographic change consistent with pneumoconiosis The cumulative exposure index was calculated in order to reflect quantity and duration of workers monthly dust exposure. From this the mean (monthly) exposure index was calculated. Statistical analyses were performed between change per year of FVC, FEV_1 or FEF_{25-75} and cumulative exposure index or total years of exposure.
5.2	**Results and discussion**	Significant findings included a positive relationship between pack years of smoking and coughing/ dyspnea; and radiographic changes in workers with a previous history of working in a limestone mine or a soda ash plant using crushed limestone. No worker only exposed to PAS had any evidence of pneumoconiosis, including some workers exposed for 35 years. There was no correlation between change per year of FVC, FEV_1 or FEF_{25-75} and cumulative exposure index or total years of exposure.
5.3	**Conclusion**	The study found that respiratory symptoms correlate with smoking and not exposure to PAS and that serial pulmonary function testing parameters and chest radiographs are not adversely affected by long term exposure to PAS.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that no post-mortem analysis could be carried out for obvious reasons. Also urinalysis, haematology and clinical chemistry parameters were not measured. However, with the other measured parameters showing no effects or a negative correlation, this does not appear to adversely affect the study. However, as this study uses humans as the test subjects and the study was conducted for up to 35 years of exposure (which is considered a significant portion of lifespan for the purposes of a carcinogenicity study) and no adverse effects were shown suggesting that this length of time is adequate, it is deemed appropriate for adding information to the risk assessment. The test material used in this study is not identical to that as given in Section 2. The specification of the substance has not been reported. However, this study is deemed appropriate for consideration in the risk assessment as the test substance was stated as precipitated amorphous silica, the same as the notified substance. Despite the deficiencies in this study, it gives an indication that exposure to PAS alone is unlikely to cause any symptoms, but workers who smoke or have been exposed to limestone may experience clinical symptoms.

Section A6.7 **Carcinogenicity (Inhalation 2 of 3)**

Annex Point IIA6.7 **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	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 ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7 — **Carcinogenicity (Inhalation 3 of 3)**

Annex Point IIA6.7 — **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.32 in Annex V of Directive 67/548/EC.
2.2	**GLP**	Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.

3 MATERIALS AND METHODS

3.1	**Test material**	Hi-Sil (hydrated silica pigments) and Silene (precipitated, hydrated calcium silicate pigment).
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	White powder.
3.1.2.2	Purity (%)	Not reported.
3.1.2.3	Impurities (%)	[redacted]
3.1.2.4	Density	Hi Sil 0.12-0.25 g/cm^3 (8-15 lb/ cu ft) Silene 0.19 g/cm^3 (12 lb/ cu ft)
3.1.2.5	Particle Size	Hi-Sil 0.022-0.030 µ Silene 0.030 µ
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	Human
3.2.2	Strain	Not applicable.

Section A6.7 — Carcinogenicity (Inhalation 3 of 3)

Annex Point IIA6.7 — **Section 6: Toxicological and Metabolic Studies**

3.2.3	Source	Not applicable.	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	21 to 67 years, average 34 ¼ years.	
3.2.6	Number of animals per group	78	
3.2.6.1	at interim sacrifice	Not applicable. No interim sacrifice carried out.	
3.2.6.2	at terminal sacrifice	Not applicable. No terminal sacrifice carried out.	
3.2.7	Control animals	No.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	1 year to 16 years 7 months, average 4 ¾ years.	
3.3.2	Interim sacrifice(s)	Not applicable. No interim sacrifice carried out.	
3.3.3	Final sacrifice	Not applicable. No interim final carried out.	
3.3.4	Frequency of exposure	Not specified.	
3.3.5	Postexposure period	Not applicable.	
		Inhalation	
3.3.6	Type	In air	
3.3.7	Concentration	At bag packer:	3.5 x 10^{-7} to 0.0002 mg/cm^3 (0.01 to 5.77 mg/cu ft)
		General air in production plant:	3.5 x 10^{-7} to 5.8 x 10^{-5} mg/ cm^3 (0.01 to 1.65 mg /cu ft)
3.3.8	Vehicle	Not applicable.	
3.3.9	Concentration in vehicle	Not applicable.	
3.3.10	Total volume applied	Not specified.	
3.3.11	Controls	Not applicable.	
3.4	**Examinations**		
3.4.1	Body weight	No.	
3.4.2	Food consumption	Not reported.	
3.4.3	Water consumption	Not reported.	
3.4.4	Clinical signs	Yes	
3.4.5	Makroskopic investigations	Yes (case study)	
3.4.6	Ophthalmoscopic examination	Yes (case study)	

