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Talc Inhalation in Rats and Humans

A Review and Appraisal of Available Evidence

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Background: Current information on the health effects and toxicology of talc suggests that this may lead to a specific target organ toxicity arising from repeated exposure (STOT-RE) classification. **Objective:** To provide an assessment of the currently available inhalation toxicity data on talc and to put these data in the perspective of other poorly soluble low-toxicity particles. **Methods:** A database of 177 articles was gathered from different sources. **Results:** Relevant animal data sets were subjected to a quality review, and epidemiological studies on talc and lung effects published since 2016 were reviewed. **Conclusions:** Of nine original inhalation studies reviewed, only one study using rats and mice met the criteria that are needed to include for a reliable evaluation for STOT-RE. Together with the pulmonary effects observed in exposed talc miners, a STOT-RE 1 classification is warranted.

Keywords: cancer, classification, inhalation, pulmonary effects, review, talc

Talcum powder is made from talc, a mineral made up mainly of the elements magnesium, silicon, and oxygen. Talc is a common metamorphic mineral, consisting of hydrated magnesium silicate that is processed into a white, fine, crystalline powder. Mineral talc is (by weight) composed of 31.7% magnesium oxide, 63.5% silicon dioxide, and 4.8% water. As a powder, it absorbs moisture well and helps reduce friction, making it particularly useful for keeping skin dry and helping to prevent rashes. It is widely used in cosmetic products, such as baby and adult powder, and other consumer products such as facial powder. Mineral talc and talc used in cosmetic products vary in purity depending on the source and application. In addition, talc varies in associated minerals, particle size, and shape morphologies.[1] Talc may contain asbestos and nonasbestos fibers. In its natural form, some talcs may also contain minor traces of asbestos, a substance known to cause different lung and mesothelial cancers in humans. In a study among 22 retail talcum products, samples were found to have fiber contents ranging from 8% to 30% by count, with an average of 19%. Fibrous particulates were generally under 1.0 μm in diameter with lengths ranging from 1.5 to 6.0 μm and was predominantly talc with minor amounts of tremolite, anthophyllite, and chrysotile, as these are often present in fibrous talc mineral deposits.[2] The purest talc from Italian mines was reported to have only a few nonasbestiform tremolite and no serpentine (chrysotile) or amphibole detectable fibers. The numerical concentration of tremolite fibers in the talc sample was 3.687×10^6 fibers/g, corresponding to a mass concentration of 0.722 parts per million.[3]

Workers involved in mining, milling, and processing of talc ore have elevated incidences of fibrotic and nonmalignant respiratory diseases (NMRDs), which are also seen among workers exposed to talc in secondary industries.[4] Most public and regulatory concerns relate to a possible link between talc and cancer in women who regularly apply talcum powder in the genital area, who show an increased risk of ovarian and/or endometrial cancer. A final source of debate is the occasional presence of asbestiform minerals in cosmetics that contain talc.[5] These latter issues are not subject of this current review.

This review focuses on available animal inhalation studies performed with talc powder in different animal species and test conditions, and tries to frame these studies within current toxicological paradigms such as lung particle overload and test guidelines for inhalation studies, such as those defined by Organization for Economic Cooperation and Development (OECD).[6,7] In addition, we reviewed epidemiological studies and checked for updates of several talc-exposed cohorts that were evaluated by the International Agency for Research on Cancer (IARC) during its monograph meeting on talc in 2006.[5] The main purpose of the article is therefore to use the current data to estimate its impact on likely future classification within the Global Harmonization System, including the specific target organ toxicity (STOT) assessment following repeated exposure (RE). STOT-RE consists of two hazard categories—STOT-RE 1 (H372, signal word "Danger") and STOT-RE 2 (H373, signal word "Warning"). STOT-RE 1 means definitely toxic to humans or toxic effect as determined in animal experiments after repeated exposure. STOT-RE 2 includes substances presumed to be toxic after repeated exposure on the basis of evidence from studies in experimental animals.

MATERIALS AND METHODS

The review was started with the database kindly provided by EUROTALC,* which consisted of 113 articles on the adverse effects of talc and published between 1968 and 2005, and 22 articles published between 2006 and 2009. The primary focus of the selected articles was the effects of inhalation on the pulmonary system. The database was complemented by an additional PubMed search for articles published between 2006 and 2021 using search items including talc (particles), inhalation, and toxicology for animal inhalation studies. For human studies, the terms epidemiology, lung cancer, and respiratory diseases were added to the talc inhalation search term string. This delivered a further 36 articles from this period and six relevant articles that were published earlier. A breakdown of studies into subcategories is listed in Table 1, but it should be noted that a large proportion of the epidemiological articles were on ovarian and endometrial cancer, which are not considered in this review. Therefore, for the epidemiological studies, the focus was on investigations evaluating lung cancer risk or NMRDs, such as chronic obstructive pulmonary disease and lung fibrosis. Animal inhalation studies were judged on study quality and reliability based on their Klimisch score categories,[8] and varied between 1 and 4. In this scheme, 1 means "reliable without restriction"; 2, "reliable with restrictions"; 3, "not reliable"; and 4, "not assignable."

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* EUROTALC is the talc industry's representative body for all regulatory and scientific matters. It is also a forum where people with an interest in talc can find information and support, and exchange views. The association has an established tradition of research and information in its main areas of interest: health and safety, environment, and standardization.

TABLE 1. Breakdown of Database Used to Source Animal Studies, Human (Epidemiological) Studies, Reviews, and General Studies

Interval	Animal	Human (Epi)	Reviews/Meta-Analysis	General and Background	Analytical + Case Reports	Total
1968–2005 EUROTALC database	14	37	18	26	18	113
2006–2009 EUROTALC database	1	8	7	3	3	22
2009–2021 PubMed search	8	12	11	8	3	42
Total	23	57	36	37	24	177

Epi, epidemiological.

