Chemical Substances and Biological Agents

Studies and Research Projects

REPORT **R-750**

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Summary

In recent years, many authors have questioned the level of protection that the accepted threshold value for beryllium (Be) and its salts provides to workers. This value is the TWAEV (Time-Weighted Average Exposure Value, sometimes simply called the TWA), and referred to in Québec as the VEMP; the TWA (Time-Weighted Average) is equivalent to the TLV (Threshold Limit Value) developed by the ACGIH (American Conference of Governmental Industrial Hygienists). The predominant effects associated with beryllium suggest that the risks are based on its speciation. Moreover, it appears that its fine particles constitute the part that is of interest in the occurrence of such effects. The objective of this research is to evaluate the toxicity of Be according to its chemical form and particle size.

For each chemical form covered by this study (Be, BeO, BeAl), toxicity was assessed following sub-chronic exposure, through oro-nasal inhalation, to either fine or total particles. For this purpose, an animal model (mouse) was used. A total of 245 mice were used. They were divided into seven groups, each of which had 35 mice. One group served as a control group, while each of the other six were exposed to either fine particles or total particles for each of the three chemical forms of Be. The duration of exposure for each group extended over a three-week period consisting of 15 days of exposure (5d/w, 6h/d). At the moment of sacrifice, multiple tissues (lung, spleen, liver and kidney) and blood samples were collected and immediately frozen until they were analyzed to determine their Be content. In addition, certain lungs and spleens were analyzed to evaluate immunological sensitivity and lung inflammation.

Many correlations were established between the tissue concentrations of Be and effects normally observed in workers following exposure. The histological sections showed that mice exposed to Be had levels of lung inflammation similar to those observed in patients with chronic beryllium disease (CBD). The findings also show an association between particle size, lung concentrations, lung inflammation, production of certain cytokines and the expression of certain lymphocytes.

The effects also depended on the chemical form of Be. Thus, Be metal and BeO were thought to be the most toxic forms. It became clear that aerodynamic diameter and solubility or insolubility played a significant role in deposition and lung retention.

The research findings will contribute to an understanding of the role of particle size and chemical form in Be toxicity. Combined with other findings, they will also guide preventive action relating to Be exposure, and may include a revision of the threshold limit value and the establishment of limit values based on the chemical form and particle size.

Table of Contents

List of Tables

List of Figures

1. The problem

In recent years, many authors have questioned the level of protection provided to workers by the accepted threshold value for beryllium (Be) and its salts (TWAEV [Time-Weighted Average Exposure Value], referred to in Québec as the VEMP[1]; this TWA (Time-Weighted Average) is equivalent to the TLV (Threshold Limit Value) of the ACGIH [American Conference of Governmental Industrial Hygienists]). Recently, certain workers in Quebec and elsewhere in the world have been sensitized to beryllium or have developed berylliosis (beryllium disease), and this has challenged the scientific community to better understand the underlying problem of exposure to Be and its salts.

The first case of Chronic beryllium disease (CBD) diagnosed in Quebec arose in 1998, involving an employee in a factory using Be. In February 1999, the *Commission de la santé et de la sécurité du travail du Québec* (CSST, Quebec's occupational safety and health commission) accepted a claim for compensation made by a foundry worker who had been diagnosed with berylliosis in 1998. Subsequently, a number of claims were submitted to the CSST. By the end of 2000, an initial analysis of the scientific literature had demonstrated that the situation in Quebec was problematic. About 40% of the workers in foundries were exposed to concentrations higher than 0.2 μg/m³. In Quebec, about 100,000 workers were exposed to these concentrations (CSST, 2004, Brousseau, 2006). Cases of CBD were discovered even in some schools that complied with the standards (Noranda, 2001).

In early 2001, the CSST and some of its partners (the *Réseau de la santé* [Health Network], of the *Institut de recherche Robert-Sauvé en santé et sécurité du travail* [IRSST] and the joint sector-based associations that were affected) established Operation Beryllium to take stock of the use of this metal and worker exposure to it.

[1] The value-weighted average exposure for a period of 8 hours / day, based on a 40 hour week.

2. The state of scientific knowledge

2.1. Characteristics of beryllium

2.1.1 Origins

Beryllium was discovered in France in 1798 by Louis Nicolas Vauquelin. The name "beryllium" was given to the metal in 1949, at which time it replaced the original name "glucinium", the designation it had acquired due to its sweet taste. It is distributed ubiquitously in the earth's crust at concentrations generally ranging from 2.8 to 5.0 mg/Kg (Kolanz, 2001). Be does not exist in a pure state in nature. It is found together with 45 natural elements, five of which are important in terms of industrial exploitation. These are beryl, bertrandite, phenacite, chrysoberyl and gadolinite. The two main beryl-bearing ores are bertrandite, composed of less than 1% Be, and beryl in which the proportion goes up to 4% Be. World reserves are estimated at approximately 80,000 tonnes, of which 65% are in the United States (INRS, 2003). Be mines are also located in Brazil, South Africa, China, Madagascar and Zimbabwe (Williams, 1994).

2.1.2 Physico-chemical properties

Be is a shiny grey metal, very hard, very light and having great elasticity. It has an atomic number of 4 and, with a molecular weight of 9.01218 Daltons, is the second lightest metal. It has the same density as magnesium, is three times lighter than aluminum and six times stiffer than steel. It is resistant to pressure, heat, vibration and corrosion. For example, copper is six times more resistant in the presence of 2% Be (Plante et al., 2002). The melting point of Be, 1287 ° C, is very high, and it retains its shape despite very large variations in temperature (Bruce and Odin, 2001). Transparent to X-rays and microwaves, Be is both a transmitter and a reflector of neutrons by alpha bombardment (Billen, 1983; WHO, 1990; Babadzhanova and Bosanko, 1997). It is an amphoteric whose crystal structure is hexagonal, and is an excellent fluorescent product (Bruce and Odin, 2001). Thus, it is a metal whose physical and mechanical properties are much sought-after, especially in the aerospace, energy, electrical and electronic sectors. In general, Be and its compounds are very slightly soluble in water, with a solubility coefficient of 0.2 for some of these compounds (INSPQ, 2004). However, when in salt form, it is more soluble in water.

2.1.3 *Uses*

There are many Be compounds, including beryllium oxide (BeO), beryllium sulphate (Be (SO4)), beryllium fluoride (BeF2) beryllium phosphate (BeHPO4) and beryllium hydroxide (Be (OH)) (Schepers, 1964), and alloys such as beryllium copper (Be-Cu), aluminum-beryllium (Be-Al) and nickel-beryllium (Be-Ni) (ATSDR, 2002).

Metallic Beryllium, alloys of Be and oxides of Be, are the most important products for trade obtained from the transformation of beryllium hydroxide, which results from acid extraction of the ore (Bruce and Odin, 2001). Beryllium metal is mainly used as a neutron moderator and reflector in nuclear reactors, in windows for X-ray tubes and in the nuclear weapons industry (INSPQ, 2004). Alloys, mainly copper and, to a lesser extent, aluminum, magnesium, nickel, zinc and zirconium, have many applications in the aerospace, electronics and engineering industries, as well as in the manufacture of sporting goods such as golf clubs (INSPQ, 2004). True Beryllium-aluminum alloys contain between 20% and 62% Be. Their use is still restricted to specialized applications such as structural materials for aircraft and aircraft parts, brake callipers for Formula-1 cars, and precision equipment parts. Other aluminum alloys contain Be, though with a much lower content (less than 0.07%). Among other things, they are used as a structural material in the aerospace industry, as well as in many other applications requiring greater strength.

Be oxides are used as moderators in nuclear reactors. They are also used to manufacture ceramics in the electronics, automotive, microelectronics, laser and microwave tube sectors (INSPQ, 2004). The soluble salts of Be sulphate and Be fluoride are mainly intermediate products in production processes (Bruce and Odin, 2002).

2.2 Sources of exposure

Exposure to Be may be environmental or occupational (Willis and Florig, 2002). In the air, concentrations hover around 0.0002 µg/m^3 in an urban environment (ACGIH, 1998). CBD cases of environmental origin are still rare (Eisenbud et al. 1949). On the other hand, the risk of developing Be-related health problems has challenged scientists and policy makers to combine their efforts to regulate this form of exposure. Thus, the standard of environmental exposure was set by the United States at 0.01 µg/m³ (ATSDR, 1993, USEPA, 1998). It should be noted that no environmental standard has been set in Quebec (INSPQ, 2004).

In soil, average ambient concentrations of Be range from 2.8 to 5 mg/kg (Reeves et al. 1986) while concentrations found in water range from 0.001 to 0.01 µg/L (EPA, 1980). Cultivable soils contain from one to 7 mg/Kg, with a mean of 0.6 mg/Kg. There are a few areas where average concentrations may reach up to 60 mg/Kg, while the maximum is about 300 mg/Kg. Be is found in foods at concentrations ranging from 0.2 to 2200 µg/kg, first and foremost in a particular variety of the French bean and in soybeans. Based on calculations made in 1987, the daily intake of Be in the general population is estimated at about 0.423 µg, of which 0.12 µg is via food and 0.3 µg via water. It has been determined that the concentration of Be in water should not exceed 4 µg/l (USEPA, 1998).

Cigarette smoke is another potential source of exposure. Thus, Be concentrations were estimated at, respectively, 0.47, 0.68, and 0.74 µg/cigarette in tobacco found in three different brands of cigarettes. If one estimates that 10% of these

concentrations are transmitted into the smoke, then a person who smoked 20 cigarettes a day would be exposed to concentrations of about 1.5 micrograms of Be/day, equivalent to three to four times the exposure associated with ambient air, water, food or the ingestion of dust (Bruce and Odin, 2001).