Section A6.7 **Carcinogenicity (Inhalation 3 of 3)**

Annex Point IIA6.7 **Section 6: Toxicological and Metabolic Studies**

3.4.7	Haematology	Yes (case study)	
		Number of animals:	2 (1 reported)
		Time points:	Not reported
		Parameters:	Red and white blood cell counts.
			Haemoglobin.
			Differential: segmented band forms, lymphocytes, monocytes, eosinophils, basophils.
3.4.8	Clinical Chemistry	Yes (Blood chemistry of case study)	
3.4.9	Urinalysis	No.	
3.4.10	Pathology	No.	
3.4.10.1	Organ Weights	No.	
3.4.11	Histopathology	No.	
		from:	Not applicable.
		from:	Not applicable.
		Organs:	Not applicable.
3.4.12	Other examinations	Review of complete health records, chest x-rays, extensive physical examinations and pulmonary function examinations on case studies.	
3.5	**Statistics**	Not reported.	
3.6	**Further remarks**	Two case studies were carried out, of which one was reported.	
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	Not reported.	
4.2	**Food consumption**	Not reported.	
4.3	**Water consumption**	Not reported.	
4.4	**Clinical signs**	No effects.	
4.5	**Macroscopic investigations**	No effects.	
4.6	**Ophthalmoscopic examination**	Pupils equal and react to light, normal vessels and discs, extraocular muscle normal.	
4.7	**Haematology**	Red blood cell count: 5090000	
		White blood cell count: 6750	
		Haemoglobin: 15.0 gm	
		Differential: segmented 44%, band forms 3%, lymphocytes 47%, monocytes 3%, eosinophils 2%, basophils 1%.	
		Slight anisocytosis and hypochromasia.	
4.8	**Clinical Chemistry**	Blood chemistry: blood sugar 83 mg/100 cc, blood NPN 35 mg/ 100 cc, blood creatinine 1.5 mg/ 100 cc.	

Section A6.7 — **Carcinogenicity (Inhalation 3 of 3)**

Annex Point IIA6.7 — **Section 6: Toxicological and Metabolic Studies**

4.9	**Urinalysis**	Not reported.
4.10	**Pathology**	Not reported.
4.11	**Organ Weights**	Not reported.
4.12	**Histopathology**	Not reported.
4.13	**Other examinations**	X-rays consistently without change. Health records showed no abnormalities, physical examinations and pulmonary function of case studies were normal.
4.14	**Time to tumours**	No evidence of tumours.
4.15	**Other**	**Case Study** The subject of the study is a 42 year old well developed, well nourished, white male. Occupational history includes previous exposure to chlorine, calcium carbonate, sodium carbonate (aged 22-25 years), silica (irregular, in open pit for 3 years) and exposure to Silene and Hi-Sil for the last 12 years. Physical examination showed BP, pulse and respiration normal. Eyes, nose, ears and mouth showed no abnormalities in form or function. The back, neck, chest, heart and abdomen were normal with no palpable masses. The heart sounds were slightly distant and soft, but there were no audible murmurs and it showed normal sinus rhythm. Both lung fields were clear to auscultation and percussion. The back showed no scoliosis, kyphosis or costovertebral angle tenderness. The extremities showed no deformities. The peripheral pulses were palpable and bilaterally equal. No evidence of edema or varicosities. Rectal examination showed no palpable masses. The prostate was of normal size and the sphincter of normal tone. There was no tenderness recorded in this area. Neurological examination showed slightly more active tendon reflexes in lower extremities but were bilaterally equal. There were no pathological signs and sensory examination was intact. As in all workers chest x-rays showed a radiographically normal appearance. The clinical laboratory data; maximum breathing capacity, lung volume and arterial blood gases were within normal limits. The alveolar-arterial oxygen gradient was slightly high, but this was due to hyperventilation. In summary, there was no evidence in this case study of interference with respiratory or circulatory apparatus. There was no evidence of abnormalities associated with this type of employment, namely emphysema and increase in membrane thickness separating the blood from the gas in the lungs.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The purpose of this study was to determine the health effects of occupational exposure to Hi-Sil and Silene. A test group of 78 male workers employed in the manufacture and processing of Hi-Sil and Silene were reviewed between October 1941 to December 1959. During this period visits to the plant dispensary were recorded and split into 2 groups; the first covering injuries, illnesses and

