In vivo Study with Quartz-Containing Ceramic Dusts: Inflammatory Effects of Two Factory Samples in Lungs after Intratracheal Instillation in a 28-Day Study with Rats

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Abstract. As various quartz polymorphs react differently in lungs, a differentiation of effects is needed while setting occupational exposure levels. The objective of this European Collective Research Project SILICERAM was to characterize differences in biological activity of four quartz species, i.) 2 quartz-containing materials collected at typical ceramic manufacturing sites (Tableware granulate, TG and Tableware cast, TC) versus ii.) a designed ceramic dust sample (Contrived Sample, CS) and iii.) ground quartz DQ12 (well-characterised standard quartz (Positive Control, PC) and TiO₂ (negative control). TG and TC had been selected as the most promising two candidates based on a preceding in vitro screening of 5 factory samples. Total doses of 5 mg per rat of the TG and TC, 1.1 mg of the CS and 0.33 mg of the PC corresponding to 0.29, 0.16, 0.29 and 0.29 mg quartz per rat, respectively, were administered to rats by intratracheal instillation. After 3 days, bronchoalveolar lavagate (BAL) analysis resulted in polymorphonuclear neutrophil (PMN) levels of 15%, 25%, 0.6% and 25% in the TG, TC, CS and PC groups, respectively. At 28 days, the values were 29%, 20%, 7% and 45%. Histopathologically, the TG and TC groups showed very slight to slight effects, the PC group, however, stronger effects after the same period. In conclusion, the following ranking was found: PC > TG > TC > CS > TiO₂ > Vehicle Control. Thus, a clear differentiation of effects for TG and TC, CS and PC was found. From a regulatory point of view, the substance-specific toxic potentials of TG and TC may need to be considered when devising occupational exposure limits.

1. Introduction
The traditional ceramics industry is divided into several subsectors: tiles, tableware, sanitary ware, refractory, bricks and roofing tiles. In order to manufacture these products powdered crystalline silica is used. The inhalation of respirable crystalline silica (RCS) can lead to silicosis, which ultimately results in to ill-health through breathing difficulties and even death. A new input to the debate has given the International Agency on Research in Cancer (IARC) in 1997 when classifying inhaled quartz or cristobalite from occupational sources as a category 1 carcinogen (human).
At present different EU states have different limits for RCS. Within Europe the Scientific Committee on Occupational Exposure Limits (SCOEL) is working towards identifying a common EU position on mg/m³ levels. One of the difficulties associated with setting common exposure limits for workers is the fact that different forms of crystalline silica appear to pose different threats. A Social Dialogue between trade-union and employers agreed on that i.) quartz concentrations at various workplace sectors should be held as low as technically possible and that ii.) toxicological experiments should continue to generate data allowing a differentiation of industrial quartz varieties. Setting a single low limit to encourage continual improvement, but allowing concessions based on proven reduced risks associated with certain RCS forms is seen as a pragmatic way forward.

Against the above background, the overall aim of this project was to provide legislators with useful data for defining RCS in air limits. The project focused on different industries of the ceramic sector: tiles, tableware, sanitary ware, refractory, bricks and roofing tiles.

**Objectives:** This non-clinical *in vivo* rat study was designed to expand the database obtained in preceding *in vitro* screening assays using sensitive cytotoxic and genotoxic endpoints (see accompanying extended abstract by Ziemann et al.):

- To validate the endpoints established in an *in vitro* screening in a 28-day *in vivo* study.
- To characterize dust-dependent lung effects by using bronchoalveolar lavage (BAL) on day 3 and day 28 and histopathological parameters on day 28 of the post-instillation period.
- To determine if there were differences in the toxicological profiles of the TG and TC sample, the CS sample and the well-characterized reference quartz DQ12 and to rank the observed effects.

2. Materials and Methods

2.1. Particle samples

2.1.1. **Test materials** Two high-volume sampling apparatus were developed for RTDs’ use and dust amounts of approximately 1000 mg were collected at various industrial sectors (Table 1). Two test items were investigated in this study: TG and TC have been collected at various workplaces of the tableware sector under the responsibility of the Instituto de Tecnología Cerámica (ITC), Spain and the Société Francaise de Céramique (SFC), France, respectively.

2.1.2. **Reference materials** The reference materials are shown in Table 1. Three reference items were investigated in this study, TiO2, the contrived sample (CS) and the positive control quartz DQ12 (PC). CS was prepared in a wet process mixing DQ12, feldspar and clay and lyophilising the mixture subsequently (tables 1 and 2).