People with certain dental alloys (nickel-beryllium) have an additional source of exposure to Be. The level of exposure of these individuals is not known, but the dosage of these alloys indicates that they may contain from 0.5 to 2% Be (US OSHA 2002).

In view of its advantageous properties, Be is increasingly used in high technology industries. Thus, countries are increasingly facing problems associated with worker exposure to this metal and its compounds.

In the workplace, inhalation is the primary route of exposure of workers; skin does not absorb Be very well. Be in the workplace is found mainly in particulate form -- in powder, dust or fumes. Workers with significant potential exposure are those working in Be mines, Be alloy manufacturers, missile and nuclear reactor technicians, and workers in electrical and electronic equipment (NIEHS, 2003).

The United States was the first country to establish exposure limits in the workplace. Prior to 1950 and the implementation of emission control systems, it was not uncommon for workers to be exposed to high Be concentrations of up to one mg/m^3 in the air (Eisendud and Lisson, 1983). Concentrations subsequently declined, even though in the 1960s workers in metal extraction plants could still be exposed to over 50 $\mu g/m^3$; in the 1970s, to over 30 $\mu g/m^3$; and in the late 1970s, to less than 2 $\mu g/m^3$ (Kriebel et al., 1988). Recently, a study revealed that about 134,000 workers in private and public sectors across the United States were potentially exposed to Be (Henneberger et al. 2004).

The nature of the task and the means employed to protect workers are also factors that may influence exposure. For example, the workstations and techniques used to perform various tasks are important in the etiology of the disease. Thus, machinists have a higher exposure risk than other workers in factories using Be, since crushing, sawing and polishing processes generate dust and aerosols that are small in size or mass. Some studies have also demonstrated that in smelters and foundries Be concentrations can be high, with average values of about 0.87 $\mu g/m^3$ (Johnson et al. 2001).

In fact, many factors can influence the level of exposure. The main ones are related to the diameter of the particles, their number, their morphology, their chemical form, and the duration and route of exposure (Henneberger et al. 2001, Kent et al. 2001, Kreiss et al. 1996; Paustenbach et al. 2001).

Aside from workers assigned to the extraction and production of Be, in which exposure is much more controlled nowadays, the workers currently most at risk are

those who perform machining operations on parts or components made out of Be or alloys containing Be. In some Quebec smelters, several workers were exposed for several years, without protection, at levels well above the standard.

In addition, many studies have demonstrated that sensitization and CBD may occur in industries where exposure levels are well below 2 μg/m³ for 8 hours (Cullen et al. 1987, Kreiss et al. 1996; Kreiss et al. 1997). Thus, it is clear that various factors influence both the exposure and the risk that results from this exposure.

2.3. Reference values and standards

The limit value of 2 μg/m^3 for occupational exposure to Be was decided in 1949 by the Atomic Energy Board (U.S.). It was not until 1959 that this same value was adopted, first, by the American Conference of Governmental Industrial Hygienists as a TLV ® (Threshold Limit Value ®), and then, in 1972, by the Occupational Safety and Health Administration of the United States (OSHA) (Kolanz, 2001).

In Quebec, the Regulation Respecting Occupational Health and Safety establishes exposure standards in the workplace for many substances. In January 2007, the TWAEV (= TWA = VEMP for Quebec) for Be was set at 0.15 μg/m^3. Before then the exposure limit was 2 μg/m^3.

In the late 1980s, the introduction of the blood lymphocyte proliferation test for Be (BeLPT), as well as more sophisticated diagnostic tools, allowed much earlier identification of cases of sensitization and disease. Incidence rates similar to those that existed before the adoption of the standard, or about 2% of exposed workers, were recorded (Newman, 1996). A study published in 2001 noted that it had found cases of CBD in which, theoretically, there had not been exposure to concentrations greater than 0.5 μg/m^3 (Strange et al., 2001). The results of a study spanning 4 years showed that workers exposed to Be levels higher than 0.01 μg/m3 had BeLPT values much higher than those of workers exposed to lower levels (Yoshida et al., 1997).

It is possible to achieve and even exceed the TLV if particles of material containing Be are emitted into the air, even if the Be content of the material or the emission rate of the particles is low. Thus, doubt was cast on the relevance of the TLV. Some researchers had already suggested reducing it to 0.1 μg/m^3 (Wambach et al, 2000). Also, the ACGIH (2009) adopted a new threshold of 0.05 μg/m^3 for inhalable particles (<100 μm in diameter), while in 2007 the Quebec government adopted a limit value of 0.15 g / m^3 as a TWAEV (2007).

2.4. Toxicokinetics

2.4.1. Absorption

Absorption into the body occurs primarily via the respiratory tract following inhalation of smoke or dust. Deposition in the lungs depends on particle size (especially aerodynamic diameter), form and solubility. For insoluble compounds, pulmonary clearance is very slow, and whatever has not been eliminated rapidly by mucociliary activity and phagocytosis is retained for several months in the lungs and gradually released into the blood. For soluble compounds, pulmonary clearance occurs rapidly through dissolution into lung fluids, while a variable proportion enters the bloodstream.

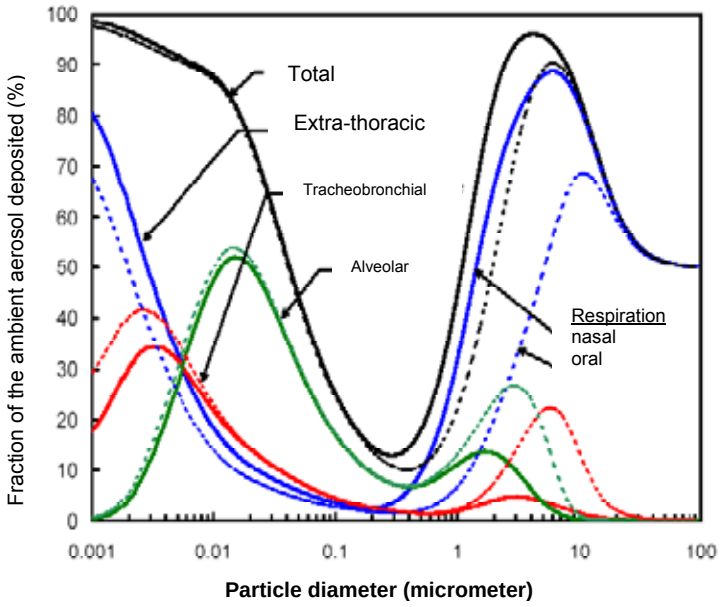
Be is very poorly absorbed through intact skin because it binds to constituents of the epidermis, namely, proteins and nucleic acids, to form complexes that are poorly diffusible. However, it is thought that skin contact may play a role in sensitization, especially following exposure to fine particles. Tinkle et al. (2003) have shown that fine particles of fluorescent dextran (0.5 µm-1 µm) in combination with a motion of the skin can penetrate the *stratum corneum* (horny layer of epidermis), the dermis and epidermis of the skin and initiate an immune response.

Be is very poorly absorbed (less than 1%) through the gastrointestinal tract. The amount absorbed depends on the dose and solubility of the compounds and is limited by the formation of insoluble colloidal phosphate in the intestine.

2.4.2 Pulmonary deposition of dust in humans

The lungs are the main routes of entry of dust into the human body. The deposition of dust along the pulmonary tree varies greatly according to the size distribution of the dust and its behaviour in the air. Normally, for coarser dust, there is an increase in the proportion of inhaled dust deposited in the alveoli with each decrease in particle diameter; this proportion reaches maximum values of around 20% and 15% for particles of 3 µm and 2 µm respectively. This percentage then declines gradually. On the other hand, it is not the smallest particles that will necessarily settle in the alveoli (IRSST, 2006).

Figure 1 shows the rate of deposition in different lung regions according to particle size. This figure clearly illustrates that no particle of one nm in aerodynamic diameter, or 0.001 µm, reaches the alveoli, whereas 80% are deposited in the nose and pharynx, with the other 20% located in the area of the trachea and bronchi. For inhaled nanoparticles of this size, retention is nearly 100% (IRSST, 2006).

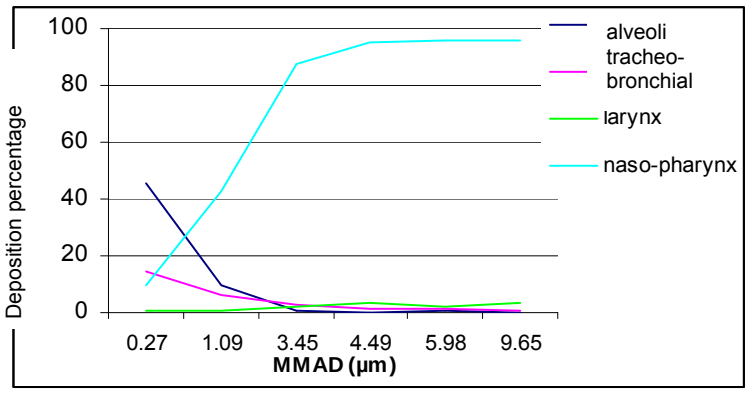


Source: IRSST, 2006

Figure 1 - Prediction of total and regional deposition of particles in the respiratory tract of humans according to particle size.

2.4.3. Pulmonary deposition of dust in mice

Raabe et al., (1988) have published a study on the deposition of monodisperse aerosol of aluminosilicate particles on the lungs of mice (CF1) of 25 to 35g, aged 10 to 15 weeks. The study showed an increased rate of deposition in the region of the nasopharynx for particles of at least 3µm, with a parallel decrease in the rate of alveolar deposition for these particles. For particles measuring at least 10µm, the alveolar deposition was almost zero, while depositions in the nasopharynx and larynx were significant (see Figure 2).



Based on data from Raabe et al., 1988.