The application of Klimisch scores to individual articles was done by the author after detailed analysis of the individual articles. Category 1 articles had to include complete data information on exposure, including size distribution of particles, and animal housing; all details of postexposure follow-up; and detailed histopathology of the control and test animals after exposure. Some studies did not provide details of the size distribution or pretreatment of the studied talc, or it was not possible to retrieve further details of the animal groups investigated. In addition, quality was scored based on evidence available from systematic lung burden investigations (score, 1), parallel studies on kinetics (score, 2), or alternative approaches to evaluate lung overload (score, 3). In some cases, the documentation of particle elimination was insufficient (score, 4) or not relevant (score, 0) for the type of study. A third measurement of quality was applied to the animal studies, and that was whether the design of the study is adequate for current STOT-RE classification purposes.[9] For this, the data in article were considered to be adequate, incomplete but adequate, or not adequate.

As noted previously, STOT-RE category 1 is used when evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans after repeated exposure. Category 2 is used for substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health after repeated exposure. Evaluation is not on quality but on suitability to use the data for classification, based on length of exposure, number of concentration levels used, follow-up time after exposure, and number animals per group as defined in the regulation.[9]

Animal Studies

The respiratory toxicity of talc in animals has been investigated by inhalation exposure to mostly three types of talc. These talc types are Johnson & Johnson's Baby Powder,[10,11] an Italian 00000 grade talc,[12,13] and MP 10-52, which is a high-purity industrial talc used as an additive in paint.[14] All previously mentioned sample types were reported asbestos free, and their composition and sources were reviewed recently by Johnson.[15] An overview of inhalation animal studies is listed in Table 1. It is interesting to note that the early studies by two different research teams in the 1970s observed only minor effects in inhalation studies with rats and hamsters at concentrations around 10 mg/m^3. These 30-year-old studies[10–13] are still used as a "benchmark" in many reports, including the EU REACH dossier.[16]

As early as 1977, Wehner et al[10] exposed groups of 50 male and 50 female Syrian golden hamsters to talc aerosol (9.8-mg/m^3 Vermont talc) for 3, 30, or 150 minutes/day, 5 days/week for 30 days, or for 30 or 150 minutes/day either until they died naturally or for a maximum of 300 days. The Vermont talc was milled and processed to reach a bimodal particle size distribution with diameter peaks at 0.6 and 2.6 μm and a mass medium aerodynamic diameter (MMAD) of 4.9 μm. Therefore, this preparation should be regarded as a mixture of two distinct particle populations, and the majority of particles on a mass basis are in the smaller fraction. Control animals (25 males and 25 females) were exposed to air only, and there was no positive dust exposure group. In addition, 200 female 7-week-old hamsters were randomly divided into groups and were exposed to talc aerosol for 30 or 150 minutes/day, respectively, for 300 days, unless they died sooner. The mean total aerosol exposure was 27.4 mg/m^3 with a respirable fraction of 8.1 mg/m^3. Most of the animals exposed to talc for this extended period died before completion of the exposures. The survival times of the exposed groups did not differ, nor were they different between the exposed groups and the controls. However, the mean survival time of the males in all groups was significantly ($P < 0.05$) longer than that of the females. At the age of approximately 12 months, these differences became statistically significant, but no treatment-related effect was observed. No trend was seen in the type, incidence, and severity of the observed pathological lesions with dose, and no significant differences between the exposed groups and the controls were seen. Interstitial pneumonia was the most common lesion seen with the highest incidence after short-term exposure (30 days) and the lowest in long-term (300 days) exposure and controls. Focal alveolar cell hyperplasia was associated to duration of talc inhalation, as the highest incidences were found in animals exposed 300 days to either 30 or 300 mg/m^3. However, this was not confirmed in a statistical analysis, using a two-way analysis of variance of focal alveolar cell hyperplasia to the number of exposure days and the number of exposure minutes per day.

Moreover, in 1977, Wehner et al[11] determined the clearance of talc upon inhalation in hamsters. They exposed hamsters for 120 minutes to an atmosphere of talc particles with concentrations between 39.7 and 74.7 mg/m^3 and with an MMAD of 6.6 μm in diameter. This particle size is considerably larger than the earlier study using 4.9 μm (and with a different particle size distribution). They reported a biological clearance half-life of talc in the lung of 7 to 10 days, but this is not adequate to describe pathological events previously described in their extended inhalation study.[10]

Furthermore, in the 1970s, Wagner et al[12,13] tested Italian grade talc by Wistar rats using three uptake routes being intrapleural inoculation, inhalation, and oral ingestion. Animals exposed to chrysotile asbestos and untreated controls were included for comparative evaluation and interpretation of findings. For inhalation experiments, equal numbers of male and female Wistar rats were exposed to a mean respirable dust concentration of 10.8 mg/m^3 of talc (Italian 00000 grade). The remaining fraction (60% of the bulk sample) was nonrespirable talc, and this sample was reported to have an upper particle size of 70 μm and a mean particle size of 25 μm. Groups of rats (eight for fibrosis, 24 for neoplasm evaluation) were exposed for periods of 3, 6, and 12 months for 7.5 hours/day, 5 days per week, and they were examined immediately and 12 months after the end of the exposure. The pathological examination revealed the presence of minimal to slight degrees of pulmonary fibrosis in talc-exposed rats compared with control animals. The fibrosis was more pronounced after longer exposure intervals and also increased during the postexposure period (12 months). No lung tumors were seen in animals ($n = 24$) exposed to talc for 6 months, whereas one adenoma was found after 12 months of exposure to talc, and no tumors were found in the control group. As expected, chrysotile

inhalation led to adenoma formation (3/24), adenomatosis (2/24), and adenocarcinoma (1/24) after 12 months of inhalation, and one (1/24) adenomatosis after 6 months of inhalation. The pleural inoculation experiments showed 18 of 48 rats with mesothelioma injected with SFA chrysotile (20 mg/rat), whereas no mesothelioma was seen in 48 talc-treated rats (20 mg/rat) or 48 saline controls. Ingestion experiments showed only incidental tumors that were not related to treatment.

