Harlan Project B52020

Beryllium

Expert Study Evaluation of the Epidemiological and Toxicological Scientific Literature on Carcinogenicity of Beryllium Metal & Beryllium-containing Alloys

Expert Statement

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CONTENTS

1 PURPOSE

The REACH Beryllium Consortium commissioned Harlan Laboratories Ltd., Switzerland, to prepare an analysis of the scientific literature on beryllium metal and beryllium-containing alloys relative to carcinogenicity. Studies dealing with animal testing were evaluated by toxicologists from Harlan Laboratories Ltd, and epidemiological aspects of the scientific literature were analysed by epidemiology experts of the REACH Beryllium Consortium. Additional testing of beryllium metal was also performed by Harlan Laboratories Ltd. These tests were performed according to OECD guidelines and under GLP. The outcome of the evaluation of existing scientific literature and new studies has been compiled into the present document.

This analysis is a fact-based analysis of the classification history. In addition, the scientific literature was screened for study data relevant for carcinogenicity and the data evaluated for its quality and its significance/adequacy regarding human health effects.

2 CLASSIFICATION HISTORY

Insoluble forms of beryllium include beryllium metal, beryllium aluminum composites (AlBeMet), beryllium oxide (BeO) and alloys containing beryllium such as copper beryllium (CuBe). These insoluble forms comprise nearly the entire commercial market for beryllium. As with many metals, the toxicity profile of soluble beryllium compounds differs from that of pure beryllium metal and differs also from that of beryllium-containing alloys due to the physicochemical differences.

However, in the European Community and later European Union, beryllium metal and beryllium compounds were classified and labelled as being toxicologically identical. An extensive search was undertaken to locate the documentation as to the basis used and the studies employed for determining the classification of beryllium metal and beryllium compounds. Except for the sparse information on the environmental aspects that was documented during the latest step in classification (2001), no other supporting documentation was found.

Classification and labeling (C&L) of beryllium was introduced in the European Community in 1967 (EC 1967). Beryllium and beryllium compounds were classified for the first time in the Council Directive 67/548/EEC. Changes of the classification and labeling of beryllium and beryllium compounds were made by the 19. ATP Commission Directive 93/72/EEC in Sept. 1993. Changes of the classification comprised Carcinogenicity Category 2; R49, “May cause cancer by inhalation” which is the current classification. Both of these classifications appear to accept the earlier classification since no separate documentation could be located that would indicate that a new evaluation was performed. Beryllium is also classified according to the Globally Harmonised System of Classification and Labelling of Chemicals (Regulation 1272/2008). The current GHS classification with regards to carcinogenicity is H350i (Category 1B: presumed to have carcinogenic potential for humans, classification is largely based on animal evidence) which is a translation of the R49 classification (Annex VII of Regulation 1272/2008).

Because no documented rationale could be found, several sources were evaluated in an attempt to determine the rational for the existing classification. The European Chemicals Bureau (ECB) and the German Federal Institute for Occupational Safety and Health (BAuA) were contacted by Harlan Laboratories Ltd. to determine whether such a rationale exists and if it is available. BAuA responded that their documentation is limited to protocols of sessions and that they do not have a documented rationale for the changes of the classification as carcinogenic (if any exist). Furthermore, the information was given that due to disagreement among the Member States with respect to the classification as carcinogenic the Specialized Experts (a Scientific Committee of the EU Commission) were charged to deal with this issue. The Committee unanimously voted for the category C2. This advice was finally adopted by the Member States. The response of ECB was similar to that of BAuA. The only documentation they have were summary records of the meetings of the C&L Working Group and the discussions between the delegations of the Member States.

The documentation delivered by ECB is also not complete since the final classification of beryllium metal and beryllium compounds deviates from the “provisional” classification

which was agreed and which was noted in the summary record from 13.8.1990 with respect to some of the risk phrases: The provisional classification of beryllium and beryllium compounds does not include the risk phrases R 25, R 26, R 36/37/38, R43 which are contained in the final C&L. It should be noted here that the phrases R25, R43, and R36/37/38 are appropriate for soluble beryllium compounds, but have not been investigated by appropriate animal studies and from the physico-chemical properties are unlikely to be appropriate for beryllium metal or beryllium-containing alloys. Appropriate animal studies were therefore performed and are summarized in 3.4.4 of this document. These studies do not support these classifications.

In summary, there is no documentation that clearly articulates the basis for the current classification of beryllium metal, beryllium-containing alloys and compounds or any of the studies utilized to support the cancer classification within the EU. Even though the toxicity of beryllium metal and beryllium-containing alloys differs from beryllium compounds, the international agencies and national institutions appear to not recognize these differences.

3 HUMAN HEALTH EFFECTS

The following section provides a basic overview of the health effects associated with beryllium and beryllium compounds. The different forms of "beryllium" are often not specified in the literature. The differentiation of beryllium metal, beryllium-containing alloys and beryllium compounds is important because of different physicochemical properties of the different forms, resulting in different toxicity profiles. Accordingly, these compounds should be evaluated separately and classified as such. Toxicologically relevant exposure to beryllium is almost exclusively confined to the work-place. The following health effects have predominantly been reported in workers exposed to beryllium:

- Acute beryllium disease - a form of severe respiratory disease associated primarily with exposure to soluble compounds;
- Dermatitis and skin sensitization have been associated with soluble beryllium compounds;
- Chronic beryllium disease – forms can include subclinical CBD (asymptomatic) and clinical CBD (symptomatic);
- Lung cancer

Dermatitis and skin sensitization have also been associated with exposure to soluble beryllium compounds.

3.1 Acute Beryllium Disease

Acute Beryllium Disease (ABD) is an acute toxic chemical pneumonitis resulting from high exposure to soluble beryllium compounds (beryllium salts such as beryllium fluoride and beryllium chloride) or low-fired beryllium oxide. Due to the current high standard of worker protection measures during processing of beryllium, ABD has very rarely been diagnosed during the last decades and low-fired beryllium oxide has not been commercially available since 1950 (Eisenbud et al, 1955).

The onset of symptoms of ABD was usually immediate, but could be delayed from several hours up to 3 days. Symptoms included dyspnea, fatigue, fever, night sweats and cough. Pulmonary function tests revealed obstructive lung disease with impaired gas exchange. Most of the cases of ABD usually resolved completely. However, some were fatal or were followed by development of chronic beryllium disease (Ordstrand et al, 1945). Cases of ABD have only been shown to occur when airborne concentrations of soluble beryllium salts or low fired beryllium oxide exceed 100 µg Be/m^3 (Eisenbud, 1982). Airborne exposures to beryllium metal, high-fired beryllium oxide or beryllium-containing alloy fumes or dust are not associated with acute or short-term respiratory reactions (Ridenour et al., 1991; Eisenbud, 1982, 1984[1]).

[1] Although Eisenbud named a potential of metallic beryllium to induce ABD in his first publication (1955), this hypothesis was not repeated in later publications. In the latest publication (1984), he claimed that diagnosis of ABD after beryllium metal exposure was questionable.

3.2 Beryllium sensitization

Beryllium sensitisation (BeS) is an immunologically mediated reaction to repeated Beryllium exposure (allergic reaction). Although the sensitisation itself is not a disease and is symptomless, it is a critical factor for development of CBD. Beryllium has been demonstrated to bind to proteins and form antigens (Vacher, 1972), thereby triggering the immune response responsible for the allergic reactions. A relatively weak beryllium-sensitisation was detected in humans by the beryllium skin patch test (BePT, Curtis 1951) for powdered beryllium metal (3 of 13 workers), however no sensitization was observed with a beryllium metal disk. This study demonstrated a clear difference to the soluble Be compounds as these elicited a much stronger reaction in almost all workers tested. The use of the BePT was curtailed because simultaneous experimental application of multiple tests sensitized members (positive patch test) of control populations and because it was suggested that the test itself might trigger BeS (Epstein, 1991) or exacerbate existing cCBD (Waksman, 1959).

3.3 Chronic beryllium disease

Chronic beryllium disease (CBD) is a systemic granulomatous disorder that requires a beryllium-specific immune response (Deubner DC et al., 2007). A latency period of several weeks to more than 20 years is characteristic for CBD. Granuloma formation can exist with no symptomology or physical impairment of health, this form of CBD being called subclinical CBD (sCBD). If symptoms are present, the form of CBD is termed clinical CBD (cCBD). The clinical course of cCBD is considered highly variable since the symptomatic disease may not develop or it may develop slowly over time. The earliest manifestations of clinical chronic beryllium disease (cCBD) are the symptoms of shortness of breath, dry cough, or wheeze, and in some, night sweats or fatigue. Chest radiographs can be normal, but often range from small nodular opacities, with an upper level predominance, to formation of conglomerate masses (Mueller-Quernheim, et al, 2005). Progression may lead to weight loss, cor-pulmonale with heart failure, disability and death. In addition to cCBD, these symptoms may be found in persons with other lung diseases and in persons with no diagnosable disease (Murray JF et al.).

Chronic Beryllium Disease was diagnosed, before the late 1980s, when clinical symptoms were observed along with changes in chest X-rays or lung function tests. In the late 1980s a change in the criterion for diagnosis of CBD was first suggested (Kreiss, H, et al., 1989), applying abnormal lymphocyte proliferation tests for beryllium sensitisation in blood or lung fluid (BeLPT, Beryllium lymphocyte proliferation test) and the presence of non-caseating granulomas in lung biopsy. The BeLPT has never been validated correctly, and there is evidence of bad performance (Cher 2006, Borak 2006, Donovan 2007), leaving doubts on the reliability of the test.

It is due to the change of diagnostic methods that before the late 1980s all reported cases of CBD were symptomatic, while afterwards due to improved diagnostics a big part of the described cases were asymptomatic. This is important when reviewing epidemiological data. The BeLPT needs careful validation. A positive BeLPT leads to severe psychosocial and socio-economic consequences, because workers are often advised to cease beryllium-exposure, with the consequence of giving up their jobs and subsequent loss of income.

Not all persons exposed to beryllium develop BeS, and even fewer develop CBD. Additional factors specific to individual persons (genetic polymorphisms, underlying infections, lifestyle factors?) seem to play a crucial role.

3.4 Lung cancer

Since about 1950, a series of *in vivo* animal studies on the carcinogenicity of beryllium and its compounds have been conducted. Most of these studies investigated the inhalation route because this route appears to be most relevant for humans in workplaces. Only in rats and sensitive mouse strains a clear carcinogenic response to beryllium metal-exposure could be demonstrated, while no or equivocal response was observed in wild-type mice.

According to published epidemiologic reports, there is no evidence or studies to suggest the beryllium containing alloys are carcinogenic to humans. A number of epidemiology studies using the same population have been conducted to assess the carcinogenic potential of beryllium compounds and its soluble salts. These studies were analyses and re-analyses of cohorts at the same beryllium producing plants in the United States. Increased incidences of lung cancer deaths were reported in retrospective cohort mortality studies of these workers. No clear correlation between the incidence of lung cancer deaths and levels or duration of exposure has been established because historical exposure levels were not reported. A positive association between length of latency and lung cancer deaths was reported with the highest cancer risks associated with a latency of more than 25 years. This was later proven to be inaccurate by Deubner and Levy. The evidence for excess lung cancer in beryllium workers is weak, both because it is derived primarily from short tenure workers, known to have relatively high SMRs for lung cancer. Also, because of the findings that the excess is fragile and disappears with reasonable variations of the study approach, evaluating the available literature on the potential carcinogenicity of exposure to beryllium along with the history behind some of that literature is important to a complete understanding as to whether findings, statements and conclusions provided in the cancer references are relevant when there is new knowledge that either supports, supersedes or invalidates earlier findings or interpretations. The studies by Levy (2002, 2007, 2009), Brown (2004) and a new study by Deubner (2007) provide new evidence that exposure to beryllium does not convey a significant risk of cancer to humans. In addition, both Levy 2007 and Deubner 2007 identified a significant methodological error in the Sanderson study which they suggest negates the use of this study as a dose/response cancer link for beryllium. The latest study, Schubauer-Berigan 2010, did not find a significant increase in risk of cancer following working with beryllium.

A manuscript prepared by the European Commission on beryllium in relation to occupational diseases (Information Notices on Occupational Diseases: A Guide to Diagnosis, 2009) states that “the causal relationship between prolonged or repeated exposure to beryllium and the occurrence of bronchial cancer has not been firmly established, and due to the multicausality of the occurrence of this type of cancer, the recognition of the occupational origin must lie on a thorough assessment based on rigorous scientific criteria taking into account all possible aetiologies. Each case must therefore be considered separately”.

The newer studies are rigorous and they deal with sizeable cohorts exposed to very high levels of beryllium. Failure to find convincing evidence that beryllium workers have excess rates, combined with clear evidence that in beryllium workers lung cancer is not related to degree of exposure, supports a reclassification of beryllium as non-carcinogenic in humans.

3.4.1 Literature Review Protocol

The scientific literature was screened for publications addressing potential genotoxicity and carcinogenicity of beryllium.

The reliability of the data is a key initial consideration in a weight-of-evidence analysis and provide an indication of which studies should be evaluated more closely. Without knowledge of how a study was conducted all other considerations and potential conclusions drawn from this study may be irrelevant. An approach used to assist the initial screening of study reports is that developed by Klimisch et al. (1997). Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs. This approach was developed as a scoring system for reliability as follows:

> 1 = reliable without restrictions: "studies or data generated according to generally valid and/or internationally accepted testing guidelines (preferably performed under Good Laboratory Practices (GLP)) or in which the test parameters are based on a specific (national) testing guideline, or in which all parameters described are closely related/comparable to a guideline method."
>
> 2 = reliable with restrictions: "studies or data (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."
>
> 3 = not reliable: "studies or data in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment."
>
> 4 = not assignable: "studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.)."