Figure 2 – Lung deposition in mice

2.4.4. Distribution

The absorbed Be is carried into the body on plasma proteins in the form of colloidal phosphate. In the short term, the Be accumulates in the liver, especially in cases of significant exposure. In the long term, it is found mainly in the lymph nodes and the bones. Be has also been identified in the blood of the umbilical cord and maternal blood.

2.4.5. Metabolism

Be is not metabolized. In the lungs, soluble salts of Be are partially transformed into insoluble salts. The immunological action seems to depend on the formation of a beryllium-protein complex, due to the low molecular weight of Be.

2.4.6. Excretion

For compounds absorbed in the body, excretion occurs mainly via the urinary tract. For non-absorbed compounds, excretion is primarily through feces or by mucociliary clearance, and depends on the solubility of the compounds. Be has also been identified in milk and in the colostrum. Mobilization and excretion may continue for several years and persist long after cessation of exposure.

2.4.7. Half-life

There is no precise data on humans. Based on animal data, clearance in the lung tissue occurs in a biphasic manner for insoluble, slightly soluble and soluble compounds. Initially, 30% to 50% of the Be is eliminated, with a half-life of several days. A second phase, which varies according to the degree of solubility of the Be, suggests that the half-life for soluble compounds is of the order of a few weeks or less, whereas for compounds with little or no solubility it can be a few months or even years. The half-life in the whole body can be several years.

2.5. Pathologies attributable to Be

2.5.1. Sensitization

This phase, characterized by the proliferation of blood lymphocytes that react to exposure to Be (BeLPT) is of greater or lesser duration and is asymptomatic (Rosenberg, 1993). The test can be performed on liquid collected by bronchial lavage (BAL-BeLPT). Some individuals develop the disease even if exposure is stopped, while others do not. Sensitization reveals, among other things, an increased susceptibility of the individual to developing the disease. In some cases, symptoms and signs consistent with beryllium disease are present even when the sensitization tests are negative.

2.5.2 Chronic beryllium disease

Chronic beryllium disease (CBD) is characterized by the development of granulomas, coupled with the formation of interstitial fibrosis, both resulting from an immune response specific to Be. In patients with CBD, the Be acts as a class II antigen and stimulates local proliferation and accumulation in the lungs of Be-specific CD4 + cells. The discovery of Be-sensitive cells in the lung was a significant contribution to demonstrating that Chronic beryllium disease is an immune lung disease (Rosenberg, 1993).

2.5.3 Acute beryllium disease

Acute beryllium disease reflects the direct irritant effect of Be on mucous membranes - including those of the respiratory tree - as well as on the skin, and arises when there is exposure, even brief exposure, to relatively high concentrations. In the respiratory system, it is chemical pneumonitis, which, nowadays, is no longer observed except in accidental situations (Rosenberg, 1993). Acute beryllium disease is often completely reversible; it results from a toxic process rather than an immunological process. It is diagnosed by chest radiography, and through evaluation of pulmonary gas exchange, in workers exposed to high concentrations of Be. No cases of acute beryllium disease have been observed in workers whose exposure was not greater than 15 µg/m^3.

2.5.4 Cancer

In Quebec, the regulation respecting health and safety classifies Be as a carcinogen found in humans (Class C1). According to the Environmental Protection Agency (U.S. EPA, 1998) Be is classified in Class B1 (probable carcinogen), while the International Agency for Research on Cancer (IARC, 1993) has classified it as carcinogenic to humans (Group 1). Animal studies on several species and through different routes of administration have demonstrated the carcinogenic properties of this substance (INSPQ, 2004).

2.6. Risk factors

Many factors can affect the probability of workers developing a disease attributable to Be. Among these factors are genetic predisposition, the types of work related to various industrial processes, the chemical forms of Be associated with different toxicities, particle size and routes of exposure (Kolanz, 2001).

2.7. Toxicity based on animal models

Several animal studies have helped to document Be toxicity. These began as early as 1940 (Vorward, 1950) and rapidly demonstrated both acute and chronic Be toxicities (Hyslop et al., 1943). However, studies that are more recent have revealed several similar responses in animal models and humans.

For example, Barna et al. (1981) exposed Guinea pigs to Be oxide (BeO) through intratracheal instillation and found that certain strains developed granulomas. Hayley et al. (1989) conducted studies on acute toxicity by exposing dogs to BeO and found sporadic positive responses to BeLPT. In addition, several researchers have attempted to develop a rodent model to reproduce the CBD encountered in humans. Thus, Votto et al. (1987) have described granulomatous lung injury induced by beryllium sulphate ($BeSO_4$) in rats exposed through intratracheal instillation.

The U.S. Agency for Environmental Protection has reviewed studies on different animal models and concluded that none of them reproduced exactly all the effects observed in humans (EPA, 1998). However, recent studies conducted on mice suggest that this species may be a suitable model. First, Huang et al. (1992) have described a granulomatous lung disease in a strain of mice (A/J) and a slight BeLPT response was observed. Other strains of mice such as C3H and HeJ were subsequently investigated by Finch et al. (1998 and 1996) and by Nikula et al. (1997) who observed the presence of lymphocytic aggregates and pneumonia with granulomatosis.

These studies thus show that in several ways a number of pulmonary responses observed in mice exposed to Be compounds are similar to those observed in humans (Table 1). These include the presence of micro-granulomas and mononuclear infiltrates, the general proliferation of lymphocytes and a specific accumulation and growth of T cells in response to Be (Finch et al, 1996). This parallel with the response observed in humans enabled the authors to consider developing a promising and significant mouse model. In their view, this model should facilitate not only studying the cellular and molecular mechanisms of the responses involved in the CBD, but also examining the influence of the physicochemical form of Be and its mode of exposure (Finch et al. 1996).

Table 1 - Comparison of responses: CBD in humans and two mouse strains (C3H/HeJ and A/J)

Responses	Humans	Mice
Microgranulomas	+	+
Lymphocyte proliferation	+	+
Increase in T cells	+	+

Adapted from Finch et al. 1996

3. Research objectives and hypotheses

The research objectives are to assess and compare the toxicity of Be among Québécois based on the main chemical forms of Be found in industry (Be, BeO and BeAl) and on particle size. These objectives are based on several hypotheses: 1) The different chemical forms of Be and the variable morphology of its particles do not have the same risk potential; 2) compliance with the threshold values does not protect against all chemical forms of Be or all Be particles regardless of their size distribution.

4. Research method

The toxicity evaluation was carried out using an animal model, with nine-week-old male mice (C3H/HeJ) (The Jackson Laboratory, Bar Harbor, ME). All animal protocols were in accordance with rules laid down by the CIPA (*Comité institutionnel de protection des animaux* ["Institutional Committee on Animal Care"]) at the *Université du Québec à Montréal* (UQAM) in Montreal.

For each chemical form, subacute toxicity was evaluated following exposure through inhalation (oro-nasal) to fine particles "F" and total particles "T". Note that the classes F and T were used to compare the two MMAD (mass median aerodynamic diameter) having the same chemical form.

The duration of exposure for each group was 3 weeks, including 14 days of actual exposure (5d/wk, 6h/d). In addition to exposure, the mice were placed in individual cages and could eat and drink ad *libitum*. These cages were kept in depressurized Canadian Cabinet safety cabinets, fitted with air inlet filters of the HEPA type.

A total of 245 mice were used. The mice were divided into seven groups, each of which had of 35 mice. One group served as a control, while the other six were exposed to either fine particulate matter (F) or to total particulate matter (T) for each of the three chemical forms of Be. The sample size took into account a possible loss 5 mice/group and was based primarily on the need to "pool" the blood of 3 mice at a time for assessment of the effects (see more on this later in the method section).

The level at which the mice were exposed was derived from the only sub-chronic toxicity study based on immunological and lymphocyte effects. The Stiefel et al. study (1980) facilitated observation in rats of increased T-cell activity following exposure to 500 µg/m^3 for 10 weeks, 6 hrs per day. To derive an exposure level for mice, it was necessary to take into account the difference between rats and mice in terms of pulmonary deposition rate based on particle size, and in terms of inhalation rate and body weight. By proceeding in this way, the derived target exposure level to which the mice were exposed was 250 µg/m^3.

4.1. Oro-nasal exposure

The mice were exposed to particles of Be, BeO and BeAl obtained from the supplier Brush Wellman Inc. (Product Stewardship Department) Mayfield Heights, Ohio 44124. The particles had not been treated before being used.

The exposure was carried out using 22.8 L nose-only inhalation chamber (Model 04-1100, Intox Products, Albuquerque, N. Mex.) and an exposure system equipped with 48 windows to accommodate 48 rats or mice, as originally described by Raabe et al (1973).

The Be particles were generated by a particle generator (fluidized bed apparatus, model 3400 TSI Inc., St. Paul, MN), mixed with filtered air (HEPA) and pumped into the inhalation chamber at a rate of 22 L air/min, equivalent to a rate of 60 air changes per hour during the exposure. To obtain fine particles, an ASME stainless steel tank (Model 73, McMaster-Carr, NJ) was added to the generator to act as a cyclone.

Be concentrations in the inhalation chamber were checked continuously using a Dust Track brand aerosol monitor (Model 8520, TSI Inc.). This device is a portable laser photometer that measures, in real time, dust particles of between 0.001 $\mu g/m^3$ and 100 mg/m^3. A double check was performed by taking biweekly air samples. Sampling was carried out using a Gilian pump (Gilian Corp., West Caldwell, NJ) and a particle collection device (filter cassettes). For the fine fraction, a Dorr Oliver 10 mm nylon cyclone was used, while a closed cassette with 4 mm inlet orifice was used for total Be. Filters made of mixed cellulose ester (MCE) (Omega M-083700AF) with a diameter of 37 mm and a porosity of 0.8 microns were used. The pumps were calibrated before and after each sampling with a Gilibrator (Gilian Corp.). A constant flow of 1.7 L/min was used for respirable particles and another of 1.5 L/min for total particulate.