Around two decades later (1993), the National Toxicology program (NTP) talc study was published.[14] As a standard preparation for this lifetime study, a 4-week subchronic toxicity range-finding inhalation study in F344 rats, equivalent to a chronic inhalation of 0, 2.8, or 8.4 mg/kg per day for male rats and 0, 3.2, or 9.6 mg/kg per day for female rats, was conducted. These studies were the basis for the dose selection in the lifetime inhalation study done in male and female F344 rats (0, 6, or 18 mg/m^3, 6 hours/day, 5 days/week), which continued exposure until mortality in any exposure group reached 80% (113 weeks for males and 122 weeks for females). In parallel, lifetime inhalation studies were done with B6C3F mice (0, 6, and 18 mg/m^3, 6 hours/day, 5 days/week) for up to 104 weeks.[14] Absolute and relative lung weights of male rats exposed to 18 mg/m^3 were significantly greater than those of controls at the 6-, 12-, and 18-month interim evaluations and at the end of the lifetime study, whereas those of female rats exposed to 18 mg/m^3 were significantly greater at the 1-, 18-, and 24-month interim evaluations and at the end of the lifetime study. Inhalation exposure of rats to talc produced a broad array of inflammatory, reparative, and proliferative processes in the lungs. Nearly all exposed rats showed granulomatous inflammation, and with longer exposure duration and higher concentrations, the severity increased. Hyperplasia of the alveolar epithelium and interstitial fibrosis were present in many exposed rats near foci of inflammation. Accumulation of talc containing macrophages (histiocytes) was seen in the peribronchial lymphoid tissue of the lung and in the bronchial and mediastinal lymph nodes. The incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) were significantly greater in female rats of the high-exposure group (18 mg/m^3) than in controls. Such an effect was not seen in male rats, because numbers of pulmonary neoplasms in exposed male rats were similar to those in controls.[14]

In the upper respiratory tract, minor alterations attributed to talc exposure were observed. A concentration-related increased incidence of hyperplasia of the nasal mucosa in male rates and accumulation of cytoplasmic, eosinophilic droplets in the nasal mucosal epithelium in male and female rats occurred in talc-exposed groups. Interestingly, adrenal medulla pheochromocytomas (benign, malignant, or complex [combined]) were seen in both male and female rats, and the incidences in the 18-mg/m^3 groups were significantly greater than those of controls. Finally, lung function was found to be decreased in F344 rats,[12] more specifically as smaller lung volume, lung capacity, and reduced forced expiratory flow in both sexes at 18 mg/m^3 starting at 6 or 11 months (lung capacity) of exposure. In mice, chronic active inflammation and accumulation of alveolar macrophages at both levels of talc (6 and 18 mg/m^3) were observed in both sexes. In contrast to rats, no increased numbers of alveolar hyperplasia, squamous metaplasia, or interstitial fibrosis or pulmonary neoplasms were seen in exposed or control mice. Accumulations of particle-loaded macrophages were also present in the mice bronchial lymph nodes, and cytoplasmic eosinophilic droplets were significantly increased in the nasal mucosal epithelium of exposed male and female mice.

Some limitations regarding the NTP study have been raised.[17] First, as noted earlier, a subchronic 4-week range-finding inhalation study using concentrations of 6 and 18 mg/m^3 of talc was performed before initiating the chronic study, but it was observed by Oberdorster[17] that such chronic studies should not be performed simply at the highest technologically feasible concentration but three concentrations should be used, of which only the highest should show some interference with lung-defense mechanisms, that is, clearance impairment, and the two lower concentrations should show no interference with clearance and particle accumulation. Despite the similar findings from the range-finding study, the subsequent chronic study was performed at the two concentrations for which markedly impaired lung clearance had been detected in the 4-week study; that is, the maximum tolerated dose may have been exceeded at both exposure concentrations. Thus, the purpose and use of the 4-week talc inhalation study remains unclear because the results apparently were not applied to selecting dose levels for the chronic study in rats.[14] Second, the NTP did not consider the overload paradigm that was published earlier in 1988 by Morrow,[18] which stated that beyond a certain volumetric load (around 1-μg/g lung) in the rat lung, pulmonary retention is increased and subsequent pathological responses are typical for any low-toxicity particle. The retained lung burden in the NTP study exceeds the overload threshold level, and therefore, the findings on tumorigenicity in female rats need to be qualified as most likely produced secondary to particle overload and related chronic toxicity.

In 1989, Pickrell et al[19] reported a study in which they exposed groups of rats (F344/Crl, $n = 20$) and B6C3F1 mice ($n = 20$) to three different concentrations of asbestos-free talc for 6 hours/day, 5 days/week for 4 weeks. In each group, 10 female and 10 male rats were included, and controls animals were exposed to filtered air using the same schedule. The MMAD of the talc aerosol was 3.0 μm, and the mean airborne exposure concentrations for exposures were 0, 2.3, 4.3, and 17 mg/m^3 of talc. After a 20-day inhalation exposure, lung burdens in rats were 0-, 0.07-, 0.17-, and 0.72-mg talc per gram of lung, respectively. From this, it is clear that the amount retained in the lung per unit of exposure concentration increased more in linear with increasing concentration. Similar saturation of lung clearance was seen at similar exposure conditions in the mice. Only a modest increase of talc-containing, free macrophages was seen in alveolar spaces of both rat and mouse groups exposed at the highest levels of talc for 20 days. The lung burdens were measured according to a method[20] that is based on acid-insoluble magnesium determined by flame atomic absorption.