Nevertheless, in case no high-quality studies to address endpoints required for risk assessment were present, all available material was evaluated. A search strategy of relevant data bases (Appendix 1) was developed for the literature search and the results for "beryllium" produced 1531 hits after non-relevant articles were discarded. The literature hits were manually screened by toxicology experts for relevance in regard to genotoxicity and carcinogenicity of beryllium metal, alloys and compounds. If the information provided in title and abstract was not sufficient to judge the relevance of the study, full articles were ordered and evaluated. By expert judgment, 38 publications containing experimental study data for carcinogenesis and 21 publications containing

experimental study data for genotoxicity of beryllium and/or its compounds were identified.

Additional new studies on beryllium metal were performed by Harlan Laboratories Ltd. These studies were all performed according to OECD guidelines and under GLP conditions. These studies are therefore summarized separately in Section 3.4.4.

3.4.2 Summary of Literature Review: Animal and *in vitro* Studies

The complete listing of the studies assessed, including a short description of the evaluated parameters and their Klimisch rating, are presented in Appendix 2.

It must be stated that most, if not all of the animal and *in vitro* studies, do not comply with modern requirements of study conditions, e.g. described in OECD Guidelines. None of these studies were conducted according to GLP, in particular many of the older studies do not have an appropriate control group or the study conditions and/or results are not adequately reported. Thus, the reliability of many studies was rated according to Klimisch et al. (1997) as not reliable (Rating 3) or not assignable (Rating 4). The great majority (84%) of the studies were performed using soluble beryllium compounds with a few performed using beryllium metal or alloys.

Some studies have been heavily criticized by associated staff, claiming that conduct and reporting of studies was inadequate. Study results were partly published by authors other than the experimentator without correspondence about potential weaknesses of the data; partly only by oral communication/in abstract or tabular form without any description of the used methods, and technicians conducting the studies were not adequately trained (Reeves, 1987). Furthermore, results of an inhalative monkey study conducted by Vorwald (Vorwald, 1968) have been reported in a misleading way. The intention was not to prove carcinogenicity of beryllium, but to demonstrate that the monkey in general was a model for carcinogenicity. Animals were not only exposed to beryllium, but to cigarette smoke and other “exposures that were expected to be co-carcinogenic” as well. Thus, the study cannot be taken into account for risk assessment (protocol of the public hearing on 31st of August, 1977 at the US OSHA).

The intention of this document is to clearly distinguish the discussion of beryllium metal and alloys from the discussion of soluble beryllium compounds. Due to the different physico-chemical behavior of the different forms of beryllium (e.g. solubility under physiologic conditions, with great impact on bioavailability), this separation seems appropriate. Furthermore, this document should give a statement on the quality of the study data being the basis of classification of beryllium.

In vitro studies

The predictiveness of *in vitro* studies has been shown and validated for genotoxicity. Among the 21 identified studies, there were no reliable *in vitro* studies on beryllium metal or beryllium-containing alloys. A complete listing of these studies can be found in Appendix 2. Most of the studies were conducted with soluble compounds, while testing of extracts from the metal would be appropriate to test these insoluble compounds.

Additional *in vitro* studies on Be metal were therefore performed by Harlan Laboratories Ltd. These studies are summarized separately in Section 3.4.4. As a pre-test, a study on cytotoxicity of beryllium metal powder extract (10% test item in saline for 24 hours) has been performed in L929-cells. No signs of cytotoxicity were observed when the pure extract was applied to the cells, indicating either no relevant ion formation (= no bioavailability) or non-toxicity.

In vivo Animal Studies

Since about 1950, a series of studies on the carcinogenicity of beryllium and its compounds have been conducted. Most of these studies investigated the inhalation route because this route appears to be the most relevant for humans in workplaces.

Soluble beryllium compounds
A large number of studies addressing carcinogenicity have been performed with soluble beryllium compounds. The complete listing of the studies assessed and their Klimisch rating are presented in Appendix 2.

Beryllium metal
There are only twelve (12) animal studies available addressing carcinogenicity of inhaled beryllium metal or alloy (see table below) [2]. These studies are summarized in Appendix 3. Five studies suggest carcinogenicity of inhaled beryllium metal in the rat, three studies suggest carcinogenicity in sensitive mice (A/J or p53-knockout), four studies suggest non-carcinogenicity in wild-type mice and one study suggests non-carcinogenicity in guinea pigs.

Table 1 Summary of *in vivo* studies assessing the carcinogenicity of beryllium metal

Test Animal , Route of Exposure	**Carcinogenic response identified**	**Data Quality** [Klimisch 1-4]	**Reference**
Rat inhalation	rats: yes	2 (Reporting of the study is fragmented into several publications. Although the level of detail in the individual publications is clearly not sufficient for an overall judgement on data quality, the combination of information from all publications is considered to give sufficient proof that the study was adequately conducted	Finch et al., 1994, 1995 and, 1996 ; Belinsky et al., 1994 ;Nickel-Brady et al., 1994; Nikula et al., 1995 ; Belinsky et al., 1997
Rat intratracheal instillation	yes	4 (study with high mortality and relatively low animal numbers	Groth et al., 1980

[2] One additional study by Hueper et al. (1954) addressed carcinogenicity of beryllium metal, but the route of delivery (intrapleural and intrafemural bolus application) is not considered physiologically relevant

		per time point)	
Rat intratracheal instillation	yes	4 (too little experimental details given to evaluate the study)	Litvinov et al., 1983
Mouse inhalation	sensitive mouse strains: weakly, wild-type mice: no	2 (well-documented, guideline-comparable study)	Finch et al., 1995, 1996 and 1998b
Guinea pig intratracheal instillation	no	4 (no details on experiment)	Schepers et al., 1961

The studies by Groth and Schepers are not inhalation studies. Beryllium metal and alloy were administered by intratracheal administration. By this method, a small sample of the test substance is once or repeatedly instilled as a suspension or solution into the lung. This route has two disadvantages: the upper respiratory tract is not exposed, and the bolus application results in unavoidably high local concentrations of beryllium metal or alloy in the lower lobes of the lung (Vorwald, 1959). Accordingly, studies of this types are not considered being suitable for classification or risk assessment issues.

Other studies with beryllium metal and alloys address acute and chronic toxicity as well as histopathological changes in mice and monkeys, but do not allow direct conclusions on carcinogenicity (Curtis 1951, Ferraris 1952, Haley et al., 1990/92/94; Finch et al., 1991/93/94/98; Zissu et al., 1996; Nikula et al., 1997; Benson et al., 2000).

An association between beryllium metal exposure and lung tumors was only observed in rats and sensitive mouse-strains, but could not be demonstrated in wild-type mice (Nikula, 1995; Finch et al., 1995; Finch et al. 1996) and guinea pigs (Schepers, 1961)[3].
This observation was also documented by Finch and Hoover (History of the LRRI Beryllium Research Program, August 1999) in a document "Overview and Publications of the Lovelace Respiratory Research Institute Beryllium Respiratory Research Program." The investigator staff at the Lovelace Institute was comprised of Belinsky, Benson, Finch, Hoover, and Nikula whose studies were evaluated in this analysis. The authors stated:

"Published reports by other workers on the issue of beryllium carcinogenicity are contradictory, plagued with experimental design problems and inconclusive for predicting carcinogenicity of beryllium in humans. An additional factor has been the controversy surrounding the cancer epidemiology studies in beryllium workers. Our results demonstrated that inhaled beryllium metal is a potent lung carcinogen in rats. We subsequently extended these studies to mice to further our understanding of beryllium-induced carcinogenic process, and relevance to humans. Results indicate beryllium metal at similar lung burdens is not a pulmonary carcinogen to C3H mice and is weak in A/J mice."

The disconnect between carcinogenicity in the rat (the robust response at relatively low lung burdens) versus the missing response in wild-type mice maybe explained with the long-term chronic-active inflammation in rat lung, as stated by Dr. Gregory Finch (who

[3] It should be noted that study period was limited to 3 months

was part of the team investigating inhalation toxicity of beryllium in the biggest test battery ever performed on this issue):

"[…] an obvious fundamental question is how relevant are animals to humans. For beryllium, given the body of evidence that Be is not genotoxic, the mechanism of carcinogenesis is not clearly known. Thus, […], the presumption would be that the mechanism is also relevant to humans. However, where Be has always "hung up" for me is […] the disconnect between carcinogenicity in the rat [the robust response at relatively low lung burdens] versus the weak- to non-existing response in mice [A/J, C3H, and the p53 +/- TGs we did]. For the rat, we always had the view that the long-term chronic-active inflammation in rat lung [with relatively strong neutrophilic "foreign body-type" response] was sufficient to lead to neoplasms, whereas for the mouse, the inflammation had more of a lymphocytic ["immune"] versus neutrophilic response, and they didn't have much of a carci signal." [4]

On the basis of these experimental results, the species-specific specialities of the rat should receive a closer look before risk extrapolation to humans is made. An age-dependent increase of chronic active inflammation in rats has been noted by the US National Toxicology Program (NTP, 1996). A review of 24 chronic inhalation studies conducted by NTP over 10 years revealed that mean incidence of chronic active inflammation in unexposed control mice was low (1.4%), the incidence was about factor 7 higher in rats (10.2%) (Sivulka, 2006).

If there is a predisposition of rats to develop inflammation while they age, this may have severe impact on carcinogenesis if particulate matter –like insoluble metal-compounds- is present in the lung. This can be supported by an analysis of inhalation studies conducted at NTP (reported effects were limited to those considered of greatest biological significance (i.e., any form of inflammation, fibrosis, proteinosis):

Table 2 Summary of inflammation incidences observed in NTP studies

Compound	Effect	LOAEC [mg compound/m^3]		Incidence (%)		Severity[5]	
		Mice	Rats	Mice	Rats	Mice	Rats
MoO_3	Chronic Inflammation	>100[6]	30	N/A	50-86	N/A	1.5-1.7
$CoSO_4$	Granulomatous inflammation, fibrosis, and proteinosis	> 3[7]	0.3	N/A	30-100	N/A	1.2-2.0
$NiSO_4$	Chronic active inflammation (rats and mice), fibrosis (rats only), and	0.25	0.25	12[7]	23-92	1.3[8]	1.4-2.1

[4] Note to Marc Kolanz, 12th of Dec 2002
[5] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[6] Mice showed no evidence of inflammation at any doses tested. The highest dose tested was 100 mg MoO_3/m^3. Any LOAEC would presumably have been higher then this dose.
[7] Mice showed no evidence of inflammation, fibrosis, or proteinosis at any doses tested. The highest dose tested was 3 mg $CoSO_4/m^3$. Any LOAEC for these effects would presumably have been higher than this dose
[8] Chronic active inflammation seen in female mice, only.

	proteinosis (rats only)						
NiO	Chronic inflammation (rats and mice).	1.25	0.6	31-65	98-100	1.4[9]	1.6-1.7
Ni_3S_2	Chronic active inflammation, fibrosis, and proteinosis	0.6	0.15	12-97	68-100	1.0-2.9	1.7-2.5

Rats appear to be considerably more sensitive to the non-neoplastic respiratory effects of these compounds than mice. The solubility of the metal compound seemed to make no difference in the effects seen in rats versus mice. Respiratory toxicity effects tended to manifest themselves at lower doses, at higher incidences, and greater severity in rats than in mice.

The reason for the difference between rat and mouse/guinea pig can only be speculated. However, there is increased evidence that rats are particularly sensitive to inhalation effects of poorly soluble particles due to reduced lung clearance rates relative to other species. This is related to both the airway geometry of the rat lung and the reduced macrophage capacity. The impaired alveolar macrophage function leads to accumulation of particles and focal accumulation of particle-laden alveolar macrophages. In rats, these events may lead to inflammation and cell proliferation, with the final consequence of lung tumors. There is an ongoing scientific debate regarding how to interpret data, and the relevance to human exposure, from rat inhalation studies of poorly soluble particles (e.g. ILSI Risk Science Institute Workshop, 2000). There is scientific support that neoplastic effects seen in rat studies of non-genotoxic poorly soluble substances under overload conditions may not be relevant for the human risk assessment.

An overall evaluation of the carcinogenicity studies with beryllium-compounds (including studies with soluble beryllium compounds) suggests that the induction of pulmonary cancer by beryllium metal and beryllium compounds maybe species-specific. A listing of the inhalation studies available is given in Appendix 3.

While rats were susceptible, no pulmonary tumours were observed in wild-type mice (Finch et al., 1996), guinea-pigs (Reeves, 1987) and hamsters (Wagner 1969). Only one case of a pulmonary tumor was described in rabbit (Dutra et al., 1951) upon inhalation of beryllium oxide[10]. A number of studies with intravenous, intraossal, intrathoracical or intraocular administration of beryllium compounds have been conducted in rabbits. As those routes of administration are not reflecting the exposure situation but an artificial model, they were fully evaluated, but not regarded as relevant (listed in appendix 2).

Although several studies with monkeys were performed (Schepers 1991/64, Vorwald 1968[11], Conradi 1971, Wagner 1969), none can be considered to reliably cover the

[9] Both sexes.

[10] It should be noted that only 19 animals, divided in 3 dose groups, were exposed under highly variable experimental conditions to beryllium oxide, and that the animal bearing the tumor was from the medium dose group. No tumors were observed in the high dose group.

[11] The intention was not to prove carcinogenicity of beryllium, but to demonstrate that the monkey in general was a model for carcinogenicity. Animals were not only exposed to beryllium, but to cigarette smoke and

endpoint carcinogenesis due to either very low animal numbers or low experimental quality/ insufficient reporting.

Oral studies

Oral exposure to beryllium has not been found to cause cancer in animals. No studies with beryllium metal are available, but even under the worst-case of dietary exposure of dogs, rats and mice to beryllium sulfate (a soluble beryllium compound), no significant increases in the number of lung reticulum cell carcinomas/tumors/neoplasms were observed (Morgareidge et al., 1975, 1976; Schroeder and Mitchener 1975a, 1975b).

other “exposures that were expected to be co-carcinogenic” as well. Thus, the study cannot be taken into account for risk assessment (protocol of the public hearing on 31st of August, 1977 at the US OSHA).

3.4.3 Summary of Literature Review: Epidemiological Studies and Data

The epidemiological studies on the association of occupational beryllium exposure and cancer can be divided into different phases and issues due to different size or type of study populations, quality of exposure assessment and other factors that determine the validity and limitations of epidemiological studies.