The particle-size distribution for the aerosols in the inhalation chamber was determined by evaluating the mass median aerodynamic diameter (MMAD). It was also carried out once/exposure-week using a seven-staged cascade impactor (Marple Personal Cascade Impactor, Series 290). In addition, for fine particles, sampling was also carried out once/week using the MOUDI (Micro-Orifice Uniform Deposit Impactor), Model 100, MSP Corp., Minneapolis, Minn.), which is a low-pressure impactor. This impactor allows measuring mass distribution as a function of a MMAD of between about 0.05 microns and 10 microns.

4.2. Sacrifice

The sacrifice of the mice took place one week after the end of exposure. In addition, five mice per group were sacrificed 3 weeks after the end of exposure. Several tissues (left lung, spleen, liver and kidneys) were collected and immediately frozen until analysis for determination of their Be content. In addition, blood samples (1-2 mL) were collected at the femoral artery with a heparinized syringe.

4.3. Determination of beryllium in biological matrices

Analyses of Be in various tissues were carried out via ICP-MS using two methods developed and implemented in IRSST laboratories and inspired by Krachler et al. (1996). For the determination of Be metal and BeAl, biological matrices were digested in the presence of 6 ml of a mixture of nitric and perchloric acid (3:1). To do this, the samples were heated on a plate until the acid mixture completely evaporated and then reconstituted in a final volume of 10 mL of 1% nitric acid. For BeO, 500 µL of sulphuric acid were added to 6 mL of the nitric and perchloric acid mixture (3:1) to ensure complete dissolution of the BeO particles. The samples were heated on a plate until complete evaporation of the acid mixture (except for the sulphuric acid, which does not evaporate), then reconstituted in a final volume of 14 mL of 1% nitric acid (1% sulphuric acid).

4.4. Assessment of effects

After sacrifice, each mouse was assessed to determine its immunological sensitivity and lung inflammation. The immunological sensitivity was determined by using the test for lymphocyte proliferation induced by Be, the BeLPT (Beryllium lymphocyte proliferation test); this is a standard test used to diagnose CBD in humans. This analysis was performed on the spleen cells and on the infiltrating lymphocytes through bronchoalveolar lavage (BAL) of the specimens.

For the bronchoalveolar lavage, a volume of 5 mL of saline water was used to prepare between 5 and 8 aliquots of about 0.8-1 mL each. The lavage was performed on the right lung using five aliquots of saline water, successively injected intratracheally and then removed. This allowed approximately 4 mL to be removed. The liquid thus obtained was centrifuged and the cell pellet separated from the supernatant, which was frozen at -80 ° C for future analysis. Then the cell pellet was re-suspended with complete medium (RPMI 1640 with 20% fetal bovine serum, penicillin/streptomycin, 1-glutamine and HEPES).

The cells were placed in a culture medium at a concentration of 2.5 x 10^4 /mL in 96 well plates. The BAL or spleen cells were cultured in a medium with three doses of $BeSO_4$ (1 mm, 10 mm and 100 mm), while maintaining the standard protocols for clinical diagnosis of CBD. A positive control concanavalin A (CON A) was also included. All operations were performed in triplicate. The cells were cultured in a humidified incubator at 37° C, 5% CO_2 for a period of 6 days. On the 5th day, 1µCi/mL of tritiated thymidine (3[H]thy) was added and the cells incubated for 18 hours. Cell proliferation was measured by adding tritiated thymidine using an automated harvester and a beta-scintillation counter completing one count per minute (CPM) of 3[H] thy.

A stimulation index was calculated by measuring the CPM (counts per minute) or SI (stimulation index, equal to the ratio of CPM of stimulated cells compared to non-stimulated cells) and control samples, referring to the “mp” protocol of the US

Government Department of Energy (available on the website: http://tis-nt.eh.doe.gov/be/). An index stimulator ≥ 3 is considered positive for sensitization to $BeSO_4$.

The lymphocytes obtained from the spleen cells were cultured *in vitro* and exposed to a solution of Be sulphate to stimulate their proliferation. Studies have shown that only Be salts whose concentrations vary between 0.1 and 100 µM, and not the salts of other metals, stimulate the proliferation of lymphocytes. The use of tritiated thymidine thus boosted test performance. Counting with a scintillation camera measures the incorporation of DNA precursors marked with a radionuclide and is an indicator of cell proliferation. Thus, BeLPT enables the detection of sensitization and has also been recommended as a screening tool for CBD because it can identify individuals at risk before they develop symptoms.

Following the bronchoalveolar lavage, the right lung was distended with an OCT (Optimal Cutting Temperature) fluid and the lung was divided into sections. One part was placed in wax paper and the other part frozen at -80 ° C. The part placed in the wax paper was cut into sections stained with hematoxylin and eosin, or, for eosinophils, with the Giemsa mixture. These histological sections were then examined, and classified according to presence of granulomas, inflammation of infiltrated cells, collagen deposition and pulmonary fibrosis. The part that was kept frozen was also cut and its sections were identified by way of immunocytochemistry for the presence of CD4+ T-lymphocytes, CD8 + T-lymphocytes, CD19 and interferon gamma (INF-γ).

4.5. Statistical analysis

The quantitative results are presented in tables and figures using means and standard deviations (SD). In the figures, the vertical bars represent standard deviation. The qualitative results are presented using percentages.

For the quantitative parameters, the comparison of groups was performed using one-way analysis of variance. Following a significant ANOVA, contrasts comparing two groups were estimated and tested using the Tukey procedure to control the overall probability of committing a type I error. The basic assumptions, such as the normality of the residual values and the homoscedasticity of variance were verified using the Shapiro-Wilk and Levene tests. This revealed that no results were significant.

The significance level was set at 0.05 for all tests. All statistical analysis was performed using an SPSS software package (SPSS Inc., 233 South Wacker Drive, Chicago, IL, USA).

5. Findings

5.1. Concentrations et characteristics of beryllium

The concentrations obtained in the inhalation chamber (Table 2) were close to the target concentration of 250 µg/m^3. The temperature and relative humidity values obtained were, respectively, 22-25 ° C and 25-40%.

The MMAD obtained for the various chemical forms varied between 0.4 µm and 6.5 µm (Table 2). Moreover, contrary to expectations and considering that the MMAD obtained for BeO-F and BeO-T were identical and included only in the fine fraction, the data for this chemical form will henceforth be grouped together with the presentation of the findings as a whole. In addition, for purposes of validation, the BeO-F dust was analyzed by electron microscopy. The findings are presented in Appendix 1. They converge with the analysis of data obtained by the impactors that allows us, in particular, to consider as plausible the hypothesis of a bimodal population of particles in which a lognormal function was utilized to represent a polydisperse distribution of the large particles.

Table 2 - Mean concentrations of Be and MMAD

Chemical forms	Density	MMAD (µm ± SD) (n=5)	Concentration (µg/m^3 ± SD) (n=5)
Be-T	1.85	4.1 ± 0.71	285 ± 32
Be-F	1.85	1.5 ± 0.12	254 ± 31
BeO-T	3.01	0.4 ± 0.14	267 ± 27
BeO-F	3.01	0.4 ± 0.03	248 ± 10
BeAl-T	2.30	6.5 ± 1.96	275 ± 08
BeAl-F	2.30	4.4 ± 1.64	253 ± 49

Such particle sizes (diameters) result in rates of lung deposition in mice and humans that are quite different (Table 3). Note that in contrast to the wide variability in mice, the deposition rates in humans vary only slightly: 5% to 15%.

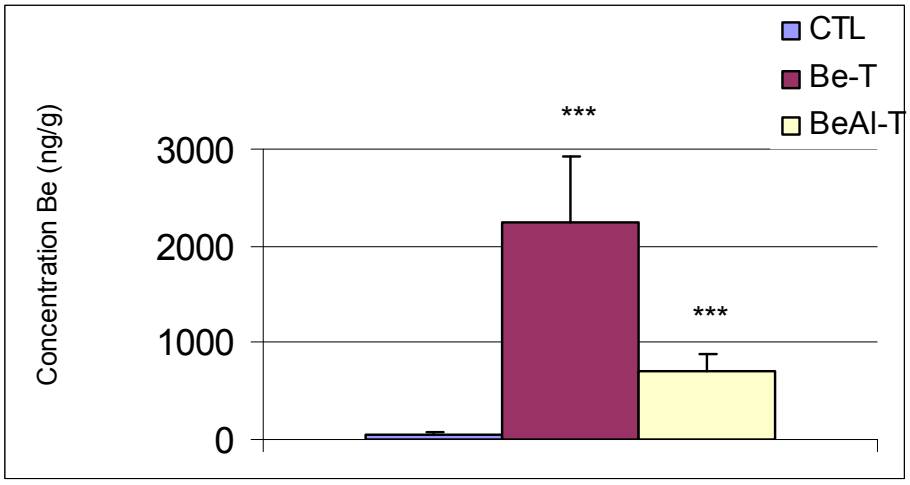
5.2. Blood and tissue concentrations

For all tissue concentrations, there were significant differences between the control group and the exposed groups. To avoid making the figures too cumbersome, these differences were not shown. Figures 3, 4, 5 and 6 show the tissue concentrations of Be in the exposed groups and the control group.