In the most recent inhalation study published in 2015,[21] male and female Sprague-Dawley rats were exposed by inhalation to respirable talc (MMAD, 3.88 μm; Rex Materials, Seoul, Korea) at 5, 50, and 100 mg/m^3 for 6 hours daily, 5 days/week during 4 weeks. Infiltration of macrophages on the alveolar wall and spaces near terminal and respiratory bronchioles was seen, as well as upregulation of oxidative stress genes. Superoxide dismutase 2 was significantly induced in both the low and high talc inhalation groups of male rats ($P < 0.05$) as compared with sham controls. Expression of superoxide dismutase 2 in the lungs of male rats exposed to the highest concentration of talc was more than twice the level in the control rats, whereas female rats at the highest concentration of talc showed superoxide dismutase 2 expression 4 times that of the control group. Somewhat different results were obtained with glutathione peroxidase: in male rats, more than double levels of glutathione peroxidase were measured than those in control rats, although only the difference between the control group and the low exposed group was statistically significant. Glutathione peroxidase tended to be increased in all female rats exposed to talc but did not reach statistical significance.

Lung Particle Overload as a Confounder

Previously, Oberdorster[17] pointed out that the findings in the NTP study[14] can be explained by the particle lung overload effect, which is a concept that was published by Morrow[18] in 1988. Oberdorster[17] showed, by further analysis of particle accumulation kinetics, that lung overload had been reached at both concentrations in both species, resulting in talc accumulation leading to high lung burdens. As such, the carcinogenicity observed after talc inhalation in female rats in the NTP study could be considered as a secondary effect due to particle load in the lung and the chronic inflammatory response that is associated to it in the rat.[22,23] It is now well documented that, in rats, lung particle overload is associated with inflammation, epithelial hyperplasia, and, in extreme cases, lung cancer. Although the human relevance of rat lung tumors occurring with particles under such lung overload has been questioned,[22,23]

recent EU regulatory decisions, such as that on TiO_2,[24] have considered these outcomes as evidence for a possible human hazard.

To evaluate the occurrence of particle lung overload in earlier published animal studies (Table 2) as a potential mechanism in talc-induced lung adverse responses, information is required on lung burden or lung clearance before and during inhalation exposure.[22] Unfortunately, these data are only partly available in most studies (Table 3). In the early studies in the '70s, lung burden has been measured in parallel to the rat long-term inhalation studies,[9,10] and these studies indicated rapidly increasing lung burden, but critical details are missing to draw a clear conclusion regarding whether or not lung overload occurred. However, a companion inhalation study[8] with a single high (70 mg/m^3) exposure to talc did not show signs of lung overload. In addition, Pickrell et al[19] noted that talc lung burdens, normalized for lung weight and exposure concentration, in rats exposed to the two higher exposure concentrations were greater than those in rats exposed to the lowest talc exposure concentration and suggested that clearance of talc in rats was reduced after continued exposure and accumulation, following the reduced clearance particles accumulation modeling published by Morrow[18] some years before their studies. Interestingly, they conducted pharmacokinetic modeling to show that the calculated doses for rat and mice were lower than the actual lung burdens.

Oberdorster[17] had already concluded that the lung tumors found after talc inhalation were due to a general particle effect after lung overload. Since the early '90s, it is well documented and accepted that, in rats, lung particle overload is associated with inflammation, epithelial hyperplasia, and, in some cases, lung cancer.[22,23] The relevance of these findings to human risk assessment for PSLTs has been the subject of scientific debate over the last few decades[24] and has led to the publication of a number of workshop reports. In one such recent expert workshop,[23] expert consensus agreement was reached that the rat is a sensitive model for PSLT inhalation toxicology, but also, most importantly, agreement was reached that rat lung cancer occurring only under conditions of lung particle overload does not imply a cancer hazard for humans under nonoverloading exposures.

Weight of Evidence Analysis

As can be seen from Table 1, inhalation studies with talc have been published between 1977 and 2015, which encompasses a time span of almost 40 years. During this period, internationally accepted test guidelines for the scientific conduct of inhalation animal studies have been updated and specified,[6,7] animal ethical approval was introduced, and the lung overload phenomenon has been better recognized as an emerging issue based on the early work of Morrow[18] and has been used in recent EU regulatory activities.[24] It is the purpose of this section to describe the approach taken to evaluate the weight of evidence of the inhalation studies on talc, based on three factors:

1) The study quality categories based on Klimisch[8] vary between 1 and 4 in which 1 means "reliable without restriction," 2 means "reliable with restrictions," 3 means "not reliable," and 4 is "not assignable"; this last category describes studies or data from the literature in which description of the test conditions does not give sufficient experimental details, which are only listed in short abstracts or are mentioned by other reviews without the ability to verify (secondary sources).
2) Scoring the used experimental design by comparison with the current OECD TR 412[23] or 413[24] design, in which 1 means equivalence to current testing demands, 2 is incomplete but adequate, and 3 means the test protocol and data analysis do not meet these current OECD test guideline criteria.

TABLE 2. Experimental Animal Studies Using Talc Inhalation Exposure Relevant for STOT-RE and Carcinogenicity Classification