- Early cohort studies on workers employed at two major beryllium processing plants in the USA;
- Cohort studies using data of the Beryllium Disease Registry;
- More recent studies using improved assessments of confounding, type of employment and exposure.

Table 3 Summary of human epidemiology studies on beryllium*

Type of study	Relationship observed	Data Quality [Klimisch 1-4]	Reference
Retrospective cohort mortality study	Authors state that altered risk of cancer can not be evaluated by the study	4 (not corrected for confounding, no detailed statistical analysis)	Mancuso et al, 1969
Extension of Mancuso et al (1969)	Risk of lung cancer was highest among workers employed 1-2 quarters (3-6 months). There was also a higher risk of lung cancer among workers with prior occupational respiratory illness	4 (not corrected for confounding, no detailed statistical analysis)	Mancuso et al, 1970
Extension of Mancuso et al (1969)	Elevated risk of lung cancer for beryllium workers relative to viscose rayon employees	4 (not corrected for confounding)	Mancuso et al, 1980
Retrospective cohort mortality study	Excess risk of lung cancer in workers with 15 or more years since onset of beryllium exposure	4 (study population compared to general population, correction for smoking questionable)	Wagoner et al 1980
Retrospective cohort mortality study	Excess risk of lung cancer in beryllium workers with 15 years of onset since exposure	4 (not corrected for confounding)	Infante et al 1980
Cohort mortality study (extension of Infante et al (1980))	Excess risk of lung cancer in beryllium workers	4 (smoking data was only available for part of the study population, correction is unclear)	Steenland et al 1991
Retrospective cohort mortality study	Excess risk of lung cancer identified for beryllium workers	3 (exposed individuals mainly living in cities were compared to rural population)	Ward et al., 1992
Re-analysis of the study by Ward et al (1992)	No association between occupational exposure to beryllium and lung cancer	2 (comparing the study population to adequate (city) controls)	Levy et al., 2002
Re-analysis of the	No association between time worked	2 (well-documented re-	Levy et al., 2009

study by Ward et al., 1992	and age at hire with lung cancer	analysis using Cox proportional hazards analysis)	
Mortality and autopsy study of subjects in the UK beryllium registry	No tumours identified	2 (Short but well reported study of workers with proven beryllium exposure)	Williams, 1996
Nested case-control study (to Ward et al., 1992)	Excess risk of lung cancer in beryllium workers when 10- or 20-years lag was included	2 (well-documented study; restriction of the database questionable)	Sanderson et al., 2001b
Re-analysis of the study by Sanderson et al., (2001b)	No association between occupational exposure to beryllium and lung cancer	2 (well-documented reanalysis, valid transformation, same criticism as for Sanderson et al regarding restriction of database)	Levy et al., 2007
Re-analysis of the study by Sanderson et al., (2001b)	Increased risk of lung cancer following average exposure (10 year lag) to beryllium, no increased risk following cumulative exposure to beryllium	2 (well-documented reanalysis including adjustments for birth year and age at hire)	Schubauer-Berigan, 2010
Comment on the current knowledge on occupational exposure to beryllium and risk of lung cancer	Beryllium data was not analysed in this study	4 (brief comment with no presentation of new data or analysis)	Deubner et al., 2009

*Studies in light-grey font colour are older work with methodological shortcomings. Bold-print studies are original work. All studies are summarized in Appendix 4.

The early epidemiological studies were not found reliable (indicated in grey color above) due to shortcomings in exposure reporting and potential confounding factors were not adequately addressed. They were recently reviewed in detail (Hollins et al., 2009).

Repeated cohort mortality studies of US beryllium workers suggested the rate of lung cancer compared to community rates might be modestly increased, *e.g.* standard mortality ratio 1.26, $p > 0.01$, but found no other cancer excess (Ward, 1992). The modestly higher lung cancer rate in beryllium workers appears to be fragile. Using the same data as Ward, the standard mortality ratio has been estimated as low as non-significant 1.01, $p > 0.05$ when variations in the community groups to which workers are compared, methods for smoking rate correction, and different methods for summing results across the beryllium production facilities are considered, (Levy & Roth, 2002).

A subsequent study, based on the same data as Ward et al., 1992, estimated exposure and analyzed exposure-response directly. This study found that beryllium workers with lung cancer did not have higher average, maximum or cumulative exposures to beryllium (Sanderson et al., 2001). When exposure was adjusted for latency, the assumed time from lung cancer induction to death, workers with lung cancer appeared to have had higher beryllium exposure. However, it has been demonstrated that this result is an artifact of the statistical method. When the artifact was corrected, workers who developed lung cancer did not have higher beryllium exposure than other workers

whether or not exposure was adjusted for latency (Levy et al., 2007). This artifact was verified empirically by a simulation study (Deubner et al., 2007). The two latest studies on beryllium occupational exposure and risk of cancer (Levy et al., 2009 and Schubauer-Berigan et al., 2008) were also based on the original dataset used in Ward et al. 1992. The Schubauer-Berigan study used date of birth and age at hire as covariates and did not find that time worked with beryllium nor cumulative beryllium exposure was associated with lung cancer.

The main observation regarding these studies is that the outcome is largely dependent on the statistical approach, including the use of confounders and latency period. As evidenced by the number of publications discussing the occupational exposure to beryllium and risk of lung cancer over the last 10+ years, there is an ongoing scientific debate regarding the appropriateness and suitability of the different approaches. It should be emphasized that the majority of these studies are nevertheless based on the same dataset originally presented by Ward et al. 1992. Accordingly, the reliability of these studies depends on the quality of this dataset.

It was suggested over two decades ago that there was not a beryllium exposure – lung cancer response relationship in beryllium workers. To paraphrase Sir Richard Doll (Doll, 1985), "If beryllium workers were indeed exposed to lung cancer risk by their work with beryllium, it was not manifest in the trend of lung cancer with duration of employment." In the US beryllium worker populations studied, most of the lung cancer observed is in workers who stayed in the beryllium workplace less than one year, with no increase in rate in persons who stayed longer (Ward et al., 1992).

IARC (1993) hypothesized an exposure-response relationship by comparing the magnitude of standardized mortality ratios (SMRs) for lung cancer in different worker groups assumed to have greater or lesser exposure to beryllium. This approach to examining exposure response was deemed to be problematic due to: 1) intrinsic lack of comparability of SMRs, which are indirectly standardized, 2) *ad hoc* method of selection of groups to be compared, 3) lack of exposure data on the groups compared, 4) lack of tests of statistical significance of the difference between the SMRs, 5) lack of control of confounding and 6) the interpretation of the consistent results from redundant observations (repeated studies on the same worker populations) as validating the studies.

It is apparent that the issue of the short-term workers being the most susceptible and the fact that Sanderson did not match on employment years has raised questions and skepticism regarding the carcinogenicity of beryllium.

3.4.4 Summary of new studies performed on Beryllium metal

Additional studies have been performed by Harlan Laboratories Ltd. on beryllium metal. These studies were all according to guideline and performed under GLP.

1) Acute oral toxicity (OECD 423, GLP)
Beryllium powder was suspended in polyethylene glycol 300 (PEG 300) and administered to total of 6 female rats. The animals were then observed for the next 14 days for viability, clinical signs, and bodyweight development. At the conclusion of the study, the animals were also subjected to a macroscopic necropsy. No mortality or clinical signs indicative of toxicity of toxicity were observed. Bodyweight development was also normal. The only observation noted was feces stained grey on first day of observation. This was due to staining by beryllium powder. It is concluded that the LD_{50} following oral administration of beryllium powder was above 2000 mg/kg bw. Accordingly, no classification for acute oral toxicity is considered required for beryllium metal.

2) Skin irritation (OECD 404, GLP)
Beryllium powder (0.5 g) was moistened with water (0.5 ml) and applied to the skin of three New Zealand white rabbits for 4 hours. The test item was then removed by flushing with lukewarm water. The skin reaction was assessed 1, 24, 48, and 72 hours after exposure. There were no indications of skin irritation at any of the timepoints. Accordingly, no classification for skin irritation is considered required for beryllium metal.

3) Eye irritation (OECD 405, GLP)
Beryllium powder (0.1 g) was instilled into the conjunctival sac of the left eye of three New Zealand white rabbits. The right eye was left untreated and served as control. The eye reaction was evaluated after 1, 24, 48, and 72 hours, and 7 days after exposure. There were no reactions in the cornea or iris. There was however a mild and transient redness of the conjunctiva (mean 24-72 hour value of 1 on the Draize scale). This redness was completely gone after 7 days. No classification for eye irritation is considered required as the reactions seen did not fulfill the criteria of EU Directive 2001/59/EC and EU Regulation 1272/2008.

4) Skin sensitization (OECD 406, GLP)
Beryllium powder was suspended in PEG 300 to a 15% concentration and injected intradermally together with Freund's adjuvant in the dorsal skin of the scapular region. One week later the same area was clipped free of hair and a 50% solution (0.3 ml) of beryllium powder in PEG 300 was applied. The test item was held in contact with the skin for 48 hours. Control animals were treated the same way but with vehicle only. The animals were challenged two weeks by applying 0.2 ml of a 10% solution of beryllium powder to the left flank while the vehicle only was applied to the right flank. The test substance was held in contact with the skin for the next 24 hours. The skin reaction was then assessed 24 and 48 hours after removal of the substance. No skin reactions were observed in the control group or in the treated group. Accordingly, no classification for skin sensitization is considered required for beryllium metal.

5) Bacterial reverse mutation test (OECD 471, GLP)
Beryllium metal was extracted by shaking with physiological saline for 3 days at 37°C. The following concentrations were achieved in two separate experiments: 733.7 µg/ml

and 124.0 µg/ml. Dilutions of 2.5, 5, 10, 20, 40, 60, 80 and 100% of the beryllium solutions were used in the main experiment. The main experiment was performed in the following strains: TA 1535, TA 1537, TA 98, and TA 100. In the plate incorporation assay the bacterial strains were incubated with the test item extract, metabolic activation system (rat liver S9 mix) or not, and overlay agar. The mixture was poured on selective agar plates, allowed to solidify and incubated for at least 48 h in the dark. The preincubation assay was performed in a similar manner however with a 60 minute preincubation period before plating. The performance of the system was verified by using known mutagens (with and without metabolic activation). No cytotoxicity was observed at any of the concentrations tested. There was no increase in revertant colonies following exposure to beryllium metal extracts with and without metabolic activation.

6) Mammalian chromosome aberration (OECD 473, GLP)
As beryllium metal is relatively insoluble, it was extracted at 37°C for 3 days at a loading rate of 100 mg/l in the cell culture medium used in the assay under non-abrasive shaking. Extract concentration of 3.258/20.27 µg Be/ml culture medium with FCS and 20.83/4.434 µg Be/ml culture medium without FCS were analytically found in the extracts prepared for the first/second experiment. The extracts were used to expose the cells at 100% (pure extract), 75% and 50%. Human whole blood cultures were set up. After 72 h, the cells were incubated with the test item extracts for 4 or 22 hours in presence or absence of a metabolic activation system (rat liver S9 mix), respectively (cell culture medium of cultures with 4 hours exposure was replaced by fresh test item-free culture medium after that period). 19 hours after start of exposure, colcemid was added to the cultures to arrest cells in metaphase. Three hours later, cells were washed, fixed, metaphases stained and microscopically evaluated. All experimental conditions were tested in two independent cell cultures. The experiment was repeated for confirmation. Statistically significant increase in the number of structural chromosome aberrations was observed only at one incidence (22 h of 75% extract). As this increase was not dose-dependent (no increase at 100% extract), within the historical control data of the laboratory and was not reproducible in the repeat experiment, it was considered incidental. Beryllium metal powder, extracted at worst-case conditions in culture medium for cell exposure, was not clastogenic in human lymphocytes.

7) Mammalian mutation (OECD 476, GLP)
Due to insolubility of the test item in cell culture media, the test item was extracted at 37°C for 3 days at a loading rate of 100 mg/l in the culture medium used in the assay under non-abrasive shaking. The extracts were used to expose the cells. The following concentrations were achieved: 3.12 µg Be/ml culture medium were analytically found in the extract prepared for the first experiment; 4.477 µg Be/ml culture medium were analytically found in the extract prepared for the second experiment without FCS, and 14.02 µg Be/ml in the extract prepared with FCS. The assay was performed in two independent experiments. V79 cells were exposed to the test item extracts for 4 h with and without metabolic activation (rat liver S9 mix), or for 24 h in absence of metabolic activation. The highest concentration of the test item extract was 100%, and the lowest concentration investigated was 12.5%. Three days after treatment, cells were subcultivated. After an expression time of 7 days, cells were again subcultivated and 6-thioguanine was added to the culture medium to kill all cells that were not mutated in the HPRT-locus. Surviving cells were allowed to form colonies within 8 days. Colonies were stained, their size evaluated, and counted. Cell survival was determined after treatment (parallel cultures) and after subcultivation. The performance of the test system was

proven by parallel experiments with know mutagens. Beryllium metal powder extracts did not induce mutations in the HPRT locus of V79 cells.

8) Unscheduled DNA synthesis
Due to insolubility of the test item in cell culture media, the test item was extracted at 37°C for 3 days at a loading rate of 100 mg/l in the culture medium used in the assay under non-abrasive shaking. The extracts were used to expose the cells.
The UDS study contains the results from many different experiments. Freshly isolated rat hepatocytes were exposed to 0, 50% or 100% of beryllium metal extract with 0, 0.67, 1.19, 1.71, or 2.23 µg/ml 2-AAF for 18 hours in the presence of 3HTdR (methyl-3H-thymidine). The uptake of radioactivity was determined by autoradiography. Vehicle control groups and cultures treated only with the beryllium metal powder extract were tested in parallel. For each concentration, including the controls, 100 cells were evaluated. In the DNA repair assay the mutagen (2-AAF) alone was always able to induce relevant DNA repair synthesis (distinct increase in the number of nuclear and net grain counts as well as % cells in repair). No increase in DNA repair was observed for cells treated with the test item extract alone. When cells were treated in parallel with the mutagen 2-AAF a distinct decrease of the of the mean net grain counts and of the amount of cells in repair was found after treatment with 100 % of the test item extract. However, a clear dose dependence was not observed. It is concluded that beryllium metal extract did not cause DNA damage in this study but at the highest dose exerted an effect on the repair of pre-existing DNA damage.