2.2. Test system

Male Wistar WU rats [strain Crl:(WI)WU] were purchased from Charles River Deutschland, Sulzfeld, Germany. The age of the animals at the start of exposure was 8 weeks and the weight approx. 300 grams. The rats were exposed to the test/reference items by intratracheal instillation. Males are preferred to females because of literature data that indicate a higher sensitivity with regard to silicotic response for males [2].

2.3. Dosing scheme and investigations

TG and TC served as the basis for dosing with 5 mg per rat. These fractions consist of 5.8% and 3.1% quartz, respectively. To reach a similar quartz dose in the quartz DQ12 and in the CS groups as compared to the TG and TC samples, the contained quartz percentage was adapted. This allows a direct comparison of quartz polymorphs at similar quartz doses (see Table 2).
Each total dose was administered by intratracheal instillation of 4 equal aliquots on 4 consecutive days. Animals were sacrificed 3 days (→ BAL) and 28 days (→ BAL, histopathology) post-treatment for inflammatory endpoints. The experimental set-up is shown in Table 2.

### Table 1  Particle Size Distribution of Quartz-Containing Factory and Reference Samples

| Name/code                      | Intended use / Collected by | Geometric Mean (µm) (geom. STD) weighting by mass | Geometric Mean (µm) (geom. STD) weighting by number | Quartz Content (weight-% - XRD) |
|-------------------------------|-----------------------------|---------------------------------------------------|---------------------------------------------------|-------------------------------|
| TiO₂ Bayertitan T T           | Negative Control            | 1.80 (1.90)                                       | 0.44 (1.58)                                       | ---                           |
| Tableware (granulate) TG      | ITC Spain                   | 5.97 (1.83)                                       | 0.52 (1.95)                                       | 5.8                           |
| Tableware (cast) TC           | SFC France                  | 4.36 (1.76)                                       | 0.53 (2.15)                                       | 3.1                           |
| Contrived sample a CS         | Surrogate for a Ceramic Dust| 3.77 (1.86)                                       | 0.87(1.66)                                        | 26.1                          |
| Quartz, type DQ 12 PC mid-size| Positive control            | 3.01 (1.53)                                       | 1.72 (1.56)                                       | 87                            |
|                               |                             | 87% α quartz, 13% amorphous                       | 87% α quartz, 13% amorphous                       |                               |

* 30% DQ12, 20% feldspar, 50% china clay

### Table 2  Dosing Scheme

| Treatment                          | Cumulative Particle Dose (mg per rat) | Quartz Dose (mg per rat) |
|------------------------------------|---------------------------------------|--------------------------|
| Saline (vehicle control)           | - (0.3 ml saline)                     | -                        |
| TiO₂ - Bayertitan T (negative control) | 5                                | -                        |
| Tableware (granulate) TG           | 5                                    | 0.29                     |
| Tableware (cast) TC                | 5                                    | 0.16                     |
| Contrived sample a CS              | 1.1                                   | 0.29                     |
| Quartz DQ12 mid-size PC            | 0.33                                  | 0.29                     |

* 30% DQ12, 20% feldspar, 50% china clay

Note: Dosage of Quartz DQ12 Mid-Size has been specified on the basis of known quartz contents of the two ceramic samples. The quartz dose of the CS and DQ12 group was the same to allow a direct comparison.
3. Results
TG and TC BAL data, 3 days after the end of treatment, showed a moderate inflammatory response. The intensity of the initial response did either not change or decreased after 28 days. In contrast, the quartz DQ12 group showed a strong response which continued to intensify during the subsequent 25 days. At 3 days, bronchoalveolar BAL analysis resulted in polymorphonuclear neutrophil levels (PMNs) of 15%, 25%, 0.6% and 25% in the TG, TC, CS and PC groups, respectively. At 28 days, the values were 29%, 20%, 7% and 45%. Histopathologically, the TG and TC groups showed very slight to slight effects, the PC group, however, stronger effects after the same period. Results of endpoint analyses are given as normalized data in Table 3.

4. Discussion
The scientific approach in this study was to characterize differences in the biological activity of various quartz species showing a similar particle size. It is well-known that such differences in toxicological potency exist depending on age, treatment, composition, surface contamination and other factors representing the multifactorial toxicity aspects of quartz and quartz-containing particles [3], e.g. for crushed quartz and aged quartz [4] [5].

DQ12 mid-size used as the positive control in this study is a crushed quartz that has been widely accepted as a biologically active standard in similar toxicological studies [1]. The particularity of quartz DQ12 samples is their lack in any aging effect. Most of the DQ12 fractions nowadays used have been produced in the eighties of the last century and there exists a more than 20-year experience in various labs regarding the persistence of the intrinsic toxicity of these samples.

CS was prepared in a wet process mixing DQ12, feldspar and china clay and mimics the typical composition of a ceramic dust with high quartz content (here DQ12). The toxicological results reveal that feldspar and china clay have a strong masking effect on the toxic properties of DQ12 (Table 3).