Study Description and Findings	Type of Talc	Exposure Details	Reference
Rat inhalation study: The study noted mild fibrosis in exposed rats, as well as one case of lung adenoma. Lung burdens (2.8, 4.5, and 12.3 mg) suggest saturation of lung clearance over time.	Italian 00000 grade	10.8 mg/m^3 (MMAD missing); exposure of 3, 6, and 12 months. Follow-up at 12 months after exposure.	Wagner et al[12]
Talc deposition and effects after 20 days of repeated inhalation exposure of rats and mice to talc	MP 10–52	Exposure: 6 hr/day, 5 days/week for 4 weeks Rats: 2.3, 4.3, and 17 mg/m^3 Mice: 2.2, 5.7, and 20.4 mg/m^3 MMAD: 3 μm ± 1.9 aerosol	Pickrell et al[19]
Syrian golden hamsters' exposure to respirable talc showed little to no pathology compared with controls. Animals however did not survive to lifetime.	J & J BP	9.8 mg/m^3 (MMAD, 4.9 μm); exposure of 3, 30, or 150 minutes for 30 days, 300 days of follow-up	Wehner et al[10]
Pulmonary clearance in 10-week-old Syrian golden hamsters exposed to respirable talc. The clearance half-life was 7–10 days; pulmonary clearance was completed in 4 months.	J & J BP	40–75 mg/m^3 (MMAD, 6.6 μm); single exposure of 120 minutes, 300 days of follow-up	Wehner et al[11]
NTP lifetime inhalation study in rats and mice. In rats, the inhalation of talc resulted in a range of inflammatory, reparative, and proliferative lung responses. Lung tumor formation and reduced clearance are evident at high doses in female rats (AA, BA, CA) only. In mice, there was no evidence of hyperplasia or interstitial fibrosis, although clearance was reduced at the higher dose.	MP 10–52	6 and 18 mg/m^3 (rats: MMAD of 2.7 and 3.2 μm; mice: MMAD of 3.3 and 3.6 μm)	NTP[14]
Critical review of NTP (1993) dose selection and outcome interpretation	MP 10–52	Exposure described in NTP (1993)	Oberdorster[17]
Lung burden study of talc in rats and mice after 20 days of inhalation exposure (Pickrell et al[14]) Rats: 2.3, 4.3, and 17 mg/m^3 Mice: 2.2, 5.7, and 20.4 mg/m^3	MP 10–52	Mean lung burdens in rats were 77, 187, and 806 μg of talc per gram of lung (n = 10) for exposures at 2.3, 4.3, and 17 mg/m^3 of talc, respectively. Mean lung burdens in mice were 114, 325, and 1150 μg talc per gram of lung (n = 10) for exposures at 2.2, 6.3, and 20.6 mg/m^3 of talc	Hanson et al[20]
An animal model for inhalation exposure to talc. Respirable fraction contained 1300 particles/mL larger than 1 μm. Between 1% and 2% of these particles were fibrous.	Italian 00000	10.8 mg/m^3 (MMAD missing); exposure of 3, 6, and 12 months. Follow-up at 12 months after exposure.	Wagner et al[13]

AA, alveolar adenoma; BA, bronchiolar adenoma; CA, carcinoma (combined); MMAD, mass medium aerodynamic diameter; NTP, National Toxicology program; STOT-RE, specific target organ toxicity arising from repeated exposure.

TABLE 3. Evaluation of Animal Inhalation Studies With Talc Using Three Indices for a Weight of Evidence Evaluation

Study	Klimisch Score	Meets Current Test Criteria (STOT)	Effects Related to Lung Overload Conditions	Remarks
Pickrell et al[19]	2	2	1	20 days of exposure, no follow-up
Wagner et al[12]	2	3	2	Enriched bulk sample
Wagner et al[13]	2	NA	NA	Enriched bulk sample
Wehner et al[10]	2	3	3	Single concentration, short daily exposure; hamsters only
Wehner et al[11]	2	3	1	No connection possible with Wehner et al.[10] Other concentrations and particle size distribution.
NTP[14]	1	1	1	Dose selection based on MTD from the 4-week study
Oberdorster[17]	1	NA	1	Relates to NTP[14]
Hanson et al[20]	2	1	3	Relates to Pickrell et al[19]
Shim et al[21]	1	1	0	Focus on mode of action. No pathology documented.

Klimisch score (1–4): 1, reliable without restriction assessment purposes; 2, reliable with restrictions; 3, not reliable; 4, not assignable. STOT-RE test criteria: 1, meets current test criteria; 2, incomplete but adequate; 3, not adequate; NA, not applicable. Overload: Evidence available from systematic lung burden investigations (1), parallel studies on kinetics (2), alternative approach to evaluate overload (3), documentation insufficient (4), or not relevant for type/duration of the study (0).

MTD, maximum tolerated dose; NTD, National Toxicology program; STOT, specific target organ toxicity.

3) Whether the studies have evaluated particle clearance/lung burden to enable the assessment of the presence, or absence, of lung overload.

In addition, many of the inhalation studies have used samples containing respirable particle fractions that account for only part of the bulk sample.[7,8,15] It also should be emphasized that, during the aerosolization of bulk talc samples, the percentage of fibrous talc is enhanced by 4 to 6 times, and this also limits further interpretation of findings as compared with the original sample.[15]

In Table 3, all animal studies are listed again but, this time, with the application of the quality criteria and scoring described previously to each individual study. This analysis shows that all studies have been well designed and documented and, accordingly, have Klimisch scores that are either 1 or 2, which means that they are reliable. However, most of the studies do not meet the relevant current OECD test guidelines for repeated exposure regarding target organ toxicity because either they used a single exposure level or the parameters and follow-up time do not meet current OECD-defined criteria.[6,7] Finally, the incorporation of parameters to estimate lung clearance and overload is incidental[12] or inadequate.[10] The only study that fulfills all criteria and has provided sufficient data to evaluate lung overload is the NTP[14] study. Although the most recent animal inhalation study by Shim et al[21] meets all test and quality criteria, it has no systematic pathological follow-up. In summary, of the nine original inhalation studies described and evaluated, only one meets current criteria to classify talc using the current STOT-RE criteria. The use of the Wagner et al[12] investigation in the current EU REACH dossier can be explained as the first benchmark animal study, but meanwhile, better data are available such as the NTP study[14] or epidemiological data, as discussed in the following section.

Studies in Human Populations

Pneumoconiosis: Occupational Exposure

The first effects reported in workers exposed to high airborne levels of respirable talc dust include respiratory symptoms, decrease in lung function, chronic bronchitis, and fibrotic nodules, classified as a pneumoconiosis, and are seen both in humans[25,26] and in animals.[14,27] A significantly increased mortality from NMRDs and pneumoconiosis has been reported for talc miners and millers in various cohort studies from around the world and has been reviewed by Johnson[15] and Wild.[28] Interestingly, the mining sites that were investigated were also the source of talc used in earlier animal inhalation studies and include Vermont in the United States, Italy, France, and Norway (Table 3).