Section A6.7 **Carcinogenicity (Inhalation 3 of 3)**

Annex Point IIA6.7 **Section 6: Toxicological and Metabolic Studies**

		complaints neither caused by or aggravated by contact with Silene and/ or Hi-Sil, the second were visits resulting from, or contaminated by exposure to Hi-Sil and/ or Silene. In addition annual chest x-rays were carried out on all 78 workers and detailed case studies of 2 employees were built. The case studies included extensive physical examinations and pulmonary function studies.
5.2	**Results and discussion**	Significant findings included the lack of variation in behaviour of injuries and illnesses between those involving Hi-Sil and Silene and those not. There was no evidence of any recurring illnesses and trends towards specific complaints within the group studied. Extensive case studies on two men found there to be no abnormality in physical and pulmonary functions. Finally x-rays of the group showed no abnormalities and there was no evidence of silicosis found.
5.3	**Conclusion**	The study found no evidence that the health of workers was significantly affected by exposure to Silene and Hi-Sil.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that no post-mortem analysis could be carried out for obvious reasons. Also haematology and clinical chemistry parameters were only measured on 2 case studies. However, with the other measured parameters showing no effects, this does not appear to adversely affect the study. As this study uses humans as the test subjects and the study was conducted for up to 17 years of exposure (which is considered a significant portion of lifespan for the purposes of a carcinogenicity study) and no adverse effects were shown suggesting that this length of time is adequate, it is deemed appropriate for adding information to the risk assessment. The test material used in this study is not identical to that as given in Section 2. However, this study is deemed appropriate for consideration in the risk assessment as the test substance was sufficiently similar to the notified substance. Despite the deficiencies in this study, it gives an indication that exposure to silica is unlikely to any adverse effect on health.

Section A6.7 **Carcinogenicity (Inhalation 3 of 3)**

Annex Point IIA6.7 **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	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 ...
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	

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.

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

A6_7e – Study Summary has been removed.

Section A6.7 Carcinogenicity (mammalian cells 1 of 1)

Annex Point IIA6.7

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.10 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 OX 50 (Degussa, Germany)
3.1.1	Lot/Batch number	Not available.
3.1.2	Specification	Note: the non-relevant (i.e. not completely synthetic amorphous) compounds tested in the study have not been reported upon. Deviating from specification given in section 2 as follows (please see Appendix 1 for justification of test material used):
3.1.2.1	Description	Not reported.
3.1.2.2	Purity	≥ 99.8[1] [1]Degussa Product Information sheet for Aerosil OX 50.
3.1.2.3	Stability	Not reported. However, silicon dioxide is known to be a stable compound (melting point >1500°C).
3.2	**Study Type**	*In Vitro* mammalian transformation assay.
3.2.1	Organism/cell type	Syrian hamster embryo (SHE)
3.2.2	Deficiencies / Proficiencies	Relative cloning efficiency
3.2.3	Metabolic activation system	Not relevant
3.2.4	Positive control	No positive control used.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Up to 61 µg/cm^2