5. Conclusions
From the results of the present study the following conclusions can be derived:

- The vehicle control did not induce lung inflammation.
- The positive control quartz DQ12 produced significant and progressive lung inflammation.
- The Tableware (granulate) and the Tableware (cast) samples induced a weaker inflammatory response than the positive control (statistically significant) and this response, albeit statistically significant compared to the vehicle control, was not progressive.
- The Tableware (cast) sample showed a (partially statistically significantly) weaker response than the Tableware (granulate) sample.

Overall, the above-mentioned biological effects of the test items can be ranked in the following order combining the endpoints BAL (LDH, β-glucuronidase, total protein, PMN) and histopathology findings (interstitial mononuclear cell infiltration, alveolar inflammatory cell infiltration, granulomatous alveolitis, perivascular granulocytic infiltration): quartz DQ12 mid-size > Tableware (granulate) > Tableware (cast) ≥ contrived sample > TiO₂ (Bayertitan T) > vehicle control. Thus, a differentiation of effects for TG and TC, CS and PC was found. TG, CS and PC showed differences in their toxic potential albeit dosed with the same quartz amount. A substantial decrease was observed for CS as compared to PC. Irrespective of the contained DQ12 quartz, the wet mixing process with feldspar and china clay had drastically reduced the toxicity of the final CS. From a regulatory point of view, the substance-specific toxic potentials of TG and TC may need to be considered when devising occupational exposure limits.
Table 3 Summary of main effects after 3 and 28 days (normalized data as compared to controls)

| Test Materials                  | TiO$_2$          | Tableware granulate | Tableware cast | Contrived sample | Quartz DQ12 |
|--------------------------------|------------------|---------------------|----------------|------------------|-------------|
| - not significant (+), +++, +++ effect grading |                  |                     |                |                  |             |
| BAL Day 3 28                   | 3 28             | 2 28                | 2 28           | 3 28             | 3 28        |
| Lactate Dehydrogenase (LDH)    | -                | -                   | -              | -                | -           |
|                                |                  | 3.3                 | 3.5            | 2.1              | 2.1         |
|                                |                  | +§§                 | +§§            | +++              | +++         |
|                                |                  | $§§§$               |                |                  |             |
| β-Glucuronidase                | -                | -                   | -              | -                | -           |
|                                |                  | 2.6                 | 2.5            | 1.1              | 1.6         |
|                                |                  | ###                 | ###            | $§§§$           | +++$§$      |
|                                |                  |                     |                |                  |             |
| Total Protein                  | -                | -                   | -              | -                | -           |
|                                |                  | 2.8                 | 1.9            | 1.6              | 1.4         |
|                                |                  | ###                 | ###            | $§§§$           | $§§§§$      |
| PMN* (% in differential cell count) | 1.8 4.9         | 14.5 28.6           | 2.3 20.4       | 0.6 6.7          | 25.0 44.8   |
|                                |                  | ###+                | ###++          | +++§§           | $§§§§$      |
| Lung Weights Relative Histopathology | Day 28          | 28                  | 28             | 28               | 28          |
| Inflammation                   | not significant  | very slight         | very slight    | very             | slight      |
|                                | to slight        |                     | to slight      | slight           | moderate    |
| Interstitial Fibrosis          | not significant  | very slight         | not significant| not significant  | very slight |
|                                | to slight        |                     |                |                  |             |

Statistically significantly different as compared to vehicle controls (#, ##, ###), DQ12 (+, ++, ++++) and TG ($§$, $§§$, $§§§$) (Tukey’s test)

* Total PMN numbers resulted in relative values of 0.01 (VC), 0.06 (TiO$_2$), 0.52 (TG), 0.43 (TC), 0.08 (CS) and 1.00 (PC), thus, the outcome was similar as compared to the percentage values. TC shows the same number as TG, i.e. non-quartz is contributing to the effect observed.

References
[1] Robock K 1973 Ann. Occup. Hyg. 16, 63
[2] Honnons and Porcher JM 2000 J. Environm. Path. Tox. Onc. 19, 391
[3] Fubini B 1998 Health effects of silica The Surface Properties of Silicas ed AP Legrand (John Wiley & Sons Ltd) chapter 5 pp 415-464
[4] Castranova V, Vallyathan V, Ramsey DM, McLaurin JL, Pack D, Leonhard S., Barger MW, Ma JYC, Dalal NS and Teass A 1997 Environm Health Persp 105 (Suppl 5), 1319
[5] Vallyathan V, Castranova V, Pack D, Leonhard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR and McLaurin JL Am. J. Respir. Crit. Care Med. 152, 1003

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