9) Cell transformation assay (OECD Draft Proposal: *In vitro* Syrian hamster embryo (SHE) cell transformation assay, GLP)
As beryllium is relatively insoluble in cell culture media, the test item was extracted at 37°C for 3 days at a loading rate of 100 mg/l in the culture medium used in the assay under non-abrasive shaking. The extracts were used to expose the cells. A concentration of 22.95 µg Be/ml culture medium was analytically found in the extract prepared for the experiment. Female and male hamsters were mated, and the pregnant females sacrificed in day 13 of gestation. Embryos were isolated from the uteri, decapitated, eviscerated and delimbed and the remaining parts minced in washing solution. Cells were obtained by stirring the pieces in trypsin/pancreatin solution for 10 min., and recovery of the isolated cells from the supernatant by centrifugation. Cells were plated, dead cells removed after 3-4 hours by washing, and cells were allowed to expand for 20 hours. Thereafter, cells were trypsinized and stored frozen in liquid nitrogen until use. For the experiments, a first layer of cells was seeded on culture dishes and lethally irradiated (feeder cells). The next day, the target cells were seeded on top of the feeder cells. The cells were incubated with the test item extracts (25-100%) for 7 days continuously to allow colony formation. Thereafter, cells were fixed with methanol, stained with Giemsa and microscopically evaluated for colony morphology. No metabolic activation system was used, as the primary SHE cells have enough metabolic capacity. Performance of the test system was proven by parallel experiments with benzo[a]pyrene. No cytotoxicity was observed at any of the concentrations tested. Beryllium metal powder extracts induced a criss-cross growth of cells within the colonies, which is different compared to the normal waive-like growth of SHE cell colonies.

Summary of new studies performed with beryllium metal
Although study 1-4 is not relevant for the assessment of carcinogenic potential they have been included in this summary for the purpose of completeness. They clearly demonstrate that beryllium metal is not acutely toxic and does not cause eye and skin irritation, or skin sensitization. The remaining studies also clearly demonstrate that beryllium metal does not induce DNA damage (mutagenic or larger structural re-arrangements). Extract of beryllium metal did also not induce unscheduled DNA synthesis, indicative of DNA damage, in the UDS study. The same experiment also investigated the effects of co-incubating with beryllium and the positive control, 2-acetylaminofluorene. It was observed that beryllium had an effect on DNA repair synthesis (suppressed) at the highest concentration of 2-aminofluorene (96% of cells in repair synthesis). The cell transformation assay indicated that beryllium metal extracts can induce changes in colony morphology of Syrian hamster embryo cells. However, the mechanism behind this effect, and the relevance to human exposure, can not be determined. As the genotoxicity tests were all negative, it is excluded that the morphological change observed is due to mutation or chromosome structural changes.

4 SUMMARY AND CONCLUSIONS

According to Annex VI of Directive 67/548/EEC, No. 4.2.1, the comments regarding the categorisation of carcinogenic substances read as follows:

"The placing of a substance into category 1 is done on the basis of epidemiological data; placing into categories 2 and 3 is based primarily on animal experiments. For classification as a category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species, together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association."

For a distinction between categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in category 3, even though tumours have been induced in animals: carcinogenic effects only at very high dose levels exceeding the maximal tolerated dose. The maximal tolerated dose is characterised by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10 % retardation in weight gain, appearance of tumours, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumour formation, appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds), if the particular target is not relevant to man, lack of genotoxicity in short-term tests in-vivo and in-vitro, existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation), existence of a species-specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man. For a distinction between category 3 and no classification arguments are relevant which exclude a concern for man: a

substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man, if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories, particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence."

According to Regulation 1272/2008/EEC, the comments regarding the categorisation of carcinogenic substances read as follows:

Category 1: Known or presumed human carcinogens
A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

-human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or

-animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

Category 2: Suspected human carcinogens
The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

As presented previously, most of the animal studies are not reliable in terms of modern study design. Carcinogenicity has been observed in rats and the reliable studies on beryllium metal and beryllium-containing alloys in mice suggest that only the sensitive strains developed tumours.

The most recent epidemiological studies in man suggest that beryllium is not carcinogenic to man. These studies suggest that the issue of study design is so complex that its behavior is generally not correctly predicted *a priori* by highly qualified persons and such issues are generally only grasped by experts in the field of study design and analysis of methodological flaws.

Nevertheless, using the criteria in Annex VI of Directive 67/548/EEC would suggest that a reclassification of beryllium metal from a Category 2 to Category 3 may be appropriate based on the missing clear carcinogenicity response in a second animal species (only rat and sensitive mouse strains, no clear carcinogenicity in wild-type mice, guinea pigs and monkeys) and the convincing new evidence relative to human epidemiology. The same arguments can be made for classification according to Regulation 1272/2008 and an H351 (Category 2) classification is proposed.

Aspects of the epidemiology studies which limit their relevance:

- The relatively low excess risk for lung cancer in most of the worker populations;
- The deficiencies with regard to measurements of exposures to beryllium, in particular in the 1940s and 1950 years;
- Deficiencies to control for the contributions of confounders such as smoking habits and possible other chemical carcinogens that workers may be exposed to in work places of the beryllium industry;
- Intrinsic lack of comparability of SMRs, which are indirectly standardized;
- *Ad hoc* method of selection of groups to be compared;
- Lack of tests of statistical significance of the difference between the SMRs;
- The interpretation of the consistent results from redundant observations (repeated studies on the same worker populations) as validating studies;
- Failure to account for short term worker effect.

Some additional remarks regarding beryllium-containing alloys:

The experimental basis for beryllium-containing alloys appears to be scarce with regard to mutagenicity and carcinogenicity and needs consideration. Beryllium alloys typically contain less than 2% beryllium but every alloy is unique, with physical, mechanical and chemical properties that differ significantly from the components from which it is formed. Once formed, an alloy cannot be easily separated into its components by physical means. Consideration should be given to alloy metallurgy and the science associated with alloys with regard to any cancer designation for beryllium-containing alloys as well as other alloys.

Itingen, 28/10/2010

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Christian Strupp, Dr. rer. Nat Bjarte Furnes, PhD

APPENDIX 1

Databases Screened

PubMed

MedPilot:

- AMED
- AnimAlt-ZEBET
- BIOME
- BIOSIS PREVIEWS
- CAB ABSTRACTS
- CancerLit
- CCMed
- CCRIS (Toxikologie)
- Cochrane (DARE)
- Cochrane-Reviews (CDSR)
- Deutsches Ärzteblatt
- DIQ-Literatur
- EMBASE
- EMBASE Alert
- ETHMED
- EZB Regensburg
- GeroLit
- GLOBAL Health
- IPA International Pharmaceutical...
- ISTPB + ISTP/ISSHP
- Karger-Verlagsdatenbank
- Katalog: Deutsche Zahnärztebibli...
- Katalog: NLM
- Katalog: ZB MED Ernährung/Umwelt
- Katalog: ZB MED Medizin
- Kluwer-Verlagsdatenbank
- Krause & Pachernegg Publikations...
- Lehmanns Online Bookshop
- Link-Datenbank der ZB MED
- Link-Datenbank der ZB MED
- Lippincott Williams & Wilkins Ve...
- Medline
- Medline Alert
- OldMedline
- Pressedienste Gesundheitswesen
- PsycINFO
- Psyndex
- SCISEARCH
- SOCIAL SCISEARCH
- Springer-Verlagsdatenbank
- Thieme-Verlagsdatenbank
- VVFM Virtuelle Videothek für die...
- Xtoxline

ToxNET:

- HSDB
- IRIS
- ITER

GENE-TOX
CCRIS
Multi-Databases
TOXLINE
DART/ETIC
TRI
ChemIDPlus

TOXLINE Special

Special journal and other research literature:
- Developmental and Reproductive Toxicology (DART®)
- International Labour Office (CIS)
- Swedish National Chemicals Inspectorate (RISKLINE)

Technical reports and research projects:
- Federal Research in Progress (FEDRIP)
- Toxic Substances Control Act Test Submissions (TSCATS)
- Toxicology Document and Data Depository (NTIS)
- Toxicology Research Projects (CRISP)

Archival collection (no longer being updated):
- Aneuploidy (ANEUPL)
- Environmental Mutagen Information Center File (EMIC)
- Environmental Teratology Information Center File (ETIC)
- Epidemiology Information System (EPIDEM)
- Hazardous Materials Technical Center (HMTC)
- Health Aspects of Pesticides Abstract Bulletin (HAPAB)
- International Pharmaceutical Abstracts (IPA)
- NIOSHTIC (NIOSH)
- Pesticides Abstracts (PESTAB)
- Poisonous Plants Bibliography (PPBIB)
- Toxicological Aspects of Environmental Health (BIOSIS)

APPENDIX 2

Literature evaluated

In vivo Studies with Beryllium metal

Abr.: epid. (epidermal), i.amn. (intraamnional), i.br. (intrabroncheal), i.d. (intradermal), ih (inhalative), i.m. (intramus cular), i.p. (intraperitoneal), i.pl. (intrapleural), i.tr. (intratracheal), i.v. (intravenous), p.o. (peroral), s.c. (subcutaneous), w.b. (whole body exposure), i.c. (int racorneal), i.ca. (intracardial)

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
Be metal	influence of inhalative exposure (initial lung burden) on clearance of radiolabelled Sr-particles from the lung and histopathology of the lung/ cell populations in broncheo-alveolar-lavage cells	rat	ih	n.a.	Finch et al.	1993	**4**
Be metal	mortality, histopathology of the lung and carcinogenesis after inhalative exposure (initial lung burden)	rat	ih	936	Finch et al.	1994	**4**
Be metal	lung clearance, histopathology and broncheo-alveolar-lavage cell stimulation after single (initial lung burden) inhalative exposure (1 year observation)	rat	ih	170	Finch et al.	1994b	**2**
Be metal	whole-body retention and abnormalities in broncheo-alveolar-lavage fluid after single (initial lung burden) inhalative exposure	rat	ih	n.a.	Finch et al.	1994c	**4**
Be metal	influence of inhalative exposure (initial lung burden) on lung clearance of plutonium and mortality	rat	ih	2856	Finch et al.	1990	**2**
Be metal	influence of inhalative exposure (initial lung burden) on clearance of radiolabelled Sr-particles from the lung	rat	ih	n.a.	Finch et al.	1991	**4**
Be metal	chronic inflammation in lung/carcinogenesis (life-time study)	rat/mouse	ih	928	Finch et al. / C 1	1996	**2**
Be metal	lung toxicity after acute inhalation exposure (up to 350 d)	mouse	ih	234	Finch et al. / C 2	1998	**2**
Be metal	carcinogenicity after acute inhalation exposure (life-time study, until only 10% survivors)	mouse (p53 KO)	ih	170	Finch et al. / C 10	1998a	**2**

Be metal	lung toxicity after acute inhalation exposure (up to 171 days)	rat	ih	128	Haley et al. / C 3	1990	**2**
Compound	**Endpoint Investigated**	**Animal Species**	**Route of Administration**	**Number of animals used**	**First Author / Summary No.**	**Year**	**Klimisch Score**
Be metal	carcinogenesis after single local injection (2 years after exposure)	rat	i.m, i.pl.	40	Hueper et al.	1954	**3**
Be metal	absorption/excretion, mortality, carcinogenesis and K-ras activity after single inhalative exposure	mouse (2 strains)	ih	416	Nikula et al.	1995	**4**
Be metal	Inflammatory response in lung after acute inhalation exposure (6 months after exposure)	mouse	ih	86	Nikula et al. / C 15	1997	**2**
Be metal	carcinogenesis and K-ras, c-raf-1 and p53 protein expression/gene mutation in tumor tissue after single inhalative exposure	rat	ih	120	Nickel-Brady et al.	1994	**4**
Be metal	p53 protein expression in tumor tissue obtained from carcinogenicity study	rat	ih	n.a.	Belinsky et al.	1994	**4**
Be metal	K-ras and p53 protein/gene expression in tumor tissue obtained from carcinogenicity study	rat	ih	24 tumors	Belinsky et al.	1997	**4**
Be metal / BeCu metal	particle clearance from lung and histopathology after single intratracheal administration (up to 28 days observation)	mouse	i.tr.	224	Benson et al. / C 16	2000	**2**
Be metal, BeO	pulmonary toxicity of metal/500°C BeO after intratracheal application (up to 90 d observation)	monkey	i.br.	14	Haley et al.	1994	**2**
Be metal, BeO, BeF_2, $BeCL_2$	(article in Russian, abstract in English) carcinogenesis after single intratracheal or single/multiple inhalative exposure; difference due to calcination temperature of BeO	rat	i.tr., ih		Litvinov et al.	1983	
Be metal, BeF_2, $Be(OH)_2$, BeO, Be Carbide, Be Phosphate, Be Silicate, $BeSO_4$	carcinogenicity, histopathology, pulmonary retention and clinical biochemistry after exposure	rat, guinea pig, rabbit, dog, monkey	i.tr., ih, s.c., i.p., i.v., i.ca.	n.a.	Schepers et al.	1961	**4**
Be metal, $Be(OH)_2$, Be alloys	carcinogenicity after single intratracheal application (up to 18 months observation)	rat	i.tr.	460	Groth et al. / C 11	1980	**2**