Section A6.7 **Carcinogenicity (mammalian cells 1 of 1)**

Annex Point IIA6.7 **Section 6: Toxicological and metabolic studies**

3.3.2	Way of application	Stock suspensions in sterile tridistilled water were sonicated for 2 min. Serial dilutions, vortexed each time, were made in culture medium without serum and the treatment dilutions, double concentrated, were made in the complete medium.
3.3.3	Pre-incubation time	24 h
3.3.4	Other modifications	None.
3.4	**Examinations**	Not relevant.
3.4.1	Number of cells evaluated	Not reported.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No. See Fig. 1 for details.
4.1.2	with metabolic activation	Not relevant.
4.2	**Cytotoxicity**	No. See Fig. 1 for details.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

X-irradiated SHE feeder cells were seeded at 6 x 10^4 cells/60-mm dish in 2 mL Dulbecco's MEM medium (DMEM; Gibco), pH 7.0 supplemented with 20% preselected, heat-inactivated, fetal calf serum (Labsystems) and 2 mM L-glutamine (Flow) without antibiotics or fungistatics (complete medium). On the next day, the target SHE cells were seeded onto the feeder cells at 300 cells/dish in 2 mL complete medium. Treatment took place 24 h later. In each experiment at least three concentrations per sample were tested. The highest concentration tested was 61 µg/cm^2. Each dish, containing 4 mL culture medium, received the treatment dilution (double concentrated) in 4 mL complete medium. At least 10 dishes were used per point. After 7 days of incubation at 37°C in 10% CO_2 atmosphere, the dishes were washed (Hanks' PBS, Flow) and the colonies were fixed (absolute methanol) and stained (10% Giemsa). The colonies were examined with a 10-50x magnification stereo microscope in order to count them and to detect any transformed ones. The criteria described by DiPaulo *et al.* (1969) and Pienta *et al.* (1981) were used to define a colony as morphologically transformed. For each treatment concentration and control of an individual assay, the following values were calculated: (I) total number of colonies; (ii) cloning efficiency (CE); the total number of colonies/total number of target cells seeded; (iii) relative CE: the CE of treated cells/CE of the control x 100; (iv) cytotoxicity, determined from the reduction in relative CE; (v) number of morphologically transformed colonies; (vi) transformation frequency (TF): the number of transformed colonies/total number of colonies x100. The mean CE and TF ± SEM for each treatment concentration was determined from the results of the three to five individual assays and was compared to that of the concurrent control using Student's *t*-test. The mean CE of the control cultures was within historical control data. A $P \leq 0.05$ level of significance was considered a treatment-related transforming effect.

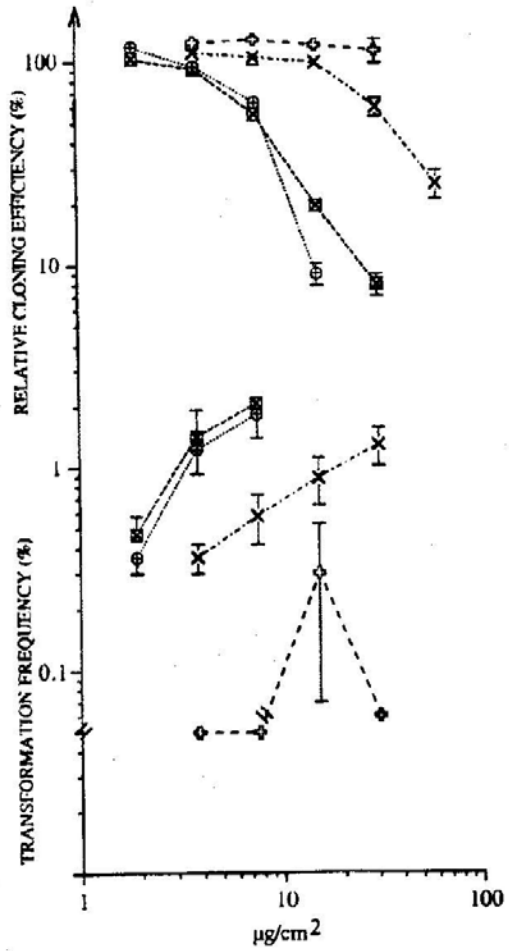
Section A6.7 Carcinogenicity (mammalian cells 1 of 1)