It is widely accepted and documented that the purest talc exposure (low quartz, low radon, low asbestiform fibers) is found in Val Chisone mine in Northern Italy. Therefore, the mortality studies of male miners and millers in this Italian mine are considered among those with the least bias regarding coexposure. These miners were subject to mortality surveys at regular intervals between 1946 and 2020,[29–31] and the study cohort consisted of 1166 miners and 556 millers. The overall standardized mortality ratio (SMR) in the cohort was 1.21 (95% confidence interval [CI], 1.14 to 1.28), and no deaths were observed from pleural cancer (311). On the other hand, mortality from pneumoconiosis was significantly increased (SMR, 9.55; 95% CI, 7.43 to 12.08), especially among the miners (SMR, 12.74). In addition, the risk of pneumoconiosis increased with employment duration in the overall cohort. For instance, the SMR for more than 25 years of employment was 15.12 (95% CI, 10.89 to 20.43).

Leophonte and Didier[32] and Wild et al[28,33] studied workers in an open cast mine and factory in the south of France (Luzenac). Their studies showed a reduction in lung function and radiological changes in lung x-rays. However, the life expectancy of the talc dust-exposed workers was not significantly different from those in the local and national reference populations. No detectable asbestos was determined in the talc ore and dust, and the mean occupational exposure to talc dust was 1.87 mg/m^3 (geometric standard deviation, 2.5). However, past exposures of up to 60 mg/m^3 were estimated in workers sorting jute bags of milled talc. Cumulative dust exposure was a strong determinant of pneumoconiosis prevalence and supported by a cross-sectional study revealing that 46 of 176 workers suffered from pneumoconiosis.[33] Workers suffering from pneumoconiosis had significantly impaired ventilatory function compared with non–dust-exposed workers. Wild[28] observed a significant dose-response relationship between cumulative exposure to talc and NMRDs in workers in French and Austrian talc mines. A more detailed analysis of the case-control studies on NMRDs showed that this increase was due to the highest exposure groups (odds ratio, 2.5). The previously mentioned findings are supported by similar findings in the Vermont[34] and Norwegian[35] investigations. There is thus consistent evidence from a number of independent studies from various locations that excessive occupational exposure to talc dust leads to NMRD, including a series of symptoms and chronic events that are typically associated with chronic exposure to other "nuisance dusts" such as coal or graphite. Occupational exposure limits as 8-hour time-weighted averages have been set to prevent these effects but vary between different countries (for most countries, it is 2 mg/m^3, and the lowest is 0.25 mg/m^3 in the Netherlands, whereas some countries such as Denmark regulate talc based on fiber exposure).[†]

[†]Further country-specific information on talc occupational exposure limits can be obtained from https://limitvalue.ifa.dguv.de/WebForm_ueliste2.aspx.

TABLE 4. Exemplary Studies and Reviews on Mortality From Nonmalignant Respiratory Diseases in Occupational Groups Exposed to Talc

Study	Location	Size	Nonmalignant Respiratory Disease (NMRD)
Fordyce et al[34]	Vermont (United States)	427	Significant elevations in NMRD (SMR, 2.73; 95% CI, 2.10–3.48) and other NMRDs (SMR, 4.13; 95% CI, 2.88–5.74)
Wild et al[33]	France (FR) and Austria (AU)	1070 (FR) + 542 (AU)	Nonsignificant excess mortality for NMRD in the French cohort due to pneumoconiosis (SMR, 5.56; three observed; 95% CI, 1.12–16.2) Increased mortality in the highest exposure groups (odds ratio [OR] of 2.5 for a cumulative exposure > 800 y.mg/m^3) with a significant trend (OR of 1.08 per 100 y.mg/m^3) with cumulative exposure to talc
Wergeland et al[35]	Norway	390	No excess mortality from NMRD, but a large healthy worker effect present
Ciocan et al[31]	Italy	1749	Increased mortality from pneumoconiosis (SMR, 9.55; 95% CI, 7.43–12.08); the mortality increased with longer (25+) years of employment with an SMR of 15.12 (95% CI, 10.89–20.43).

CI, confidence interval; SMR, standardized mortality ratio.

Lung Cancer: Occupational Exposure

Some studies of talc miners and millers have suggested an increased risk of lung cancer and other respiratory diseases, whereas others have found no such increase in lung cancer risk (Tables 4 and 5) Comparison between these studies is complicated by the fact that talc in its natural form can contain varying amounts of asbestos and other minerals, unlike the purified talc used in consumer products. In its 1987 evaluation on talc, the IARC separated talc containing and not containing asbestiform fibers in its deliberations and evaluation. On the basis of a series of epidemiological studies conducted in the populations of talc workers in New York State (United States), the IARC concluded that there was *sufficient evidence* for the carcinogenicity to humans of talc-containing asbestiform fibers but that there was *inadequate evidence* for the talc not containing asbestiform fibers. The IARC 1987 assessment on asbestos-free talc was based on four epidemiological studies among miners and millers of talc (including Rubino et al,[29] Leophonte and Didier,[32] and Selevan et al[38]). Since then, there have been a number of updates of the above cohorts, and reviews and meta-analyses have also been published that merit further discussion but are still in accord with the IARC assessment. In Table 4, there are summaries of the most recent updates of these cohorts and the outcomes of extended follow-ups.

Rubino et al[29] were the first to study the mortality of male miners and millers in an asbestos-free talc mine in Northern Italy, and this work was completed with regular follow-up studies until 2020.[30–32,39] No significant increase in mortality from lung cancer was found in this cohort of 1166 miners and 556 millers, although the exposures were initially high and subsequently lower. However, a significant increased mortality from NMRDs and pneumoconiosis was reported and discussed previously. It should be noted that, when working underground, miners can also be exposed to other substances that might affect their lung cancer risk, such as radon and respirable crystalline silica. These previously mentioned studies in Italian miners have been conducted for more than 80 years (partly retrospective) and have not revealed an increased mortality or morbidity due to malignant respiratory diseases (Table 5).