Be metal, Be Carbonate, $BeSO_4$, BeF, Be Citrate	(article in Italian) ocular lesions upon intracorneal injection	rabbit	i.c.		Ferraris et al.	1952	
Compound	**Endpoint Investigated**	**Animal Species**	**Route of Administration**	**Number of animals used**	**First Author / Summary No.**	**Year**	**Klimisch Score**
Be metal, BeF_2, $BeSO_4$, $BeCl_2$, $Be(NO_3)_2$, BeO, Be benzenesulfate, Be oxalate	human skin patch test on occupationally exposed individuals and non-exposed volunteers	human	epid.	29	Curtis et al.	1951	**3**
Be metal, $BeSO_4$, CuBe Alloy	skin sensitization (Magnussen-Kligman): induction with BeSO4, challenge with Be-Alloy	guinea pig	i.d./epid.	30	Zissu et al.	1996	**3**

In vitro Studies with Beryllium metal

Compound	Endpoint Investigated	cell type	First Author / Summary No.	Year	Klimisch Score
Be metal	cytotoxicity after exposure to particulate beryllium and dependence on the particle size	rat pulmonary alveolar macrophages	Finch et al.	1991	**2**
Be metal, $BeSO_4$	cytotoxicity	hamster ovary (CHO), rat lung (LEC) cells	Brooks et al.	1987	**4**
Be metal, BeO, $BeSO_4$	cytotoxicity	hamster ovary (CHO), rat lung (LEC) cells	Finch et al.	1988	**3**

In vivo studies with Beryllium compounds

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$Be(OH)_2$	lung retention and immunological reaction after intravenous exposure to Beryllium hydroxide in or without combination with antigens	sheep	i.v.	n.a.	Hall et al.	1984	**4**
$Be(OH)_2$	tissue distribution and lymph node proliferation after subcutaneous injection	sheep	s.c.	n.a.	Hall et al.	1984b	**4**
BeO	histopathology of the lung after 3 acute exposures (monthly intervals; observation period 2 years)	monkey, dog	ih	5 6	Conradi et al.	1971	**4**
BeO	(article in Japanese, abstract in English) carcinogenesis after repeated (15 exposures) intratracheal administration (life-time observation)	rat	i.tr.	30	Ishinishi et al.	1980	**3**
BeO	(article in Polish) osteaosarcoma after intravenous exposure	rabbit	i.v.	20	Kommitowski et al.	1967	
BeO	(article in German, abstract in English) bone tumor formation with lung metastases after intraossal implantation	rabbit	intraossal	20	Kommitowski et al.	1974	**4**
BeO	(article in Japanese, abstract in English) carcinogenesis after intrafemural implantation of hydroxymethcellulose-BeO-pellets (56 weeks observation)	rabbit	intraossal	30	Hiruma et al.	1991	**3**
BeO	histopathology, body weights, hematology and blood biochemistry after single bolus thoracic infusion	guinea pig	thoracic infusion		Shima et al	1983	**3**
BeO	histopathology after single intratracheal injection	rat	i.tr.	23	Davies et al.	1950	**4**
BeO	immunoreactions (skin sensitization, immunocompetent cell population determination) after single intratracheal exposure; strain-specific responses	guinea pigs (2 strains)	i.tr.	12 / strain	Barna et al.	1984a and b	**4**
BeO	histopathology after single intratracheal injection (up to 120 days observation)	guinea pig	i.tr.	30	Chiappino et al.	1969	**4**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
BeO	effect of reproduction/ lactation on lung toxicity after single intratracheal administration (up to 15 months observation)	rat	i.tr.	206	Clary et al.	1975	**3**
BeO	carcinogenesis in rabbits after intravenous acute exposure (up to 1 year)	rabbit	i.v.	9	Dutra et al.	1950	**3**
BeO	(article in Japanese, Title in English) tumor formation after exposure	rabbit			Araki et al.	1954	
BeO	morphological detailed description of a tumor observed in a rabbit after repeated (11 months) daily inhalative exposure (observation 17.5 months)	rabbit	ih	19	Dutra et al.	1951	**3**
BeO	absorption, distribution, excretion after acute inhalative exposure	dog	ih	n.a.	Finch et al. / C 14	1990	**3**
BeO	(abstract only) histopathology after inhalative exposure (initial lung burden) to high- and low temperature calcinated BeO (up to 22 months observation)	dog	ih		Haley et al.	1989	
BeO	lung toxicity after two inhalative exposures (up to 210 d)	dog	ih	20	Haley et al. / C 18	1992	**2**
BeO	histopathology after inhalative exposure (up to 369 h) to different grades of BeO (different post-exposure observation periods)	cat, dog, guinea pigs, rabbit, rat, monkey	ih	133	Hall et al.	1950	**4**
BeO	lung toxicity after acute inhalative exposure (up to 21 days)	rat	ih	4/group	Hart et al.	1984	**3**
BeO	lung toxicity/clearance after acute inhalative exposure (up to lifetime)	rat, hamster	ih	535	Sanders et al./ C 19	1975	**2**
BeO	skin sensitization with dermal induction/intradermal challenge	guinea pig	epid./i.d.	30	Chiappino et al.	1969	**4**
BeO	lung clearance of other particles and lung carcinogenesis after single inhalative exposure (observation 625 to 850 days)	rat	ih	303	Sanders et al.	1978	**2**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
BeO, $BeCl_2$	(article in Polish, title and keywords in English) effect on reproductive performance and offspring development	rat			Selivanova et al.	1986	
BeO, $BeCl_2$	(article in Russian, abstract in English) carcinogenesis and histopathology after chronic inhalative exposure	rat	ih	640	Litvinov et al.	1984	
BeO, $BeSO_4$	immune cell stimulation in broncheo-alveolar-lavage cells upon single intratracheal exposure after preimmunisation	mouse	i.m./i.tr.	80	Huang et al.	1992	**3**
BeO, $BeSO_4$	histopathology of the lung upon chronic inhalative exposure or three intratracheal injections	rat	ih, i.tr.	30	Vorwald et al.	1959	**4**
BeO, Be SO_4, BeF_2	blood clinical chemistry and uric acid/creatinine excretion after repeated (35 days) inhalative or single intravenous exposure	dog	i.v., ih	10	Spiegl et al.	1953	**4**
BeO, Be Phosphate, ZnBe Silicate	primary bone tumors with metastases into lung, mortality and histopathology after single intravenous exposure to suspensions of the barely soluble compounds	rabbit	i.v.	19	Hoagland et al.	1950	**4**
Be ores	lung toxicity/carcinogenesis under daily inhalation exposure (up to 28 months)	monkey, rat, hamster	w.b. ih	n.a.	Wagner et al.	1969	**3**
$^{7}BeCl_2$	whole body retention of radiolabel after single intravenous or peroral administration	dog	i.v. / p.o.	4	Richmond et al.	1964	**4**
$^{7}BeCl_2$	whole body retention of radiolabel after single intravenous or intraperitoneal administration	rat	i.v. / i.p.	24	Richmond et al.	1965	**4**
$^{7}BeCl_2$	body retention and blood cell/serum distribution of retained radiolabel after single intravenous exposure	mouse	i.v.	n.a.	Sakaguchi et al.	1988	**4**
$^{7}BeCl_2$	tissue distribution after single or repeated exposure (different routes)	rat	i.v., s.c., i.m., i.p., i.tr.	75	van Claeve et al.	1953	**4**
$^{7}BeCl_2$	(only title and keywords) effect on carrier on distribution after intratracheal exposure	rat	i.tr.		Kuzntsov	1974	
$BeCl_2$	(only title and keywords) distribution / retention and lung toxicity after inhalative exposure	guinea pig	ih		Hart et al.	1980	

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$BeCl_2$	hemotopoesis-related enzyme activity in the blood and spleen of pregnant mice after subcutaneous exposure	rat	s.c.	29	Sakaguchi et al.	1996	**4**
$BeCl_2$	skin sensitization (Magnussen-Kligman): induction with BeSO4, challenge with Be-Alloy	guinea pig	i.d./epid.	40	Bomann et al.	1979	**4**
$BeCl_2$	absorption, distribution and excretion after single intravenous exposure	rat	i.v.	n.a.	Klemperer et al.	1952	**4**
$BeCl_2$	mortality and enzyme activity in blood and tissues after single intraperitoneal administration	rat, guinea pig	i.p.	116 72	Cochran et al.	1951	**4**
$BeCl_2$	absorption, distribution and excretion after single peroral or intravenous exposure	mouse, rat, monkey, dog	p.o. , i.v.	n.a.	Furchner et al.	1993	**2**
$BeCl_2$	distribution and placental penetration after single intravenous injection	mouse	i.v.	28-36	Bencko et al.	1979	**3**
$BeCl_2$	organ distribution and excretion (bile, faeces, urine) after single intravenous application	rat	i.v.	12	Cikrt and Bencko	1975	**3**
$BeCl_2$	(only title) teratogenicity	chicken			Puzanova et al.	1978	
$BeCl_2$	histopathology and enzyme activity in liver after single intraperitoneal exposure	rat	i.p.	45	Malendowicz	1966	**3**
$^{7}BeCl_2$, $BeSO_4$, Be Citrate, Be phosphate	tissue distribution and alpha-fetoprotein production after intravenous exposure to particulate Be salts	mouse/rat	i.v.	10 / 10	Vacher et al.	1974	**4**
$^{7}BeCl_2$, $BeSO_4$, Be Citrate, Be phosphate	skin sensitization and influence of the route of exposure on this parameter; elimination from skin	guinea pigs	i.v., i.d., i.p.	94	Vacher et al.	1972	**2**
$^{7}BeCl_2$, $^{7}BeF_2$	(article in Russian) organ distribution of Be after exposure by different routes	rat			Bugryshev et al.	1984	
Be $(NO_3)_2$	influence of two chelating agents on beryllium distribution and liver toxicity after repeated (21 days daily) oral exposure	rat	p.o.	24	Flora et al.	1995	**3**
Be $(NO_3)_2$	histopathology and enzyme activity in lung after dietary exposure (25 doses)	rat	in-feed	10	Goel et al.	1987	**3**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$Be(NO_3)_2$	histopathology and enzyme activity in lung after dietary exposure (40 doses)	rat	p.o.	12	Goel et al. / C 12	1980	**3**
$Be(NO_3)_2$	(only abstract) influence of chelation/antioxidant therapy on clinical chemistry, hepatic peroxidation and metal burden in organs after blood parameters after imtramuskular bolus exposure	rat			Johri et al.	2002	
$Be(NO_3)_2$	influence of chelation/antioxidant therapy on clinical chemistry, hepatic peroxidation and metal burden in organs after blood parameters after intramuscular bolus exposure	rat	i.m./i.tr.	65	Johri et al. / C 17	2004	**2**
$Be(NO_3)_2$	influence of an ayurvedic medicine on histopathology and enzyme activity of reproductive female organs after single intravenous exposure	rat	i.v.	24	Mathur et al.	1989	**3**
$Be(NO_3)_2$	influence of chelation/antioxidant therapy on enzyme activity and organ peroxidation in kidney and liver after repeated (28 days) intraperitoneal exposure	rat	i.p.	35	Mathur et al. / C 13	2004	**3**
$Be(NO_3)_2$	effects on maternal and fetal toxicity by intravenous exposure of pregnant rats on day 14, 16, 18 and 20 post coitum	rat	i.v.		Mathur et al.	1994	**2**
$Be(NO_3)_2$	influence of chelation/antioxidant therapy on organ enzyme activity, hepatic peroxidation and metal burden in organs after blood parameters after intraperitoneal bolus exposure	rat	i.p.	24	Mathur et al.	1994a	**3**
$Be(NO_3)_2$	(only title and keywords) lung effects after single intradermal exposure	guinea pig	i.d.		Levy et al.	1961	
$Be(NO_3)_2$	influence of an ayurvedic medicine on organ histopathology and clinical biochemistry after single intravenous exposure	rat	i.v	60	Mathur et al.	1994b	**3**
$Be(NO_3)_2$	influence of dietary plant leave administration on blood glucose after single intravenous exposure	rat	i.v	40	Prakash et al.	1986	**3**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$Be(NO_3)_2$	influence of chelation/antioxidant therapy on organ enzyme activity, hepatic peroxidation and metal burden in organs after single intramuscular exposure	rat	i.m	20	Sharma et al.	2000	**3**
$Be(NO_3)_2$	influence of chelation therapy on fetotoxicity after single intramuscular exposure	rat	i.m	18	Sharma et al.	2002	**3**
$Be(NO_3)_2$	influence of chelation therapy on clinical chemistry parameters and tissue (organs/reproductive organs) enzyme activity after repeated (daily for 21 days) intraperitoneal exposure	rat	i.p.	35	Shukla et al.	1998	**3**
$Be(NO_3)_2$	investigation on beryllium contents in blood and urine and lymphocyte stimulation of exposed humans and rats/guinea pigs exposed by inhalation	rat/guinea pig	w.b. ih	10 10	Stiefel et al.	1980	**3**
Be phosphate	electron microscopic investigation of the liver after intravenous exposure to beryllium phosphate particles	rat	i.v.	36?	Dinsdale et al.	1982	**3**
Be phosphate	electron microscopic investigation of the liver after intravenous exposure to beryllium phosphate particles with or without administration of colloidal carbon	rat	i.v.	n.a.	Dinsdale et al.	1981	**3**
$BeSO_4$	carcinogenicity after inhalative exposure; EXPOSURE TO OTHER CARCINOGENIC COMPOUNDS IN ADDITION	monkey	ih	12	Vorwald et al.	1968	**3**
$BeSO_4$	blood kinetics after single intravenous administration	rat	i.v.	49	Vacher et al.	1968	**4**
$BeSO_4$	absorption, distribution and excretion after single intravenous exposure	rat	in drinking water	12	Reeves et al.	1965	**2**
$BeSO_4$	in vivo micronucleus assay in mice after single oral exposure and lung carcinogenesis in strain A mice after repeated (3 per week, 2 weeks) intraperitoneal exposure	mouse	p.o. i.p.	65	Ashby et al. / B3, C 4	1990	**2**
$BeSO_4$	skin sensitization (Local Lymph node assay)	mouse	epid.	4	Basketter et al. / C 5	1999	**2**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$BeSO_4$	carcinogenicity and chronic toxicity upon life-span dietary administration	rat	in-feed	52	Schroeder et al.	1975	**2**
$BeSO_4$	lymphocyte transformation test for sensitization, brocheo-alveolar-lavage cell populations and organ histopathology (lung, spleen, kidney) after single intratracheal exposure	mouse	i.tr.	21	Burdon et al.	1997	**3**
$BeSO_4$	toxicity to fish upon acute exposure	perca fluviatilis, rutilus rutilus	in water	n.a.	Jagoe et al.	1993	**3**
$BeSO_4$	skin sensitization/lymphocyte stimulation upon repeated (twice weekly for 6 weeks) intradermal exposure	rabbit	epid.	94	Kang et al. / C 20	1977	**2**
$BeSO_4$, $BeCl_2$, BeF	(article in Japanese, abstract in English) excretion of radiolabelled material and sensitization in footpad- and macrophage migration inhibition test after subcutaneous exposure	mouse	i.d		Sakaguchi et al.	1983	
$BeSO_4$	acute toxicity to Daphnia magna	Daphnia	in water	n.a	Khangarot et al. / C 6	1989	**2**
$BeSO_4$	21 day earthworm reproduction test, 28 day enchytracheid reproduction test and the 28 day collembola reproduction test	eisenia fetida, enchytraeus crypticus, folsomia candita	in soil	n.a	Kuperman et al. / C 7a, b, c	2006	**2**
$BeSO_4$	acute toxicity to birds after single intravenous exposure	pigeons and chicks	i.v.	26	Pham et al.	1966	**3**
$BeSO_4$	(only title and keywords) embryonic development and RNA-synthesis	lymnaea			Bose et al.	1973	