Table 3 - Percentage of lung deposition as a function of MMAD

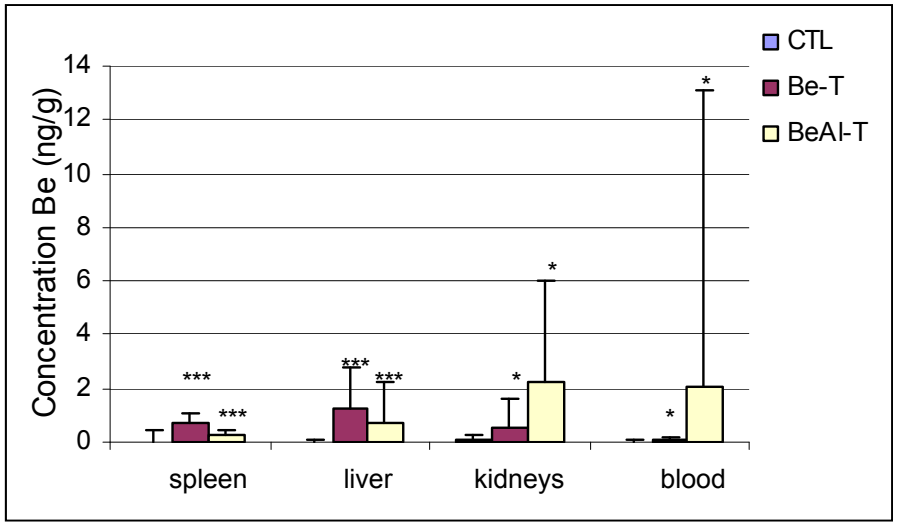
Chemical form	MMAD (µm)	Rate of pulmonary deposition in mice (%)*	Rate of pulmonary deposition in humans (%)*
Be-T	4.1	0.5	10
Be-F	1.5	11	15
BeO-F	0.4	40	8
BeAl-T	6.5	0.25	5
BeAl-F	4.4	0.4	9

Rates determined using data from Raabe et al., 1988 and IRSST, 2006.



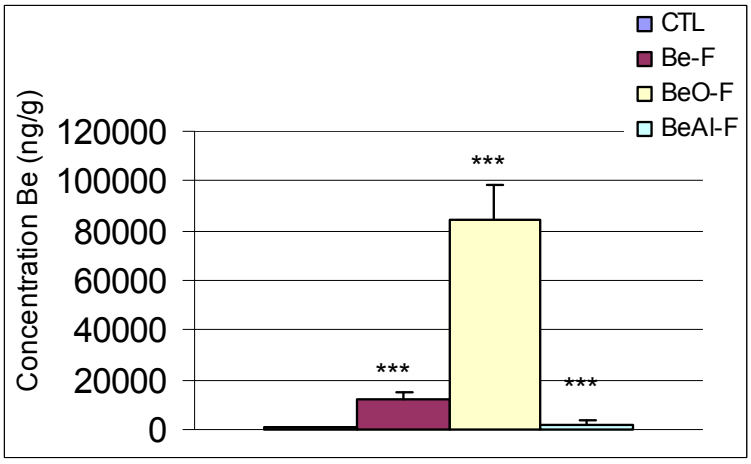
*** Significant difference ($p < 0.001$)
Concentration Be = Be concentration

Figure 3 – Mean lung concentrations (± SD) of beryllium in mice exposed to Be-T and BeAl-T compared with the control group (n = 15/group)



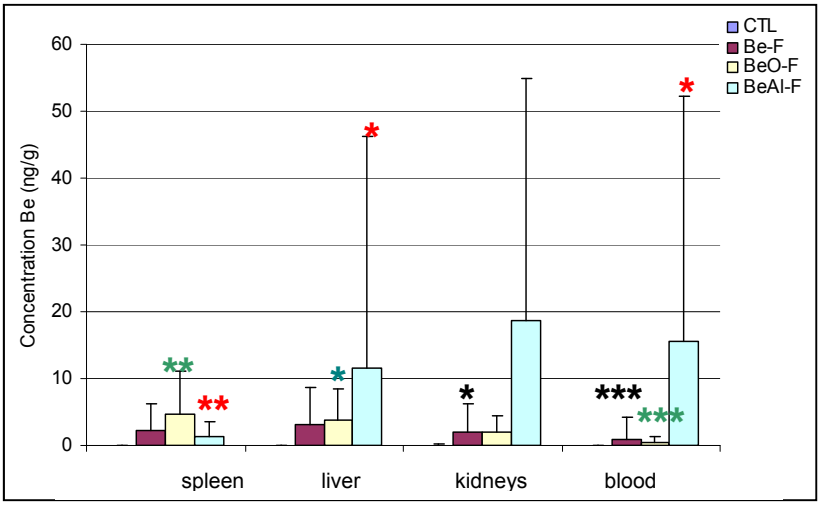
*** Significant difference ($p < 0.001$)
Significant difference ($p < 0.05$)

Figure 4 - Mean concentrations (± SD) of beryllium in the blood (n = 30) and tissues (spleen: n = 15, liver: n = 30, kidneys: n = 30) in mice exposed to Be-T and BeAl-T compared to the control group



*** Significant differences ($p < 0.001$) among all the groups

Figure 5 - Mean pulmonary concentrations (± SD) of beryllium in mice exposed to Be-F (n = 15), BeO-F (n = 30) and BeAl-F (n = 15) compared with the control group

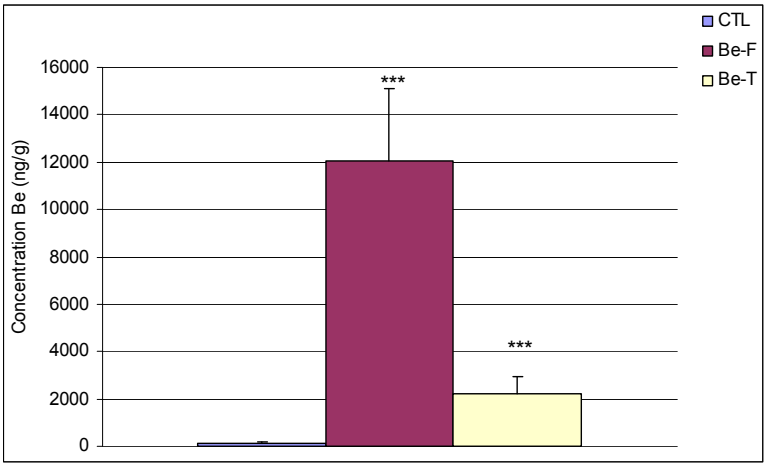


* **Significant difference** ($p < 0.05$) with Be-F
** **Significant difference** ($p < 0.01$) with Be-F
* Significant difference ($p < 0.05$) with BeAl-F
** Significant difference ($p < 0.01$) with BeAl-F
*** Significant difference ($p < 0.001$) with BeAl-F
* **Significant difference** ($p < 0.05$) with BeO-F
*** **Significant difference** ($p < 0.001$) with Be-F

Figure 6 - Mean concentrations (± SD) of beryllium in the blood (n = 30 for Be-F and BeAl, n = 60 for BeO-F) and tissues (spleen n = 15 for Be-F and BeAl-F, n = 30 for BeO-F, liver and kidneys n = 30 for Be-F and BeAl-F, n = 60 for BeO-F) in mice exposed to Be-F, BeO-F and BeAl-F compared to the control group.

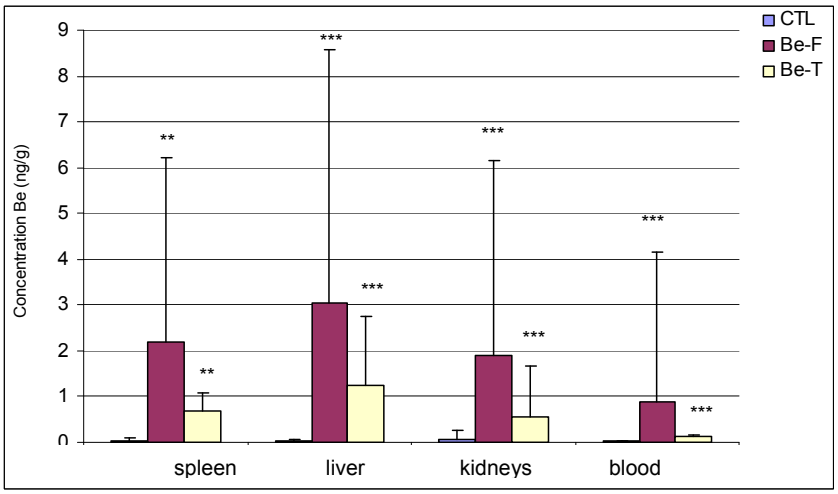
One of the most interesting findings obtained here are the lung concentrations of different chemical forms of Be as concerns their fine fraction. Indeed, the theoretical rate of lung deposition -- much higher for the group exposed to BeO -- is somewhat confirmed by the much higher lung concentration compared to the other two groups.

Figures 7, 8, 9 and 10 show tissue concentrations among the different particle sizes (total and fine) for groups of mice exposed to Be and BeAl. These findings demonstrate the preponderant role of size in their absorption and accumulation in different tissues. They are found mainly in the lungs, at levels at least ten times higher than elsewhere.