Annex Point IIA6.7 **Section 6: Toxicological and metabolic studies**

5.2	**Results and discussion**	No biological response was elicited.
5.3	**Conclusion**	The amorphous silicon dioxide used in this study does not have cytogenic potential.
5.3.1	Reliability	3
5.3.2	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, amongst other things, number of cells counted. However, as the result is negative, this does not adversely affect the outcome. Also the test strain was appropriate for the method.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
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.1 Relative cloning efficiencies (%) and transformation frequencies (%) of SHE cells treated with amorphous particles



Aerosil OX 50 = :

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 of high purity, have no crystalline content, comparable solubility and comparable particle size.

Characteristic	**Aerosil OX 50** (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]

Section A6.7 — **Carcinogenicity (Oral 1 of 1)**

Annex Point IIA6.7 — **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.32 in Annex V of Directive 67/548/EC.
2.2	**GLP**	No. Not specified in report.
2.3	**Deviations**	No. No standard test guideline used.

3 MATERIALS AND METHODS

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

Section A6.7 — Carcinogenicity (Oral 1 of 1)

Annex Point IIA6.7 — **Section 6: Toxicological and Metabolic Studies**

3.2.6.1	at interim sacrifice	See Tables 6, 11, 12.
3.2.6.2	at terminal sacrifice	See Tables 6, 11, 12.
3.2.7	Control animals	Yes. See Table 1.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Mice: 93 weeks Rats: 103 weeks
3.3.2	Interim sacrifice(s)	See Tables 6, 11, 12.
3.3.3	Final sacrifice	Mice: 93 weeks Rats: 103 weeks
3.3.4	Freqency of exposure	Daily
3.3.5	Postexposure period	Overnight.
		Oral
3.3.6	Type	In food
3.3.7	Concentration	See below
3.3.8	Vehicle	Not reported
3.3.9	Concentration in vehicle	0, 1.25, 2.5 and 5%
3.3.10	Total volume applied	Mice: Mean: (M/F) – 1.25%: 38.45/37.02g; 2.5%: 79.78/72.46g; 5%: 160.23/157.59g Rats: Mean: (M/F) – 1.25%: 143.46/107.25g; 2.5%: 179.55/205.02g; 5%: 581.18/435.33g
3.3.11	Controls	Plain diet.
3.4	**Examinations**	
3.4.1	Body weight	Yes. Weekly.
3.4.2	Food consumption	Yes. Weekly.
3.4.3	Water consumption	Not reported.
3.4.4	Clinical signs	Yes.
3.4.5	Makroskopic investigations	Tumours
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes

		Number of animals:	All animals.
		Time points:	24- and 48-weeks.
		Parameters:	Erythrocytes (RBC), haemoglobin (Hb), leukocytes (WBC) and haematocrit (Ht).
3.4.8	Clinical Chemistry	Yes.	
		Number of animals:	All animals.
		Time points:	24- and 48-weeks.
		Parameters:	Aspartate transaminase (AST), alanine transaminase (ALT), serum inorganic phosphorus (IP), total protein (TP), albumin (ALB), lactic dehydrogenase (LDH), alkali phosphatase (ALP), total bilirubin (TB), total cholesterol (T-Cho), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), blood urea nitrogen (BUN), uric acid (UA), creatinine (Cre) and calcium (Ca).
3.4.9	Urinalysis	No	
3.4.10	Pathology	No	
3.4.10.1	Organ Weights	Yes	Heart, liver, spleen, kidneys and brain.
3.4.11	Histopathology	Yes	
		from:	All dose groups.
		from:	See Table 6
		Organs:	Liver, lung, haematopoietic
3.4.12	Other examinations	Heart, liver, spleen, kidneys and brain: examined microscopically.	
3.5	**Statistics**	The mean and standard deviations of various measured parameters were calculated for each dose group. The significant difference between the control and the compound-treated groups was tested using Student's t-analysis variance test. Those means showing significant differences have been marked with asterisks ($P<0.05$;*, $P<0.01$; **). The chi-square test of significance ($P<0.05$) by Mantel-Hanszel was employed to compare the survival date exclusive of sacrificed specimens. Prevalence rates were cited as percentages of tumour groups and non-tumour groups in cases of post-mortem examination. The significance of differences between the two means of prevalence was tested by using Fisher'' exact test for fourfold tables. The percentages of the frequencies of tumour in specific tissues were analysed by using the following technique: The Cochran-Armitage test for linear trend in proportion with continuity correction.	
3.6	**Further remarks**	None.	