Wergeland et al[35] published a follow-up of a previous cohort of Norwegian talc workers. The original study covered the employment between 1953 and 2011, and all comparisons were done with the general population or subgroups within the cohort. The 24 additional years of observation increased the number of deaths from 117 to 271. This leads to an overall SMR of 0.88 (95% CI, 0.77 to 0.99), without a difference in mortality between miners and millers. The total number of observed cancers more than tripled compared with the previous follow-up. Interestingly, the SMR for all cancers combined changed from 0.90 to 1.10 (95% CI, 0.93 to 1.29; 149 cases), but the small excess (about 10%) was similar in miners and millers. Twenty-one cases of lung cancer were observed, giving an SMR of 1.17 (95% CI, 0.73 to 1.48), but no cases of mesothelioma or cancer of the pleura or the peritoneum were recorded. A statistically significant, elevated colorectal cancer incidence was observed.

Fordyce et al[34] published an update to the original Selevan et al[38] study, including 37 additional years of follow-up of the Vermont talc cohort. The initial cohort included workers employed from 1940 to 1969, and mortality from 1940 up to 1975. The update[34] expanded the cohort with workers employed between 1930 and 1940 and between 1970 and 1983. In addition, the mortality analysis was extended from 1975 to 2012, adding 37 years of follow-up time. All-cause mortality in the updated cohort was 30% higher than the US population (SMR, 1.33; 95% CI, 1.20 to 1.48), and this was mainly explained by a significant increase in NMRD (SMR, 2.73; 95% CI, 2.10 to 3.48) and other NMRDs (SMR, 4.13; 95% CI, 2.88 to 5.75). No significantly increased mortality due to lung cancer was observed (see also Table 5), although the SMR was 1.44 due to a wide CI (95% CI, 0.98 to 2.03).

Wild[28,38] reviewed all available epidemiological cancer studies focusing on occupational talc exposure as a risk factor. Their analysis considered three different populations: (1) those in which no other occupational carcinogen was identified, (2) talc miners who were also exposed to other carcinogens such as quartz or radon, and (3) general industrial populations in which talc exposure was reported and associated with potential exposure to radon, crystalline silica, or asbestos. No excess lung cancer mortality was found for the populations of talc millers exposed to high levels of talc but without any other potential carcinogen (SMR, 0.92; 42 cases). However, mortality in the second group of talc miners exposed to quartz and/or radon showed an excess SMR (SMR, 1.20; random effect relative risk, 1.85; 40 cases). Six other studies (group 3) were identified, and all reported increased lung cancer mortality. However, talc exposure was confounded with other carcinogens, and only one study was able to adjust for them. In conclusion, Wild[28] found no increased lung cancer mortality among talc millers, despite their high exposure experience.

Chang et al[37] calculated the SMR of lung cancer based on a meta-analysis of lung cancer using searches on MEDLINE, EMBASE, CNKI, and Wanfang databases up to March 2017. They identified 13 observational cohort studies and calculated a meta-SMR of 1.45 (95% CI, 1.22 to 1.72; $P < 0.0001$) for lung cancer among the study subjects exposed to talc. The analysis of a subgroup with asbestos contamination showed no significant difference in lung cancer death between subjects exposed to talc with and without asbestos ($P = 0.8680$). This indirectly suggests that nonasbestiform talc might increase the risk of lung cancer.

Mandel et al[40] reviewed occupational epidemiological studies that were published with elongated mineral particles in gold, talc, and taconite mining. Their studies consistently found pneumoconiosis in each of these types of mining. However, lung cancer is inconsistently reported in these industries and is regarded therefore as an unlikely outcome of nonasbestiform elongated mineral particle exposure. Boffetta et al[41] also concluded in a review on elongated mineral particles[41] that there is no evidence that exposure to talc without contamination with asbestiform minerals increases the risk of lung cancer or mesothelioma (see also Table 3)

TABLE 5. Overview of Lung Cancer and Pleural Mesothelioma Mortality in the Most Recent Updates of Five Cohorts Highly Exposed to Talc

Study	Period/Location	Size	Lung Cancer *N* Cases; Relative Risk (CI)	MT
Fordyce et al[34]	1940–1975, Vermont (United States)	427	32; SMR, 1.44 (0.98–2.03)	0
Wild et al[36]	1945–1996, France	1070	21; SMR, 1.23 (0.76–1.89)	0
Wild[28]	1973–1995, Austria	542	7; SMR, 1.06(0.43–2.19)	0
Wergeland et al[35]	1953–2011, Norway	390	21; SMR, 1.7 (0.73–1.79)	0
Cioccan et al[31]	1946–2020, Italy	1749	85; SMR, 1.02 (0.82–1.27)	0
Total	70+ years	4178	166; SMR, 1.13 (0.97–1.31)	0

Updates taken from Boffetta et al.[37]

CI, confidence interval; MT, number of mesothelioma cases observed; *N*, number of deaths from lung cancer; SMR, standardized mortality ratio; CI: 95 % confidence interval, MT is number of mesothelioma cases observed.

Finally, some limited research has also looked at a possible link between inhaled talc exposure at work and other cancers, such as stomach[42] and colon cancer,[35] but there is no convincing evidence of such links at this time.

DISCUSSION

An important issue that has been raised and discussed in this article is how the current evidence on adverse responses in animals and humans exposed to talc will be considered in the classification of STOT-RE. Specific target organ toxicity arising from repeated exposure is assigned on the basis of findings of "significant" or "severe" toxicity. In this context, "significant" means changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. "Severe" effects are generally considered more profound or serious than "significant" effects and are of a considerably adverse nature, which may significantly impact on health. The Regulation (EC) No. 1272/2008 on the classification, labeling, and packaging of substances and mixtures (CLP Regulation)[‡] does not require actual testing of substances and mixtures for classification purposes, and the assessment herein is based on consideration of all available adequate and reliable information, primarily evidence relating to repeated-dose animal exposures and human epidemiological studies. Available data on animal studies and human epidemiology have been evaluated by weight of evidence using a number of quality criteria and expert judgment in this article.