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
7 Be SO_4, 7Be Phosphate	selective uptake into and toxicity to liver cell populations (parenchymal/non-parenchymal)	rat	i.v.	n.a.	Skilleter et al.	1985	**4**
Be SO_4	histopathological and ultrastructural abnormalities in the liver of normal and hepatectomized rats after single intravenous administration	rat	i.v.	36	Goldblatt et al.	1973	**4**
Be SO_4	histopathology (inflammatory and proliferative response) after daily inhalative exposure (72 weeks)	rat	ih	300	Reeves et al. / C 8	1967	**2**
Be SO_4	lactate dehydrogenase activity in different lung fractions after daily inhalative exposure (72 weeks)	rat	ih	n.a.	Reeves et al.	1967	**4**
Be SO_4	accumulation/excretion of beryllium in/from lung after daily inhalative exposure (72 weeks)	rat	ih	300	Reeves et Vorwald	1967	**2**
Be SO_4	cardiovascular toxicity (telemetry) upon intratracheal exposure	dog	i.tr.	n.a.	Pham et al.	1970	**4**
Be SO_4	influence of intraperitoneal iron treatment on mortality after repeated (2h/d, 14 d) inhalative exposure	rat	ih	40	Sendelbach and Witschi	1987	**3**
Be SO_4	histopathology and cell proliferation by thymidine uptake (up to 21 days) after single inhalative exposure	rat/mouse	ih	36 40	Sendelbach et al.	1986	**3**
Be SO_4	histopathology in lung, cell proliferation by thymidine uptake and enzyme activity in brocheo-alveolar-lavage fluid one year after single inhalative exposure	rat	ih	24-32	Sendelbach et al.	1989	**3**
Be SO_4	(only title and keywords) effect of exposure on blood volume and blood cell stimulation	rabbit			Mosser et al.	1970	
Be SO_4	acute toxicity to fish	guppy	in water	n.a	Slonim et al. / C 9	1973	**2**
Be SO_4	acute toxicity to salamander larvae	A. opacum, A. maculatum	in water	380	Slonim et al.	1975	**2**
Be SO_4	weight change, mortality and histopathologic damage in lung after repeated (alternate-daily, 1month) inhalative exposure	rat	ih	80	Stokinger et al.	1950	**3**

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
$BeSO_4$	mouse ear swelling test in different HLA-DPB01 transgenic mice	mouse	epid.	n.a.	Tarantino et al.	2005	**3**
$BeSO_4$	histopathology of lung and immune cell stimulation induced by single intratracheal administration after subcutaneous sensitization	rat	s.c. / i.tr.	n.a.	Votto et al.	1987	**3**
$BeSO_4$	(only abstract) effect of exposure in drinking water on body weight and nervous system	rat	in water		Freundt et al.	1990	
	mortality, histopathology and carcinogenicity upon inhalative (up to 18 months) exposure	rat	ih	275	Schepers et al.	1957	**2**
$BeSO_4$	influence of intravenous exposure on DNA-repair in regenerating liver after partial hepatectomy	rat	i.v.	35	Witschi et al.	1970	**3**
$BeSO_4$, BeF_2, Be Phosphate	symptomatic, mortality and lung histopathology upon inhalative (up to 30 days) exposure (observation up to 300 days)	monkey	ih	20	Schepers et al.	1964	**4**
BeF_2	(only title and keywords) acute inhalation toxicity				Stokinger et al.	1953	
BeF_2	skin sensitization and its strain-specificity after dermal application	guinea pig (2 strains)	epid.	n.a.	Turk et al.	1969	**4**
7Be Citrate	excretion and blood concentration after single intravenous administration	rabbit	i.v.	6	Underwood et al.	1952	**4**
7Be Carbon	adsorption, distribution and excretion of radiolabel from weanlings and aged mice after single oral administration of radio-Be	mouse	p.o.	100	LeFevre et al. / C 21	1986	**2**
Na_2BeF_4	(article in Japanese, Title and figures in English) mortality and histopathological changes in mice after single intraperitoneal exposure	mouse	i.p.		Nomiyama et al.	1978	
Be-sulphosalicylate	effect of intravenous exposure at LD50 dose on enzyme induction in liver trigged by inducer	rat	i.v.	50	Ord et al.	1981	**3**
ZnBe Silicate	osteosarcoma-formation and metastases after intravenous exposure to suspensions	rabbit	i.v.	10	Janes et al.	1954	**3**
ZnBe Silicate	(only abstract) osteaosarcoma after intraosseal injection	rabbit	intraossal		Mazabraud et al.	1975	

Compound	Endpoint Investigated	Animal Species	Route of Administration	Number of animals used	First Author / Summary No.	Year	Klimisch Score
ZnBe Silicate	(only title and keywords) lung toxicity after intratracheal exposure	guinea pig	i.tr.		Levy et al.	1965	
ZnBe Silicate	osteosarcoma-formation and metastases after intravenous exposure to suspensions (observation 120 weeks)	rabbits	i.v.	57	Barnes et al.	1957	**3**
ZnBe Silicate	a tumor induced by intravenous exposure was transplantable into the eyes of other rabbits	rabbits	i.v.	20	Higgins et al.	1964	**4**
ZnBe Silicate	primary bone tumors with metastases into lung after single intraossal administration of suspensions	rabbits	intraossal	12	Tapp et al.	1966	**4**
ZnBe Silicate, Be Silicate	bone tumors after intravenous exposure	rabbits	i.v.	n.a.	Sissons et al.	1950	**4**
"soluble Be compounds"	(only title and keywords) absorption through injured skin	rat	epid.		Ivannikow et al.	1982	
n.a.	(article in Russian, abstract in English) organ distribution of Be after exposure by different routes	rat	i.v., i.p., i.tr		Bugryshev et al.	1976	
n.a.	influence of calcium content of diet on dietary beryllium-induced mortality and body mineral concentrations	Achatina	p.o.	40	Ireland	1986	**3**
n.a.	histopathology of embryos after single intraamnial exposure	chicken	i. amn.	n.a.	Puzanova	1980	**3**
n.a	(article in Japanese, abstract in English) influence of subcutaneous exposure on blood coagulation/hematopoesis parameters; skin sensitization by dermal exposure after subcutaneous immunization	mouse	s.c./epid.		Sakaguchi et al.	1998	
n.a.	(article in Russian) kinetics, distribution and excretion	rat, dog			Zhuravlve et al.	1974	

In vitro studies with Beryllium compounds

Compound	Endpoint Investigated	cell type	First Author / Summary No.	Year	Klimisch Score
BeO	cytotoxicity and in vitro cell transformation	rabbit embryo fibroblasts	Kommitowski et al.	1973	**4**
BeO, $BeSO_4$	(only abstract) cytotoxicity and bichemical enzyme activity after exposure	rabbit alveolar macrophages	Kang et al.	1979	
7BeO, $7BeSO_4$	cellular localisation of particulate or ionic Be	dog broncheo alveolar lavage cells	Eidson et al.	1991	**4**
BeO, $BeCl_2$, Be $(NO_3)_2$	rec-assay, Ames test with preincubation and sister chromatide exchange assay	bacillus subtilis, E. coli (2 strains) / V79	Kuroda et al. / B 6a, b, c	1991	**4**
BeO, Be-sulphosalicylate	lympohocyte (normal or Be-sensitized in vivo) proliferation in vitro in response to beryllium compound treatment	guinea pig lymphocytes	Jones et al.	1975	**4**
BeO, $BeSO_4$	cytotoxicity	dog broncheo alveolar lavage cells	Finch et al.	1988	**3**
BeO, $BeCl_2$, BE $(NO_3)_2$	rec-assay, Ames test and sister chromatide exchange assay	Salmonella sp., V79	Endo et al.	1991	**4**
$BeCl_2$	cytotoxicity	human carcinoma (KB), human embryonic lung (HEL-R66), dog kidney (MDCK), monkey kidney (VERO)	Mochida et al.	1986	**4**
$BeCl_2$	influence of exposure on calcium uptake and signalling	primary mouse peritoneal macrophages	Misra et al.	1999	**4**
$BeCl_2$	cytotoxicity	HeLa-S3, Vero, HEL-R66	Sakaguchi et al.	1984	**4**
$BeCl_2$	influence on fidelity of DNA synthesis and DNA-binding	no cells	Sirover et al.	1976	**2**
$BeCl_2$	influence on vascular wall and platelet reactivity	bovine Aorta (isoorgan) /human platelets	Togna et al.	1997	**4**
$BeCl_2$	bacterial gene mutation (plate incorporation)	E. coli	Zakour et al.	1984	**4**
Be SO_4	Ames test, chromosome aberration	S. typhimurium, hamster lung cells (CHL)	Ashby et al. / B 1 / B2	1990	**2**

Compound	Endpoint Investigated	cell type	First Author / Summary No.	Year	Klimisch Score
$BeSO_4$	cytotoxicity, cell cycle and chromosome aberration	CHO/LEC	Brooks et al.	1989	**4**
$BeSO_4$	carcinogenicity (cell transformation in vitro)	syrian hamster embryo cells	Fritzenschaf et al.	1993	**4**
$BeSO_4$	carcinogenicity (cell transformation in vitro)	syrian hamster embryo cells, BALB-3T3 cells, Rauscher Murine Leukemia Virus-Infected Fisher 344 Rat Embryo Cells	Dunkel et al.,	1981	**2**
$BeSO_4$	activation of transcription factor and interleukin-stimulation	mouse macrophages (H69.12j)	Hamada et al.	2000	**4**
$BeSO_4$	in vitro cell transformation and transplantation of transformed cells into mice; gene expression profiling of transformed cells	mouse 3T3	Joseph et al.	2001	**4**
$BeSO_4$	cytotoxicity, in vitro cell transformation and transplantation of transformed cells into mice; mRNA- and protein expression of oncogenes in transformed cells	mouse 3T3	Keshava et al.	2001	**4**
$BeSO_4$	cytotoxicity, DNA-synthesis and influence on stimulated proliferation	mouse spleen cells	Price et al.	1985	**4**
$BeSO_4$	cytotoxicity, DNA-synthesis and influence on stimulated proliferation	mouse splenocytes	Price et al.	1986	**4**
$BeSO_4$	Be-lymphocyte proliferation test in broncheo-alveolar lavage cells from chronic beryllium disease patients; induction of TNF-a production and apoptosis in macrophage cell lines	mouse macrophages (H69.12j, P388D.1), human CBD BAL macrophages (DEOHS-1)	Sawyer et al. / B 4	2000	**2**
$BeSO_4$	cytotoxicity, mRNA- and protein expression of TNF-a	mouse macrophages (H36.12a,b,d,e,j, P388D.1, RAW264.7, J774A.1), human monocytes/histiocytic lymphoma cells (THP-1, U937)	Sawyer et al. / B 5	2000a	**2**
$BeSO_4$	cell cycle arrest upon exposure	rat liver cell line (BL9L)	Skilleter et al.	1983	**4**

Compound	Endpoint Investigated	cell type	First Author / Summary No.	Year	Klimisch Score
Be SO_4	cell cycle analysis after exposure	rat liver cell line (BL9L)	Skilleter et al.	1991	**4**
Be SO_4, Be Phosphate	uptake of radio-Be into selective population of liver cells	rat primary liver cells	Skilleter et al.	1985	**4**
Be SO_4, Be $(NO_3)_2$	Ames -Test (plate incorporation and fluctuation)	S. typhimurium (5 strains), E. coli (1strain)	Arlauskas et al.	1985	**4**
7 Be SO_4, Be-sulphosalicylate	cytotoxicity and uptake	rat liver cells (ARL)	Skilleter et al.	1979	**2**
Be++ Ion beam	cytotoxicity	V79	Scampoli et al.	2001	**4**
	(article in Russian, abstract in English) DNA-repair	E. coli	Dylevoi	1990	
"Beryllium and other potentially genotoxic agents"	chromosomal aberration and sister chromatic exchange; WORKERS EXPOSED TO SEVERAL CHEMICALS IN ADDITION TO BERYLLIUM	humane lymphocytes from workers	Garry et al.	1989	**3**
"forms of beryllium in a beryllium plant"	in vitro lymphocte proliferation in response to $BeSO_4$ (in vitro BeLPT)	humane lymphocytes from workers	Kreiss et al.	1989	**3**
7Be-sulphosalicylate	Be binding to lymphocytes of guinea pigs with or without prior sensitization; binding of Be to lymphocytes from non-sensitized humans	guinea pig lymphocytes (3 strains), human lymphocytes	Skilleter et al.	1984	**4**
	(only abstract) DNA-misincorporation (isolated enzyme)	no cells	Luke et al.	1975	
	(only title) uptake into cells	alveolar macrophages	Hart et al.	1980	
	(only title) cytotoxicity	human lung fibroblasts	Hart et al.	1982	

APPENDIX 3

Summary of animal studies relevant for carcinogenicity

Groth et al. (1980)
Be metal, BeAl alloy, passivated Be metal, and $Be(OH)_2$ were demonstrated to be pulmonary carcinogens in rats. These findings are supported by successful transplantation experiments. In addition, other alloys of Be, VBe_{12}, $TiBe_{12}$, $TaBe_{12}$, $NbBe_{12}$, and Be_4B were found to produce pulmonary metaplasia, frequently a preneoplastic lesion in rats. Old rats were shown to be more susceptible to the induction of pulmonary metaplasia than young adult rats. These results indicate that a lower dose of Be would be required to produce cancer in old animals compared to young ones. A discussion on the lung cancer incidence in Be production workers is included.