4 RESULTS AND DISCUSSION

4.1	**Body weight**	Mice and rats: No effects.
4.2	**Food consumption**	Mice and rats: No effects.

Section A6.7 **Carcinogenicity (Oral 1 of 1)**

Annex Point IIA6.7 **Section 6: Toxicological and Metabolic Studies**

4.3	**Water consumption**	Mice and rats: No effects.
4.4	**Clinical signs**	Mice and rats: No effects.
4.5	**Macroscopic investigations**	Mice and rats: Positive dose-related trends were not statistically significant.
4.6	**Ophthalmoscopic examination**	Not reported.
4.7	**Haematology**	Mice and rats: No evidence of dose-related alteration (see Tables 4-1, 4-2, 9-1, 9-2).
4.8	**Clinical Chemistry**	Mice and rats: No evidence of dose-related alteration (see Tables 4-1, 4-2, 9-1, 9-2).
4.9	**Urinalysis**	Not reported.
4.10	**Pathology**	Not reported.
4.11	**Organ Weights**	Mice: Sporadic effects (atrophy or hypertrophy of organs) found sporadically. However, these were not sex- or dose-related (see Tables 5-1, 5-2, 10-1, 10-2). Rats: No evidence of dose-related alteration
4.12	**Histopathology**	Mice and rats: Positive dose-related trends were not statistically significant (see Tables 6, 11, 12).
4.13	**Other examinations**	None.
4.14	**Time to tumours**	Not applicable.
4.15	**Other**	Mice: Non-neoplastic lesions were observed in the subcutis, lungs, kidneys and liver in the treated groups but these were considered to be of no toxicological significance.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	320 mice (160 of each sex) and 320 rats (160 of each sex) were used. The animals were housed in wire-mesh cages (mice: 5 animals/cage; rats: 2 animals/cage) and prior to initiation of treatment were acclimatised to the laboratory environment for 1 week (mice; 2 weeks (rat). Tap water was available ad libitum. Animal quarters were air-conditioned with thermostats set to maintain 23±1°C room temperature continuously and 50±10% humidity; artificial fluorescent lighting was provided daily for a continuous 14-hour period. Animals were seperated according to sex and by standard randomization 5 mice were put in one cage and 2 rats per cage. Mice and rats were dined into dosage groups of 10 animals each. The test materials which were prepared weekly were administered orally each day at the prescribed dosage levels.
5.2	**Results and discussion**	The repeated oral administration of the test substance produced no significant treatment-related effects in mice and rats.
5.3	**Conclusion**	Comparison of the rates of tumours found in the study with those occurring in the controls indicate that no carcinogenic effects could be attributed to the exposure to the test substance.