A complicating factor in the design and interpretation of the animal studies is that of the concept for lung overload–induced lung cancer in rats with PSLT,[18] which has been developed and published after the time that most talc inhalation studies were conducted. This has a direct impact on the quality scoring (Table 3) of the relatively older animal investigations. As a result, most of the inhalation studies with talc powder do not meet current quality standards of testing, nor are we able to correct or evaluate their reported results to account for particle lung overload (Table 3). In addition, many older studies have used hamsters, which we now know are less sensitive than the rat to particle-induced effects.[23] In summary, of nine inhalation studies with sufficient quality, only one meets current requirements to confidently classify talc with the current STOT-RE criteria. The NTP 1993 study on talc[14] using lifetime inhalation of talc at 6 and 18 mg/m^3 resulted in a range of inflammatory, reparative, and proliferative lung responses. Tumor formation and reduced clearance were only evident at the high dose in female rats. In mice, there was no evidence of hyperplasia or interstitial fibrosis, although clearance was reduced at the higher dose. Although the dose selection has been criticized[17] and the observed renal pathology has been attributed to hypoxia occurring after concomitant fibrotic changes in the lungs,[43] this study is considered to be the benchmark for future regulatory purposes and could be used to interpolate 28-day and 90-day outcomes needed for STOT-RE classification. This also implies that no further inhalation study with talc is needed, because to establish a complete data set, one could use the 4-week range-finding study also reported in the NTP 1993 investigation for STOT-RE purposes and add results from outcomes from the first time point (6 months) of inhalation from the chronic study. Guidance values for STOT-RE classification are adverse effects up to 20 mg/m^3 in a 90-day study (STOT-RE 1) or adverse effects at 20 to 200 mg/m^3 in a 90-day study (STOT-RE 2).

The neoplastic effects of talc and two other PSLTs were discussed by the IARC Monograph Working Group that reevaluated the carcinogenic hazards of carbon black, titanium dioxide, and talc to humans at one monograph meeting in 2006.[5] The overall data from cancer studies in rodents exposed to carbon black and TiO_2 provided sufficient evidence of carcinogenicity in animals but inadequate evidence in humans, and both substances were evaluated as possibly carcinogenic to humans (group 2B). At the same time, the Working Group considered that epidemiological studies on talc miners and millers provided inadequate evidence of carcinogenicity of inhaled talc. The evidence from rodent cancer studies for talc was considered limited. The Working Group gave an overall evaluation of inhaled talc (not containing asbestos or asbestiform fibers) as not classifiable as to its carcinogenicity to humans (group 3). Since that time, TiO_2 has been classified by European Chemical Agency as a class 2 carcinogen,[§] and the process of evaluating carbon black is ongoing. The RAC Opinion on TiO_2[24] addresses some knowledge gaps with more recent studies or interpretations, and other gaps in mechanistic understanding are filled with information derived by reading from animal studies with carbon black. It is therefore reasonable to assume that any future evaluation of talc dust by European Chemical Agency will include mode of action arguments, such as those reported for PSLT for TiO_2 and carbon black. During the recent symposia, including the Edinburgh expert workshop on PSLT[21] and the London Particles & Health meeting 2021,[44] the regulatory use of this underlying mechanism as a common denominator for classification and testing was debated in more detail.

As already concluded in the most recent expert consensus report on PSLTs,[23] "prolonged exposure to inhaled particles at sufficiently high concentrations in experimental animals may lead to impairment of normal clearance mechanisms in the alveolar region of the lung, resulting in a continued build-up of particles that eventually leads to excessive lung burdens of particles accompanied by chronic alveolar inflammation. The inflammatory response may give rise to increased generation of reactive oxygen species, cell injury, cell proliferation, fibrosis, induction of mutations, and, ultimately, cancer. This latter endpoint of lung cancer is only seen in the rat and not in other rodent species. The focus of current regulatory and scientific debate is driven by the observation that many of these steps also occur in workers in dusty jobs, such as coal or talc miners and data on cancer in

[‡]https://echa.europa.eu/regulations/clp/harmonised-classification-and-labelling

[§]Category 2 carcinogens are suspected human carcinogens. This is based on evidence obtained from human and/or animal studies but is not sufficient for a category 1 classification.

animals obtained, even under conditions of impaired lung clearance, can be considered relevant to humans. On the other hand, there are several reasons to consider that these data are not relevant to humans." Among those reasons are that (a) rat overload lung tumors were concluded, in the same recent expert workshop report,[23] to be unique for the rat[31]; (b) human lung distribution of inhaled particles has a strong tendency for less inflammatory response and for a greater interstitial deposition than rats or mice[45]; and (c) inflammatory pathways between rat and human lungs are very different.[46]

For STOT-RE classification, we expect that the epidemiological data in combination with the available animal studies of sufficient quality will be the key evidence base. The human data clearly show an increased mortality due to NMRD (Table 4) but not to lung cancer in workers who have been continually exposed to high levels of talc dust over many years (see Table 5). This evidence in humans, exposed at similar levels as animals in the NTP study,[14] seems to indicate a STOT-RE 1 classification for respiratory talc powder might be appropriate. The final question is how the evidence on carcinogenicity of talc in the lung of rats versus humans may be interpreted. With regard to a potential risk of lung cancer in humans, the mode of action has been discussed earlier in this section, and it is clear that even the best available animal inhalation investigation in rats[14] has been compromised by the occurrence of lung overload[17,18] and, thus, interpretation of lung tumors observed needs to take into account the general lung overload effect seen with PSLTs in rats.

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