Finch et al. (1996)
This publication is primarily a summary of studies already evaluated for the purposes of this statement. No conflicting conclusions were reached.

Belinsky et al. (1994)
This abstract only reported on preliminary findings. Alterations in the p53 gene were studied in F344/N rats exposed to beryllium and other compounds (carbon black, diesel exhaust, X-rays). The alterations were determined by immunohistochemistry, direct sequencing and single strand conformation polymorphism (DNA) analysis. Immunohistochemistry did not reveal increase in p53 protein levels in either of the exposures. No other findings relevant to beryllium exposure were reported.

Nickel-Brady et al. (1994)
Single inhalation exposure of Fisher 344 rats to Be metal led to 64% lung tumour incidence after 14 months. The induced carcinomas were examined for genetic alterations in the K-ras, p53, and c-raf-1 genes. No K-ras codon 12, 13 or 61 mutations were detected in 24 lung tumours by direct sequencing, but a more sensitive method detected 2/12 GGT-GTT transversions, considered to be a rare and late event. No mutant p53 nuclear immunoreactivity was observed in any Be-induced tumour, and no mutations were detected within exons 5 - 8 of the p53 gene. No rearrangement of the c-raf-1 protooncogene was detected by Southern blot analysis. The results indicate that the mechanism underlying in the development of Be-induced lung cancer in rats do not involve gene dysfunctions commonly associated with human non-small-cell lung cancer.

Belinsky et al. (1997)
Pulmonary adenocarcinomas or squamous cell carcinomas were induced by beryllium metal. In these tumours, mutation of the K-ras gene was determined by approaches that included DNA transfection, direct sequencing, mismatch hybridization and restriction fragment length polymorphism analysis. K-ras activation in Be induced lung tumours had a low incidence of 2/24 and consisted of a GGT -->GTT transversion. Alteration in the p53 gene was assessed by immunohistochemical analysis for p53 protein and single-strand conformation polymorphism (SSCP) analysis of exons 4 to 9. None of the adenocarcinomas was immunoreactive toward the the anti-p53 antibody CM1. No squamous cell carcinomas induced by beryllium were available for investigation.

Finch et al. (1994)
An equal number of male and female F344/N rats (n= 936) were exposed to Be metal (MMAD= 1.4 µM) by nose only acute inhalation. The following lung burdens were achieved: 33, 84, and 420 µg. Additional control animals were sham-exposed only. Mortality was 33% and 64% (male and female, respectively) in the 420 µg group within 3 weeks of exposure. The incidence of lung tumours (animals surviving until day 365) was 2%, 62%, 89%, and 89% (males) and 0%, 83%, 96%, and 100% (females) for the control group, 33, 84 and 420 µg dosegroups. The most common primary malignant lung neoplasms were adenocarcinomas, followed by squamous cell carcinomas and adenosquamous carcinomas.

Litvinov et al. (1983)
The study part dealing with beryllium metal is reported only in words without any experimental details. Therefore, only an executive summary can be provided. Albino mongrel rats of 140-150 g were treated by single intratracheal administration of beryllium metal. Two particle size ranges of the material were tested: big and fine particles. Doses were 0.5-18 mg/kg. While little effect was seen with the large particles, dose-dependent neoplastic effects in lung were described after administration of fine particles.

Finch et al. (1995)
F344 rats and C3H or A/J mice were exposed pernasally to single doses of inhalable be metal at comparable lung burdens. Rats developed chronic pulmonary infiltration, hyperplasia of alveolar epithelium and retarded clearance of radiolabeled tracer particles at lower lung burdens and had a substantial neoplastic response. Mice showed no or minimal neoplastic response only.

Finch et al. (1998)
Mice were exposed by inhalation once to a lung clearance marker (^{85}Sr-FAP) followed by a single exposure to metallic beryllium at Be-burdens of 0, 1.7, 2,6, 12 and 34 µg per mouse and followed up for 350 days. Investigated parameters were lung clearance of the marker and beryllium, lung pathology (macro-& micropathology, broncholaveolar lavage analyses). Lung weights were increased at the 12 and 34 µg burdens, at these levels there was also a clear retardation of clearance of the ^{85}Sr-FAP marker and of beryllium. Some cellular BALF were occasionally increased in the higher burdens. While the 1.7 µg was practically without adverse effect, minor pathology findings were noted at 2.6 µg. At the burdens of 12 and 34 µg granulomatous pneumonia, lymphocytic interstitial aggregates and mononuclear infiltrates occurred with high incidence. Comparing effects and lung burdens, mice seem to be less sensitive to inhalatory Be effects than rats.

Finch et al. (1998)
Heterozygous TSG-p53 knockout mice (p53$^{+/-}$, each 15 male and 15 females/group) were exposed to Beryllium metal powder (MMAD 1.8 µm) for either 112 min. at 34 µg Be/l air (target initial lung burden of 15 µg Be), or for three consecutive days (139 min./d at 36 µg Be/l air). Concurrent controls (15m/15f) were exposed to filtered air (sham).
The same experiment was conducted in parallel with wild-type mice (p53 $^{+/+}$). In this experiment, 3 satellite animals per exposure condition were added and were killed after 7 days to determine the initial lung burden. 4-5 mice/group were sacrificed 6 months after exposure, and remaining animals were sacrificed when moribund (the rest of the

population when 90% mortality was reached). Increased numbers of neoplasm were found in p53 knockout mice, while no neoplasms were observed in wild-type mice.

Nikula et al. (1995)
The pulmonary carcinogenicity of Be metal was compared in A/J (sensitive to lung tumours) and C3H/HeJ (resistant to lung tumours) mice. Female, 6-8 week old mice (206 each srain) were exposed once, nose only, to Be metal or to filtered air (controls, 50 each strain). Despite simultaneous exposure of both strains, Be initial lung burdens (ILTs/g body weight) were 63 mcg (3.4 mcg/g) for C3H/HeJ mice and 47 mcg (3.0 mcg/g) for A/J mice. Clearance half-times were 108 and 97 days for C3H/HeJ and A/J mice, respectively. Differences between strains in ILBs and clearance half-times were statistically significant. Be exposure reduced survival significantly for C3H/HeJ mice and marginally for A/J mice. There was no difference in tumour incidence, multiplicity, or latency in Be-exposed C3H/HeJ mice compared to controls. When mice sacrificed before 11 months on study were excluded (sacrifices to determine ILBs and study preneoplastic lesions), the incidence of lung neoplasia from 11 to 22 months was slightly increased in Be-exposed A/J mice. Six percent of control and and 19% of Be-exposed A/J mice had more than one neoplasm. Analyses of K-ras mutation patterns in neoplasms from exposed and control A/J mice suggest that the increased multiplicity was due to promotion of spontaneously initiated cells. Further analyses will define whether these increases are statistically significant and if tumour latency was decreased.

Schepers et al. (1961)
The study parts dealing with beryllium metal is reported only in a table in the publication without further details. Therefore, a full summary can not be provided. No neoplasms were observed in guinea pigs three months after a single intratracheal instillation of 75 mg beryllium metal.

APPENDIX 4

Early Cohort studies on Workers Employed at the Beryllium Processing Plants at Lorain (Ohio) and Reading (Pennsylvania).

Studies by Mancuso

The retrospective cohort studies at the beryllium processing plants at Lorain (Ohio) and Reading (Pennsylvania) were initially conducted on 3685 male workers and extended in several follow-up studies. A significant increase in the mortality by lung cancer was reported in workers with a lagging of more than 15 years since the beginning of their employment (Mancuso and El Attar 1969; Mancuso 1970; Mancuso 1979; Mancuso 1980). However, these studies are characterized by a lack of exposure data, lack of consideration of confounding factors such as smoker status and other possible lung carcinogens that may occur at work places in metal processing plants. Uncertainties about the national mortality rates during the years 1968-1975 were an additional limiting factor 1. Furthermore, mortality rates of lung cancer among the workers could not be shown to be correlated with the duration of employment when dividing the cohort in short (less than 12 months; SMR 1.38, $p < 0.05$) medium (12 up to 48 months; SMR 1.06) and long duration of tenure (more than 49 months; SMR 2.22, $p < 0.01$) (Mancuso 1980).

Wagoner et al. (1980)

Similarly, in an extended study performed by NIOSH (3055 workers, 47 lung cancer deaths) the highest rates of malignancies of the lung, trachea, bronchi were found among workers that were occupied less than five years and those the start of their tenure was more than 25 years ago (Wagoner et al. 1980). When lung cancer SMRs were calculated by latency the SMRs were 0.88 (9 deaths) for less than 15 years of latency, 1.16 (18 deaths) for 15-24 years' latency and 1.68 (20 deaths) for a latency of 25 years or more (95% confidence interval, CI being 1.0-2.6 for the latter SMR which means borderline significance at the $p< 0.05$ level). However, within the latency categories there was again no pattern of increasing or decreasing SMRs by duration of employment (Wagoner et al 1980). Since the increases of mortality rates of lung cancer are not correlated with duration of tenure as a surrogate for exposure it has been assumed e.g. by IARC that high exposures may have been occurred among workers with short duration of employment, in particular during 1940s years including the years of World War II (IARC 1998). Potential confounding by a different distribution of smoking habits in the US population and in the beryllium cohort was considered and was calculated to potentially increase the lung cancer rate among the beryllium-exposed workers by 14%. On the other hand, when using the lower age-adjusted mortality rate for lung cancer among white males in the county (31.8/100 000) instead of the respective mortality rate in the US population (white males) as a whole (30.0/100 000) the lung cancer risk of the workers was underestimated by 19% (Wagoner et al. 1980).

Conclusion

Taken together, these early cohort studies are limited due to their lack of data on confounders such as smoker status and appropriately defined employment and exposure conditions. Thus, an association between exposure to beryllium and lung cancer mortality could not unequivocally be demonstrated.

Studies using data of the Beryllium Case Registry

Infante et al. (1980)
The Beryllium Case Registry (BCR) was established in 1952 to collect data on the epidemiology, diagnosis, clinical features course and complications of beryllium-related diseases. Infante et al. (1980) investigated the lung cancer mortality among 421 workers who while alive entered the Registry with a diagnosis of chronic beryllium disease (CBD) or acute pneumonitis (acute beryllium disease, ABD). From 7 lung cancer deaths observed (3.3 expected based on national mortality rates; SMR 2.12; not significant) 6 cases occurred among workers who had a diagnosis of acute pneumonitis (ABD). Only one lung cancer death was observed among the workers with a diagnosis of CBD. Although the SMR for workers with a diagnosis of acute pneumonitis (ABD) was enhanced by nearly 3-fold there was no unequivocal evidence due to the above mentioned uncertainties about the national mortality rates during the years 1968-1975. Further limiting factors of this study were the small numbers of cases and the short follow-up- time of workers who entered the Registry after 1965 (≤ 10 years). Important to this reference is a letter to the CDC, from Dr. Bayliss, objecting to having his name listed as a coauthor. He stated: ”As I view it, there is one reason and one reason only why Drs. Wagoner and Infante, over the protests of me and others including the CDC review panel, refused to use the proper death rates; they want to be able to describe the study as demonstrating a statistically significant excess of respiratory cancer in beryllium workers – which is indeed precisely how the study is described in the abstract. The manipulation of the input data to permit assertion of a pre-ordained conclusion is not, in my view, evidence of intellectual or scientific honesty.”

Steenland and Ward (1991)
An extended study was conducted on the mortality rates of 689 persons who had entered the Registry up to 1980 (Steenland and Ward 1991). Mortality follow-up was extended to 1988. For comparison the US death rates were available for all years. There were not only an excess mortality of all cancers (SMR 1.51; CI 1.17-1.91; 70 observed deaths), an excess of lung cancer but also excess deaths from non-malignant respiratory diseases (SMR 34.2; 95% CI 29.1-40.0; 158 observed deaths) and all causes of deaths (SMR 2.19; 95% CI 1.17-1.91; 428 observed deaths). The SMR for lung cancer mortality was greater among cohort members with acute pneumonitis (ABD) than among those with CBD. Smoking was reported to be an unlikely confounder of the observed excess lung cancer since the study cohort smoked less (26% current smokers) than the US referent population (32%). Since the lung cancer SMR in this study population was higher than was found in other cohort studies, particularly among the study population with acute pneumonitis (ABD), it could be assumed that the Registry population had a higher exposure to beryllium.

More Recent Studies Using Improved Assessments of Confounding, Type of Employment and Exposure

Ward et al. (1992)
A retrospective mortality cohort study of 9225 workers that were employed at seven beryllium-processing plants was conducted by Ward et al. (1992). In the total cohort, there were 3240 deaths (35%) and 269235 person-years of follow-up, of which 52% were person-years at risk 15 years or more after first employment in the beryllium industry. SMRs were calculated on the basis of US population as well as local county

mortality rates. The overall SMR for deaths of non-malignant respiratory disease was 1.48 (95% CI, 1.21-1.80), that for lung cancer was 1.26 (95% CI, 1.12-1.42; 280 observed deaths, based on US rates). At four of the six locations (the records of two plants were combined), the SMRs for lung cancer were greater than 1.00 but not significant, excess lung cancer rates were calculated for the two aforementioned plants in Lorain and Reading. The authors noted that cohorts in which there were elevated SMRs for pneumoconiosis or other respiratory diseases, presumably indicating higher exposure to beryllium also consistently had elevated SMRs for lung cancer. This correlation between non-malignant respiratory diseases and lung cancer led the authors to suggest without analysis or supporting data that the observed lung cancers were associated with high exposures to beryllium.