Section A6.7	Carcinogenicity (Oral 1 of 1)
Annex Point IIA6.7	Section 6: Toxicological and Metabolic Studies

5.3.1	Reliability	3
5.3.2	Deficiencies	Yes. It is acknowledged that this study was not carried out or reported in accordance with approved testing guidelines. There are deficiencies with the study in that urinalysis parameters were not measured. However, with other measured parameters showing no significant effects, this does not appear to adversely affect the study. There are deficiencies in the reporting as no NOAEL value is stated. The test material used in this study is not identical to that as given in Section 2. It is however acknowledged to be a synthetic amorphous silica (the same as the substance that is being supported) therefore it is felt that this is highly relevant. Despite the deficiencies in this study, it gives an indication of the level of silicon dioxide that can be tolerated without effect by mammals.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	

Section A6.7 — Carcinogenicity (Oral 1 of 1)

Annex Point IIA6.7 — **Section 6: Toxicological and Metabolic Studies**

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

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

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

Mice

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

Rats

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

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

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

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

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

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

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

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

Mean±SD was calculated by the method of rejection limit from Smirnov

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

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

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

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

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

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

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

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

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

Table 6: Incidence of Tumours in Mice fed SYLOID for two years

Table 6. Incidence of tumors in mice fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	3/10(30.0)	1/10(10.0)	2/10(20.0)	0/10	NS
	21	2/16(12.5)	8/17(47.1)	5/14(35.7)	4/16(25.0)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	1/10	1/10	1/10	0/10	NS
adenocarcinoma	21	1/16(6.25)	2/17(11.8)	3/14(21.4)	3/16(18.8)	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	21	3/16(18.8)	1/17(5.9)	0/14	1/16(6.25)	NS
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	0/10	0/10	0/10	0/10	—
	21	3/16(18.8)	3/19(15.8)	1/20(5.0)	3/20(15.0)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	2/10(20.0)	0/10	NS
adenocarcinoma	21	0/16	1/19(5.3)	0/20	1/20(5.0)	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	21	2/16(12.5)	4/19(21.1)	7/20(35.0)	2/20(10.0)	NS

NS; not significantly different —; not measured

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

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

Dosage period (M)	Group (%)	No. of animals	R B C					WBC	Platelet
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (%)	($\times10^3$/dl)	($\times10^3$/dl)
	0	8	974.3± 59.0	16.5±0.92	51.4±3.07	17.1±0.08	32.3±0.20	5.30± 2.48	642.9± 59.4
6	1.25	10	1003.0± 18.1	16.7±0.35	52.4±1.89	16.7±0.35**	31.8±0.25**	6.20± 1.16	731.6± 44.1**
	2.5	8	965.3± 29.4	16.7±0.33	50.9±1.29	17.2±0.32	32.6±0.64	5.70± 1.87	688.3± 44.9
	5.0	9	993.0± 24.8	16.6±0.79	51.9±1.97	16.9±0.20*	31.9±0.65	5.16± 1.19	686.0± 35.7
	0	10	1011.0± 41.5	16.1±0.72	64.0±2.86	16.2±0.73	25.1±0.16	3.9± 1.30	627.4± 54.8
12	1.25	10	983.6± 9.4	16.2±0.37	64.6±1.03	16.4±0.33	25.0±0.31	4.7± 0.85	631.0± 28.0
	2.5	10	979.3± 16.0	16.2±0.21	64.7±0.91	16.5±0.24	25.1±0.17	5.1± 0.94*	671.4± 25.8
	5.0	9	973.1± 41.3	15.9±0.61	63.1±2.39	16.3±0.21	25.2±0.29	4.5± 0.38	662.9± 26.4
	0	12	810.4±222.3	11.8±3.51	45.6±14.5	14.6±3.65	25.7±0.56	8.5± 6.26	991.2±374.9
24	1.25	9	740.9±142.0	12.2±3.05	48.5±12.2	16.9±0.63	25.2±0.35**	23.3±22.70	716.0±177.4
	2.5	10	775.5±286.2	12.7±5.49	48.6±20.9	16.3±4.28	26.2±1.89	4.5± 0.91	689.9± 71.4*
	5.0	13	723.1±226.6	11.8±4.21	45.8±17.3	15.6±3.25	26.2±2.38	7.2± 4.62	695.1±239.7*

Mean±SD was calculated by the method of rejection limit form Smirnov

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 11: Incidence of Tumours in Male Rats fed SYLOID for two years