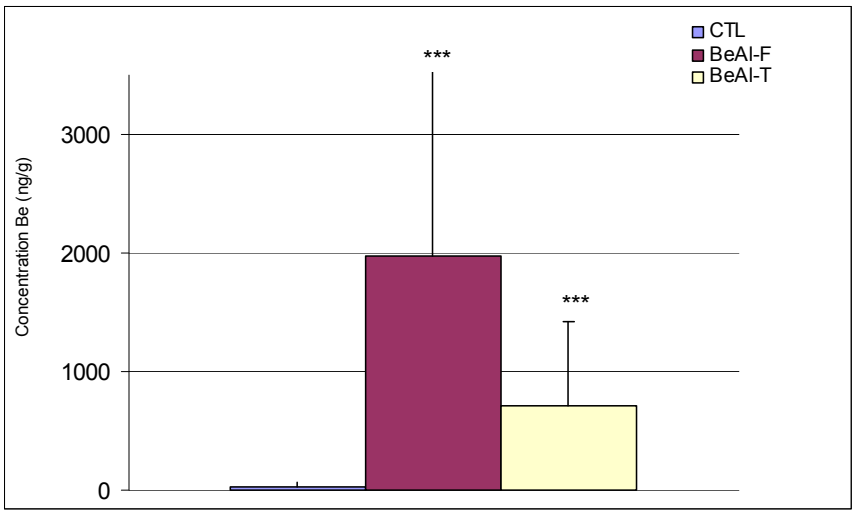
*** Significant difference ($p < 0.001$)

Figure 7 - Mean pulmonary concentrations (± SD) of beryllium in mice exposed to Be-F and Be-T compared with the control group (n = 15/group)



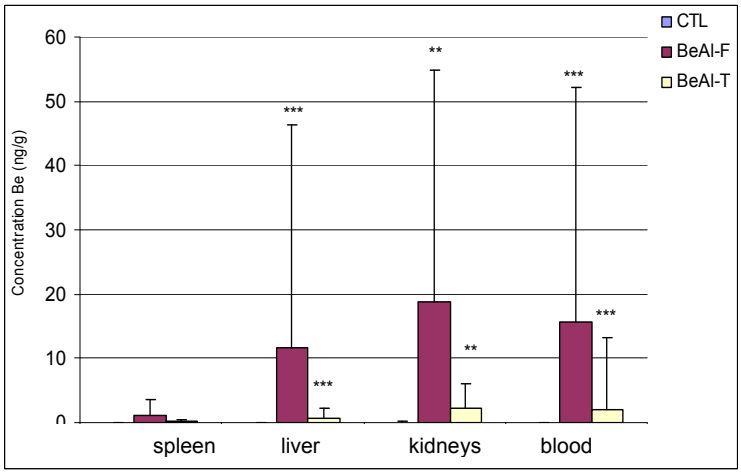
*** Significant difference ($p < 0.001$)
** Significant difference ($p < 0.01$)

Figure 8 - Mean concentrations (± SD) of beryllium in the blood (n = 30) and tissues (spleen n = 15, liver n = 30, kidneys n = 30) of mice exposed to Be-F and Be-T compared to the control group



*** Significant difference (p < 0,001)

Figure 9 - Mean lung concentrations (± SD) of beryllium in mice exposed to BeAl-F and BeAl-T compared with the control group (n = 15/group)

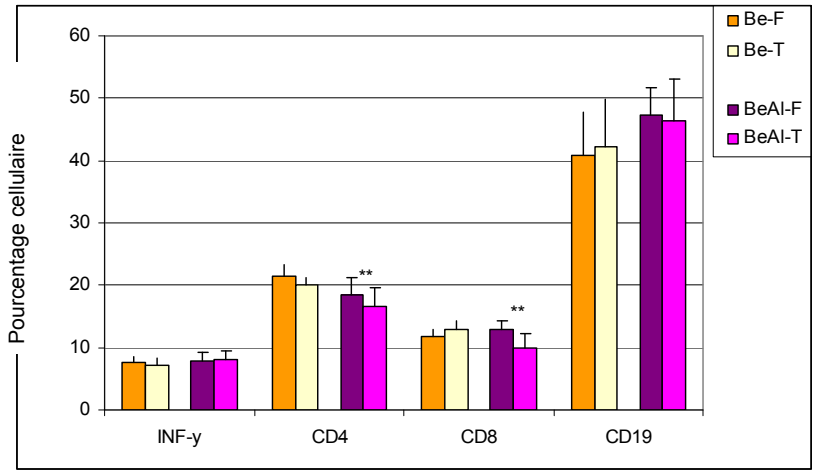


*** Significant difference (p < 0,001)
** Significant difference (p < 0.01)

Figure 10 - Mean concentrations (± SD) of beryllium in the blood (n = 30) and tissues (spleen n = 15, liver n = 30, kidneys n = 30) of mice exposed to BeAl-F and BeAl-T compared to the control group

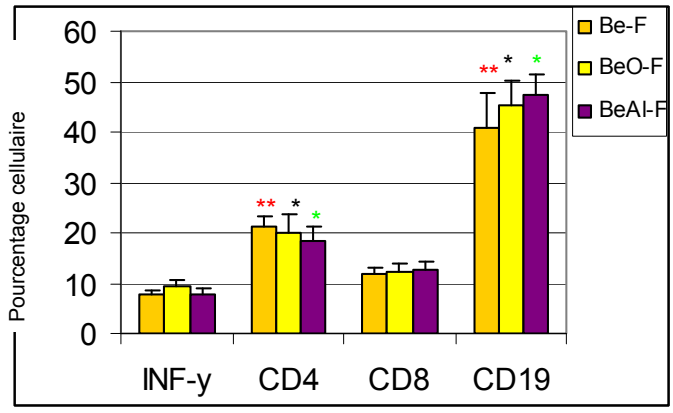
5.3. Flow cytometry

The phenotypic profiles of spleen lymphocytes among different groups of mice are shown in Figures 11 and 12, through the expression of CD4 +, CD8 +, IFN-γ, and CD19.



** Significant difference (p < 0,01) between the BeAl-F and the BeAl-T

Figure 11 - Comparative profiles of spleen lymphocytes (n = 7/group) in mice exposed to Be-F, Be-T, BeAl-F and BeAl-T
Cell percentage (± SD)

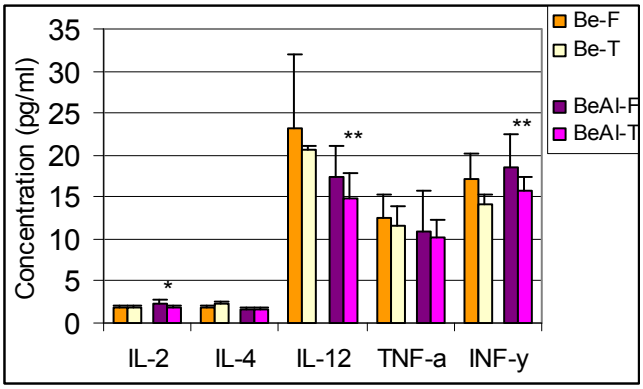


** Significant difference (p < 0.01) with BeAl-F
* Significant difference (p < 0.05) with Be-F
* Significant difference (p < 0.05) with BeO-F

Figure 12 - Comparative Profiles of the spleen lymphocytes of mice exposed to Be-F (n = 7), BeO-F (n = 14) and BeAl-F (n = 7)
Cell percentage (± SD)

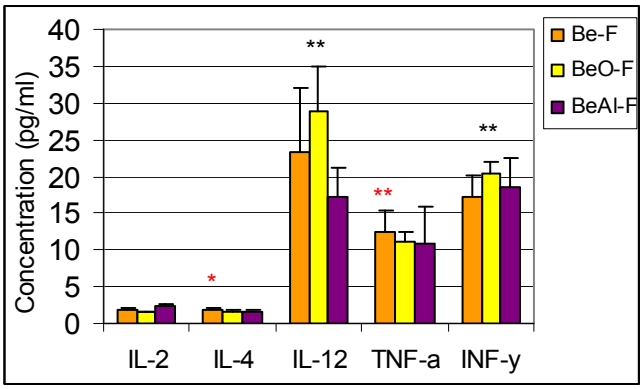
5.4. Cytokines

The measurement of cytokines (IL-2, IL-4, IL-12, TNF-α and IFN-γ) proceeded via the ELISA test on the cells of the bronchoalveolar lavage (BAL) of the mice. Figure 13 shows the cytokine concentrations assayed in the control group compared to the groups exposed to Be and BeAl. Figure 14 shows the differences between the results for the control group and those obtained in mice exposed to fine particles of Be, BeO and BeAl.



** Significant difference ($p < 0,01$) between the BeAl-F and BeAl-T
* Significant difference ($p < 0,05$) between the BeAl-F and BeAl-T

Figure 13 - Mean concentrations (± SD) of cytokines in mice (n = 7/group) exposed to Be-F, Be-T, BeAl-F and BeAl-T



** Significant difference ($p < 0,01$) with Be-F and BeAl-F
** Significant difference ($p < 0,01$) with BeO-F and BeAl-F
* Significant difference ($p < 0,05$) with BeO-F and BeAl-F

Figure 14 - Mean concentrations (± SD) of cytokines in mice exposed to Be-F (n = 7), BeO-F (n = 14) and BeAl-F (n = 7)

5.5. Histological sections and inflammation score

Figures 15, 16 and 17 represent the histological sections performed on the lungs of mice exposed to different chemical forms of Be (fine and total particles) and sacrificed 1 or 3 weeks following the end of exposure.

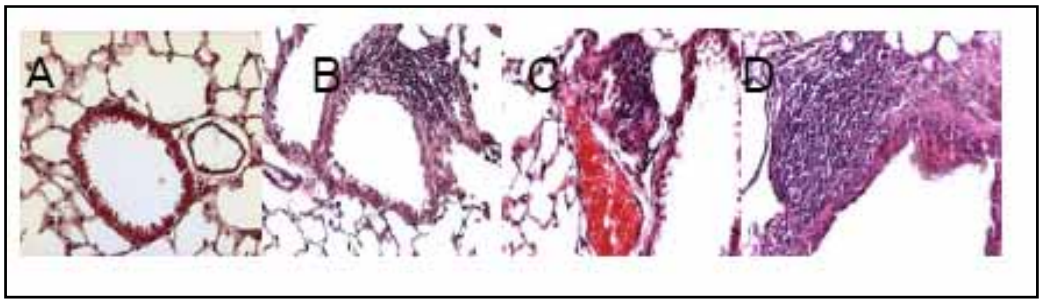


Figure 15 – Histological sections of mouse lungs (n = 8/group) exposed to Be

A: control group, B: mice exposed to Be-F and sacrificed one week following the end of exposure, C: mice exposed to Be-T and sacrificed one week following the end of exposure, D: mice exposed to Be-F and sacrificed 3 weeks following the end of exposure.