Lung cancer SMRs were stratified by latency at each plant and decade of hire. Although only three of the six locations showed higher SMRs for the 15-30 year and the >30 year category of latency, lung cancer SMRs increased stepwise for the total cohort with increasing latency. Lung cancer SMRs were significantly enhanced in three of four locations where workers were hired before 1950 (the period when exposures were also greater than subsequently). SMRs were also greater in four of the five locations where workers were hired between 1950-1959. In the total cohort, decade of hire was one of the strongest correlates of lung cancer mortality risk. Duration of employment had no effect. However, this surrogate for exposure is of minor relevance given the much higher exposures to beryllium prior to 1950 and the fact that 73% of the total cohort worked for less than five years in the beryllium industry.

Lung cancer SMRs based on US population and those based on local county mortality rates differed only slightly. The largest difference was found in the Reading cohort, Pennsylvania in which the SMR based on US rates was 1.24 and that based on the local county rate was 1.42. For all six locations, the lung cancer SMR based on US rates was 1.26 (95% CI, 1.12-1.42), whereas that based on local county rates was 1.32 (95% CI, 1.19-1.46). When lung cancer SMRs were adjusted for the effect of smoking habits at four of the plants in which a smoking survey was conducted in 1968 (covering 1466 (15.9%) of the 9225 persons of the cohort) the SMR for the total cohort changed from 1.26 to 1.12 and the SMRs in the two largest, oldest plants from 1.69 to 1.49 (Lorain, OH) and from 1.24 to 1.09 (Reading, PA). In addition, it is unclear if the assumption holds that smoking habits in the 1940s and 1950s when the exposures to beryllium were the highest were similar to those at the end of the 1960s years when the smoking survey was conducted. The authors estimated the contribution of smoking to lung cancer in the total cohort to be 13%, i.e., a SMR of 1.13 attributed to smoking compared to an SMR of 1.26 found in the total cohort.

Sanderson et al. (2001a) and (2001b)

A major limitation of the foregoing studies is the scarce knowledge of the exposure a) related to the time periods in the 1940s, 1950s and 1960s years and b) to the type of the jobs. The data from the two cohort studies using the BCR and from the study of Ward et al. (1992) suggest that workers were exposed to particular high beryllium concentration in their workplaces in the 1940s and 1950s years when there were few measures to reduce exposures. To improve the assessment of the workers' exposure to beryllium a job-exposure matrix for workers at the Reading plant was performed by the NIOSH group based on historical records on the duration of jobs and assessments on the basis of measurements in the air of workplaces (Sanderson et al. (2001a)). Since most of the measurements in the air of workplaces were carried out after 1970 and only few exist

from the time period before it is difficult to extrapolate to exposures during time periods of the 1940s and 1950s years when the exposures were probably the highest because of lacking ventilation and other measures for decreasing exposures at work places. It is known that first measurements at workplaces were initiated in 1947 after first reports had been published that beryllium may cause ABD and CBD and that further improvements of exposures to beryllium started about 1960 when a TWA of 2 μg/m^3 was introduced in some of the workplaces at the plant. Hence, it could be assessed that exposures in the 1940s and 1950s were considerably higher, probably by magnitudes, than in later decades when measures were gradually introduced. Also, between about 1960, 1970 and after about 1980 the beryllium exposures were gradually lowered by magnitudes. Despite these difficulties, the authors constructed a job-exposure matrix for more than 300 different selected jobs and different time periods. Nearly 200 measurements that had been carried out between 1947 and 1970 and nearly 7300 in the time period between 1971 and 1992 formed the basis for the job-exposure matrix. One of the main problems was the use of different sampling and analytical methods the results of which widely differed and were difficult to compare.

In a companion paper, a nested case-control study was conducted using the data of the previous cohort study of Ward et al. (1992) including a follow-up from 1988 until 1992 (Sanderson et al. 2001b), 142 lung cancer cases and 710 matched controls were identified within the cohort of 3569 male workers. Work history records including tenures were linked to quantitative, calendar-time-specific exposure estimates for each job to generate cumulative, average and maximum beryllium exposure metrics for each worker. Exposure metrics were generated by use of the job-exposure matrix (Sanderson et al., 2001a). Furthermore, tenures and exposure metrics were lagged 10 and 20 years to discount exposures that may not have contributed to causing lung cancer because lung cancer had already been induced, e.g. by earlier high exposures during short-term employments. By this method, analyses are restricted essentially to exposures that occurred early in calendar time, in the 1940s and 1950s. During this time period high exposures occurred and there were many short-term employees. Confounding by smoking was also evaluated. The majority of cases and controls were first hired during the 1940s with about 60% hired during 1941-1945. The average tenure was 3.7 years (median 5 months) for the cases and 5.5 years (median 11 months) for the controls. Almost two-thirds of the cases and over half of the controls were employed at the plant for less than 1 year. Comparison of the unlagged exposure metrics for cases and controls revealed that cases had significantly lower tenures and nearly significantly lower cumulative exposures. However, when beryllium duration of exposure and cumulative exposure metrics were lagged 10 and 20 years, the geometric mean tenures and cumulative exposure metrics were higher than those of the controls. The geometric mean average and maximum exposures were greater than for the controls and the difference was highly significant when the exposure estimates were lagged 10 and 20 years.

Analyses by quartiles of the tenure, cumulative, average and maximum beryllium exposure metrics lagging 0, 10 and 20 years showed significant increases of odds ratios ($p < 0.05$ or < 0.01) when lagging of 10 and 20 years was considered but the increases were not monotonic. The highest increases were found in the second and third quartiles. Using conditional logistic regression analysis of logs of continuous exposure variables strong positive associations were found with the log of the average or maximum exposure estimates lagged 10 and 20 years.

It is important to note that when logs were not taken, very few associations were found. The average and maximum categories of the cases and controls (including lagging of 10 and 20 years) were also compared by the exposure categories < 2 µg/m^3, 2-20 µg/m^3 , and > 20 µg/m^3. The odds ratios across these categories consistently increased with increasing exposure although not in a monotonic manner. Odds ratios for unlagged exposures increased up to 2.2-fold (not significant) whereas lagged exposures significantly increased by about 2.1- to 4.6-fold ($p< 0.05$ or < 0.01). The confounders smoking and other chemicals at the workplaces than beryllium were better controlled than in precious studies.

Levy et al. (2002)
Levy et al. (2002) performed a reanalysis of the Ward et al. (1992) study by use of the same cohort. The following changes of the outcome were achieved when different methods were applied compared to the study of Ward et al.:

SMRs for lung cancer rates were based on estimated lung cancer rates for industrialized cities instead of lung cancer rates for the US population or counties since the two largest plants of the study, Lorain and Reading, are located in highly industrialized areas and the majority of the workers that were employed in these plants resided in or adjacently to these two cities. As a result, when based on the rates for industrialized cities the SMR for Lorain dropped from 1.69 based on US rates (SMR 1.60 based on the county rate) to 1.14 (95% CI, 0.86-1.48). Similarly, the SMR for Reading dropped from 1.24 based on U.S. rates (SMR 1.42 based on the county rate) to 1.07 (95% CI, 0.89-1.28). Both calculations indicate no significant differences in lung cancer mortality between the study populations of these two plants and the lung cancer mortality in the respective highly industrialized cities

Levy et al. (2007)
Levy et al. analyzed the same population as the nested control study of Sanderson et al. (2001b) but revealed a different outcome of the odds ratios when comparing non-transformed versus log-transformed exposure metrics in particular in combination with lagging of 10 or 20 years. As a consequence, the odds ratios using non-transformed metrics were not significantly enhanced thus challenging the conclusion of the Sanderson et al. study of providing further evidence that beryllium is a human carcinogen. Levy et al. suggest that the main reason for these differences may be a bias hitherto undetected and not yet discussed in the literature that may occur by the matching procedure of cases and controls if there is a major difference in the average age of cases and controls in combination with lagging. In the case-control study, the controls were, on average, 9.7 years older than the cases (age at death or last observation when alive). The control of the imbalance is important since age itself is a major confounder of lung cancer but the imbalance found is normally directed against enhanced odds ratios if controls are older than cases. When combining the matching procedure with lagging, the situation may become more complex since also other possible confounders such as year of birth, age at hire or age at termination of employment may be imbalanced in a complex way.

Deubner et al (2007)
This study demonstrated that the Deubner et al. 2007, reported on an empirical evaluation of a complex study design that has been used repeatedly with subtle variations. The study found that empirical evaluation helped to understand the behavior

of the study design and to investigate reasons for the study design behavior that were not initially discernible when considered from a theory point of view. Using the Sanderson study, the researchers applied the design study to a closely related cohort using randomly selected probands as cases. Values for average exposures were assigned to probands equal to, greater than, and less than those assigned to controls (matches). Under certain lag scenarios the nested study design produced a finding of higher average exposure in probands compared to their matches even when this was clearly not the case. The Sanderson study design produced a biased case-control lagged exposure difference under the null hypothesis and could not distinguish qualitatively between null and alternate hypotheses. Originally interpreted as clearly establishing a beryllium exposure - lung cancer response relationship the study demonstrated that this relationship was an artifact of methods and correction of methods leads to conclusion that the slightly (and questionably) elevated SMR in this large, heavily exposed and long follow-up US beryllium worker cohort is not beryllium exposure related.

Williams, 1996
This study is an analysis of 30 people (majority were fluorescent lamp workers or machinists: died from respiratory failure) from the UK Beryllium registry (total of 69 proven cases). Autopsy data on 19 of the subjects was generated or previously published. The survival times, from onset of disease to time of death, ranged from 2-29 years. No relationship was found between length of exposure and survival time, although the individual with the longest survival time was a machinist with significant exposure. Lung cancer was not identified in any of the cases. A common observation in all workers was interstitial pulmonary fibrosis with varying degrees of cystic change and pulmonary granulomas. Fibrosis was primarily observed in the upper zones. Larger nodules was observed in some individuals and consisted of hyalinised fibrous tissue with granulomas and Schaumann bodies. Isolated Schaumann bodies were found in the majority of the cases. Extrathoracic granulomas were rare but identified in a few individuals (although limited data existed) and only observed in liver and lymph node. Although no data is presented, it is stated that atomic absorption spectrometry was performed on tissue from some individuals and demonstrated beryllium in 12/13 cases tested.

Schubauer-Berigan, 2008
The authors of this publication re-analysed the dataset by Sanderson et al., (2001b) but evaluated different potential confounding effects: adjustments for birth cohort or age at hire was specifically investigated. An increased risk of lung cancer was associated with cumulative exposure (20 year lag) and average exposure. This risk was higher in workers born before 1900 than 1900 or later. However, when adjustment for birth cohort or age at hire was included, cumulative exposure was not associated with risk but average exposure was associated with risk when a 10 year lag period was included.

Levy, 2009
The cohorts originally analyzed by Ward et al (1992) were re-analyzed in this study by using a Cox proportional hazards model. The focus of the study was primarily to look at the endpoints in the Ward et al study where an increased risk of lung cancer was found: earlier plants, increased latency, and decade of hire. Six covariates were included in the current analysis: date of birth, person-years of follow up, cohorts, employment tenure, date of hire, and age of hire. Smoking or other known risks of lung cancer was not included as confounding effects in this study. There was no increase in lung cancer in workers hired in the 1940s relative to workers in the 1950s. There was an increase in

lung cancer risk in workers hired in the 1950s relative to the 1960s; however it is speculated that the increased risk may reflect lower rates of smoking in the 1960s. An increase in lung cancers from workers at earlier plants was not found. Finally, when the covariates where taken into consideration, there was no effect of latency period on lung cancer risk.

Deubner, 2009
No new analysis is presented in this publication. It is a comment by Deubner on the current understanding of the relationship between beryllium exposure and lung cancer taken into consideration the comments by other epidemiologists. It is concluded that the Levy et al 2007 study is an improvement of the study by Sanderson et al, 2001. Finally, the Schubauer-Berrigan et al, 2008 study is considered the currently best analysis as it includes an additional confounder source. However, the author argues in this comment that more analysis needs to be done in order to determine the relationship between occupational exposure to beryllium and risk for lung cancer. The current knowledge indicates that employment duration or cumulative exposure to beryllium is not positively associated with lung cancer.

APPENDIX 5

Beryllium SMR Summary From Epidemiology Studies

Table 1 *NIOSH 2001 Beryllium Worker Lung Cancer SMR by Plant*

Facility	SMR	p	LC Cases	Population
Lorain	**1.69**	**<0.01**	**57**	**1,192**
Reading	**1.24**	**<0.05**	**120**	**3,569**
Lucky	0.82	>0.05	9	405
Cleveland	1.08	>0.05	44	1,593
Elmore	0.99	>0.05	15	1,323
Hazleton	1.39	>0.05	13	590
Multiple	1.67	>0.05	13	257
Unknown	1.33	>0.05	9	296
Total	**1.26**	**< 0.01**	**280**	**9,225**

Table 2 *Beryllium Workers SMR for Lung Cancer by Decade of Hire*

Decade	SMR	p	LC cases
1940s	**1.42**	**< 0.01**	**177**
1950s	1.24	> 0.05	85
1960s	0.62	> 0.05	18
Total	1.26	**< 0.01**	**280**

Table 3 *Beryllium Workers SMR for Lung Cancer by Years Worked*

Years	SMR	p	LC Cases
< 1	**1.32 s**	**< 0.01**	**152**
1-5	1.19 ns	> 0.05	61
5-10	1.26 ns	> 0.05	21
>10	1.19 ns	> 0.05	46
Total	**1.26 s**	**< 0.01**	**280**

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