Teble 11. Incidence of tumors in male rats fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
	12	0/10	0/10	0/10	0/10	—
hyperplastic nodule	24	0/19	1/18(5.6)	0/18	2/17(11.8)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	1/10	0/10	1/10	0/10	NS
adenocarcinoma	24	1/19	0/18	0/18	0/17	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
	24	0/19	1/18(5.6)	1/18(5.6)	1/17(5.9)	NS
Adrenal	6	0/10	0/10	0/10	0/10	—
Pheochromocytoma	12	0/10	0/10	0/10	0/10	—
	24	0/19	1/18	2/18(11.1)	2/17(11.8)	NS
Teste	6	0/10	0/10	0/10	0/10	—
seminoma	12	0/10	1/10	0/10	0/10	NS
	24	15/19(78.9)	14/18(77.8)	14/18(77.8)	14/17(82.4)	NS
Mammary gland	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	0/10	0/10	—
adenocarcinoma	24	7/19(36.8)	8/18(44.4)	1/18(5.6)@	1/17(5.9)@	P<0.01
Prepuce	6	0/10	0/10	0/10	0/10	—
fibroma	12	0/10	0/10	0/10	3/10	NS
	24	5/19(26.3)	3/18(16.7)	4/18(22.2)	4/17(23.5)	NS

—; Not tested, NS; Not significantly different (P<0.05) @; Significantly different (P<0.01)

Table 12: Incidence of Tumours in Female Rats fed SYLOID for two years

Teble 12. Incidence of tumors in female rats fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	0/10	0/10	0/10	0/10	—
	24	0/17	0/17	0/20	1/21	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	0/10	0/10	—
adenocarinoma	24	0/17	0/17	2/20(10.0)	0/21	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	24	1/17(5.9)	1/17(5.9)	3/20(15.0)	0/20(0.0)	NS
Mammary gland	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	1/10	0/10	0/10	NS
adenocarcinoma	24	8/17(47.1)	9/17(52.9)	11/20(55.0)	10/21(47.6)	NS
Clitoris						
adenoma	6	0/10	0/10	0/10	0/10	—
	12	0/10	0/10	0/10	0/10	—
	24	10/17(58.8)	8/17(47.1)	7/20(35.0)	5/21(23.8)	NS P<0.05

—; Not tested, NS; Not significantly different (P<0.05)
Numbers in panentheses are expressed as %

Section 6.8.1 Annex Point IIA, VI, 6.8.1	**Teratogenicity Study** **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 four public domain studies (published together in one report) on the lack of teratogenic effects of amorphous silicon dioxide on four separate laboratory animal species, namely mice, rats, hamsters and rabbits (see attached study summary). These public domain studies were carried out to US FDA standards, and showed that there were no adverse effects on pregnant mice, rats, hamsters and rabbits or on the development of their progeny from a range of daily oral doses of up to 1340 mg/kg bw (mice), up to 1350 mg/kg bw (rats), and up to 1600 mg/kg (hamsters and rabbits) during the critical period of organogenesis. These studies are of adequate quality, and the data is considered suitable for the risk assessment due to the similarity of the tested silicon dioxide and the silicon dioxide which will be marketed by Rentokil Initial as an insecticide (PT18). A full comparison of the test substance and the silicon dioxide marketed as an insecticide is given in the attached study summary. Given the above, and for the following reasons, it is not considered necessary to perform further testing: 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 teratogenicity 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], which is the most favourable opinion that can be reached and is reserved for substances of very low toxicity. In addition, silicon dioxide is approved for use in plastic materials 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 bw/day *. To put this exposure into context, and notwithstanding the information given above, the silicon dioxide content of raw potato is reported to be 10.1 mg/kg, and one litre of beer contains 131 mg [1].

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

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

- Operator exposure work has been carried out in humans exposed to high concentrations of silicon dioxide [10]. Such data has been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement. For example, the long term occupational exposure limit for amorphous 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 [13], and the FDA[1], who considered the health aspects of silicon dioxide as a food additive. EPA