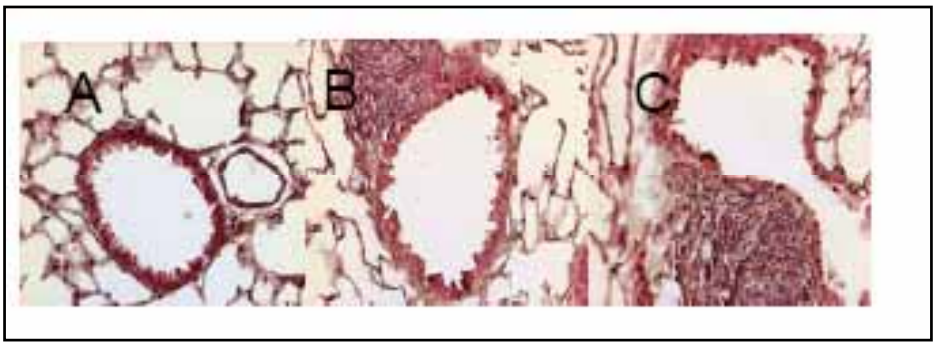


Figure 16 - Histological sections of mouse lungs (n = 16/group) exposed to BeO

A: control group, B: mice exposed to BeO-F and sacrificed one week following the end of exposure, C: mice exposed to BeO-F and sacrificed 3 weeks following the end of exposure.

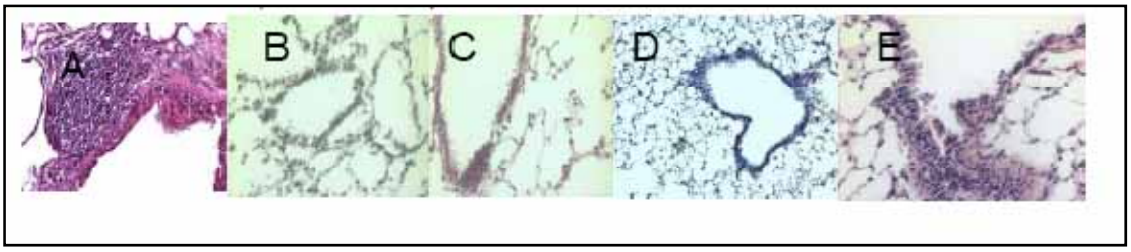


Figure 17 - Histological sections of mouse lungs (n=8/group) exposed to BeAl

A: control group, B: mice exposed to BeAl-T and sacrificed one week following the end of exposure, C: mice exposed to BeAl-F and sacrificed 1 week following the end of exposure, D: mice exposed to BeAl-T and sacrificed 3 weeks following the end of exposure, E: mice exposed to BeAl-F and sacrificed 3 weeks following the end of exposure.

Tables 4 and 5 present the histological scores assigned by two blinded investigators for mice sacrificed 1 week and 3 weeks following the end of exposure.

Table 4 - Histological score for lung inflammation in mice (n = 8/group) sacrificed one week following the end of exposure

1: no inflammation, 2: mild inflammation, 3: moderate inflammation, 4: severe inflammation

	1	2	3	4
CTL	95.5%	4.5%	0	0
Be-F	0	54.5%	45.5%	0
Be-T	22.7%	68.2%	9.1%	0
BeO-F	22.7%	63.6%	13.6%	0
BeAl-F	44.4%	55.6%	0	0
BeAl-T	61.1%	38.9%	0	0

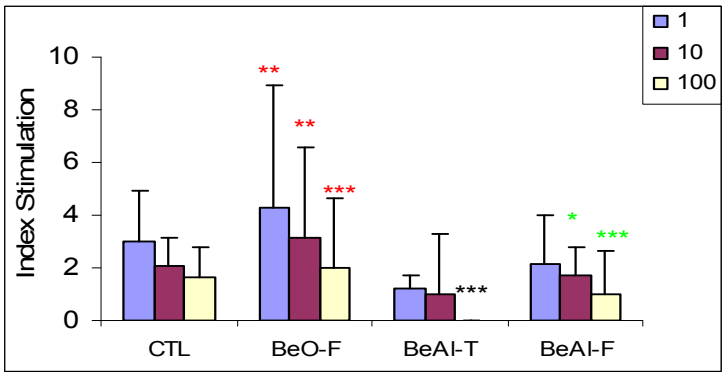
Table 5 - Histological score for lung inflammation in mice (n=5/group) sacrificed three weeks following the end of exposure

1: no inflammation, 2: mild inflammation, 3: moderate inflammation, 4: severe inflammation

	1	2	3	4
CTL	91.7%	8.3%	0	0
Be-F	0	29.4%	70.6%	0
BeO-F	0	75%	25%	0
BeAl-F	0	77.80%	22.20%	0
BeAl-T	0	100%	0	0

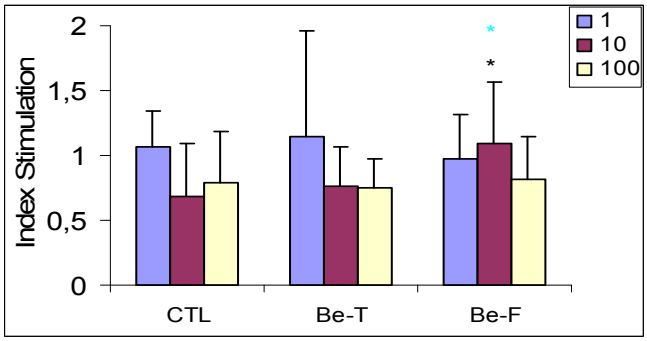
5.6. Lymphocyte proliferation assays

The lymphocytes obtained from the spleen cells were cultured *in vitro* and also placed near a solution of Be sulphate to stimulate their proliferation. The results presented in Figure 18 show that the stimulation index was generally higher at a concentration of 100 µM; the index was higher in mice exposed to BeO-F than in those exposed to BeAl. The results presented in Figure 19 show that the index obtained in mice whose cells were treated at 10 µM was higher in the Be-F group compared to the control group or to the Be-T group.



*** Significant difference (p< 0,001) between the CTL and the BeAl-T (100 µM BeSO4)
*** Significant difference (p< 0,001) between the BeO-F and the BeAl-F (100 µM BeSO4)
** Significant difference (p< 0,01) between the BeO-F and the BeAl-F (1 et 10 µM BeSO4)
*** Significant difference (p< 0,001) between the BeAl-F and the BeAl-T (100 µM BeSO4)
* Significant difference (p< 0,05) between the BeAl-F and the BeAl-T (10 µM BeSO4)

Figure 18 - BeLPT carried out using the spleen cells of mice in the control group and mice exposed to BeO-F (n = 16), BeAl-T (n = 8) and BeAl-F (n = 8) Stimulation index (± SD)



* Significant difference (p< 0,05) between CTL and Be-F (10 µM BeSO4)
* Significant difference (p< 0,05) between Be-T and Be-F (10 µM BeSO4)

Figure 19 - BeLPT carried out using the spleen cells of mice in the control group (n = 8) and mice exposed to Be-T (n = 8) and Be-F (n = 8) Stimulation index (± SD)

6. Discussion

Several risk factors are involved in Be toxicity, concerning both the sensitivity reaction and the development of CBD (Kolanz, 2001). The levels to which workers are exposed, the duration of this exposure, genetic susceptibility and the chemical form and particle size of Be are the risk factors most frequently mentioned to explain lung damage (Henneberger et al. 2001, Kent et al., 2001, Maier et al., 2003).

Chemical form and particle size play an important role in pulmonary deposition (Stefaniak et al. 2005; Rouleau et al., 2005). The retention of Be in the lung, due among other things to whether or not it is soluble, can play an important role in the development and persistence of granulomatous inflammation (Salehi et al., 2009). In fact, the specific effects are indicated, among other things, by the formation of inflammatory granulomas of the lung, the proliferation of T lymphocytes and the production of Th1-type cytokines (Amicos et al., 2006, Fontenot et al., 2002).

The present research has evaluated the effect of Beryllium's chemical form and particle size on its toxicity. It is also the first sub-chronic study on the toxicology of inhalation (oro-nasal) carried out using an animal model (mice). Without doubt, this exposure route minimized the absorption of Be via the cutaneous and gastrointestinal routes.

The results show that a decrease in particle size led to increased accumulation in various tissues and that this, consequently, seemed to have a more toxic effect than total particles. Tissue concentrations of Be (in the lungs, spleen, liver, kidneys and blood) were significantly higher in mice exposed to fine particles. The larger particles were deposited mainly in the trachea and bronchi. They were then eliminated by the mucociliary mechanism, in which the tracheobronchial mucosa are covered with hair cells, form an elevator and push the mucus containing the particles toward the digestive system (IRSST, 2006).

Depending on the MMAD obtained, 11% and 0.4% respectively of the Be-F and BeAl-F particles should theoretically be deposited in the lungs compared to only 0.5% and 0.25% for the Be-T and BeAl-T particles. As for the BeO particles (0.41 microns), about 40% of these particles should be deposited in the lungs. In fact, as illustrated in Figure 20, the lung concentrations obtained were clearly related to the MMADs. These were also weakly related to the inflammation scores in mice sacrificed one week following the end of exposure. Indeed, lung inflammation appeared to be weaker when the MMAD was higher.

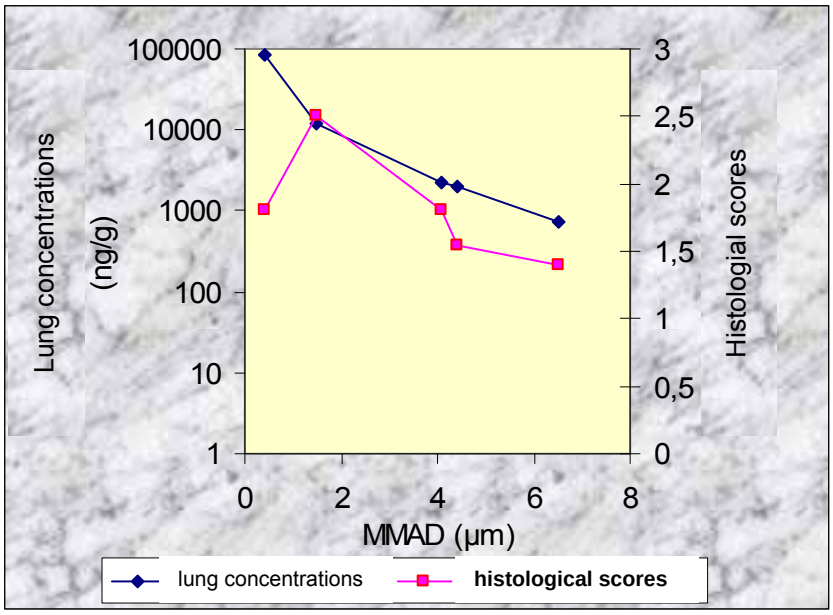


Figure 20 – Lung concentrations and inflammation scores as a function of the mass median aerodynamic diameter

In addition, the mice sacrificed 3 weeks following the end of exposure had a more severe pulmonary inflammation than those sacrificed one week following the end of exposure. The score of 3 for lung inflammation was achieved, respectively, in 45.5% and 0% (Table 4) of the mice exposed to, respectively, Be-F and BeAl-F and sacrificed one week after the end of exposure. For mice sacrificed 3 weeks after the end of exposure, the score of 3 obtained in mice exposed to the same chemical and physical forms of Be was 70.6% and 22.20% respectively (Table 5). This is of great importance since it demonstrates that the effect of Be (or of the time that elapses before measuring the response) continues even after cessation of exposure.

The production of cytokines IL-2, IL-12 and IFN-γ was also significantly higher in mice exposed to BeAl-F-T than it was to those exposed to BeAl-F, despite a slight difference in their size of their particles (Figure 13). There was a similar difference between these two groups of mice as regards their phenotypic profile of spleen lymphocytes, whereas the percentages of CD4 + and CD8 + were significantly higher in mice exposed to BeAl-F.

The importance of chemical form was also evident in this study for both fine particles and total particles. Lung tissue concentrations in mice exposed to Be-F, Be-T and BeO-F were significantly higher compared to those exposed to BeAl-T.

Similarly, blood concentration was significantly higher in mice exposed to BeAl- T or BeAl-F compared to those exposed to the Be-F and BeO-F. This may be due to the low solubility of BeAl compared to the non-solubility Be or BeO. Thus, BeAl particles tend to migrate more easily in the blood and therefore accumulate less in the lungs.

In fact, lung clearance for insoluble compounds is very slow, and whatever has not been eliminated rapidly by mucociliary activity or the phagocytosis of particles may be retained in the lungs for several months and then gradually released into the blood. For soluble compounds, clearance is faster via dissolution in lung fluids and a variable proportion enters the bloodstream (IRSST, 2006).

In addition, the mean MMAD of 4.1 microns obtained for the Be-T may partly explain its higher lung concentration compared to that of BeAl-T. The estimated rate of pulmonary deposition was around 0.5% for the Be-T, whereas it was 0.25% for the BeAl-T. Thus, the mice exposed to Be-T obtained a higher inflammation score for the mice sacrificed either 1 week or 3 weeks following the end of exposure.

This is consistent with the results obtained by Finch and colleagues, who reported that pulmonary interstitial infiltration is a dose-dependent reaction and that the severity of lung injury increases with time and the lung burden (Finch et al., 1998).

As for cytokines, the mice exposed to Be-F expressed significantly more CD4 +, TNF-α and IL-4, and less CD19, than mice exposed to BeO-F and BeAl-F (Figures 13 and 14). The concentrations of IL-12 and INF-γ are higher in mice exposed to BeO-F, whereas the concentrations of IL-4 and TNF-α are higher in mice exposed to Be-F and BeAl-F.

The pulmonary deposition rate of BeO particles (almost 40%) may explain the effects associated with it, whereas the non-solubility and 11% pulmonary retention of Be may explain its toxicity. At the alveolar level, macrophages will take care of insoluble particles via a phagocytic mechanism whose effectiveness is highly dependent on chemical form and particle size. Several studies have also shown that non-agglomerated ultrafine particles deposited in the alveoli are not phagocytosed efficiently by macrophages, which, nonetheless, are very efficient in the range of 1 to 3 microns (IRSST, 2006).

In fact, the more a particle reaching the alveoli is water soluble, the more it dissolves in the mucous membranes lining the alveoli. When there is dissolution in the mucus, there is an increased possibility of phagocytosis or of contact with type-I and type-II cells promoting transcytosis (epithelial translocation). The insoluble molecules in the mucus have an alveolar residence time that is potentially very long, unlike water-soluble particles that can be phagocytosed (depending on their size) or cross the alveolar epithelium through transcytosis (Oberdörster et al., 2007).

An examination of the more detailed results obtained in mice exposed to Be-F, BeO-F and BeAl-F, provides us with several correlations (Table 6).

Table 6 - Correlations among tissue concentrations, cytokine concentration and spleen-cell expression in mice exposed to Be-F, BeO-F and BeAl-F

Tissue concentration	Cytokine concentration	r	p
Lung	IL-12	0.615	0.002 (n=23)
	IFN-γ	0.573	0.008 (n=20)
Blood	IL-2	0.543	0.000 (n=47)
Spleen	IL-12	0.602	0.000 (n=31)
Tissue concentration	Cell expression	r	p
Lung	INF-γ	0.518	0.003 (n=31)
	CD4+	0.424	0.016 (n=32)
Blood	CD8+	0.482	0.000 (n=96)
Spleen	INF-γ	0.452	0.006 (n=36)
Cytokine concentration	Cell expression	r	p
IL-12	INF-γ	0.467	0.000 (n=62)
	CD4+	0.448	0.000 (n=64)

Considering the correlations obtained between tissue concentrations and cytokines, IL-12 was correlated with Be concentrations in the lung, spleen and liver. Moreover, if we refer to Figure 13, we see that the concentration of IL-12 increased in exposed mice and these results were significantly different from those obtained in mice exposed to BeAl-F compared to BeAl-T. From Figure 14 we can see that the concentration of IL-12 was significantly higher in mice exposed to BeO-F compared to those exposed to Be-F and BeAl-F. This pulmonary concentration of IL-12 was related to the concentration of pulmonary CD4, which, according to Figure 12, was significantly higher in mice exposed to Be-F compared to those exposed to BeO-F and BeAl-F.

Finally, it would be interesting to turn the focus of this discussion toward risk assessment for workers. To begin, the exposure level used in the present study, 250 $\mu g/m^{3,}$ can be extrapolated to humans using the following equation:

Extrapolation factor: $(VE / S_APU)_{animal} / (VE / S_APU)_{human}$
VE: Rate of ventilation

S_APU: Lung surface area

Thus, the exposure level for humans would be:

Concentration used X extrapolation factor, or,

$250\ \mu g/m^3 \times (0.052\ m^3/j / 0.295\ m^2) / (20\ m^3/j / 54\ m^2) = 121\ \mu g/m^3$

In addition, since the exposure concentration resulted in lung inflammation characterized as mild to moderate, it cannot be regarded as the Lowest Observed Adverse Effect Level (LOAEL). To establish the latter, it would be prudent to divide the exposure level derived for humans by a factor of 10. Thus, the LOAEL would be 12.1 $\mu g/m^3$.
Then, using a standard approach, this value should be divided by three other uncertainty factors: A factor of 10 for extrapolation from a sub-chronic to chronic toxicity; another factor of 10 for extrapolation from a LOAEL to the No Observed Adverse Effect Level or NOAEL; a third factor of 1.25 for extrapolation of the duration of exposure used in the present study (6 h) to the duration of exposure of 8h generally considered for the worker. In this approach, the threshold limit value that could be derived from the present study would be 0.10 $\mu g/m^3$, a value slightly below the Quebec limit of 0.15 $\mu g/m^3$.

Conclusion

This study allowed us to verify the extent to which pulmonary effects were related to the chemical form of Be and the size of its particles; several results converge with those obtained by Finch et al. (1990 and 1996) and Haley et al. (1994). Clearly, the C3H/HeJ mouse strain model used in this study proved to be very good since it faithfully reproduced many effects observed in humans.

Many correlations were found between Be tissue concentrations and effects normally observed in workers following exposure. Indeed, the histological sections showed that mice exposed to Be had levels of lung inflammation similar to those observed in patients with CBD. On the other hand, it is acknowledged that lung injuries in humans are more extensive than those observed in animals (Nikula et al., 1997, Finch et al., 1998).

The results also show an association between particle size, lung concentrations, lung inflammation, production of certain cytokines and the expression of certain lymphocytes. The effects also depend on the chemical form of Be. Thus, Be metal and BeO are the most toxic. Clearly, aerodynamic diameter and solubility played a significant role in deposition and lung retention.

The roles of size and chemical form in Be toxicity raise a fundamental question. Should we not set specific exposure limits, taking into account these parameters? If, for pragmatism, the use of multiple limit values would be difficult to apply operationally, it would nonetheless be desirable, as a minimum, that measures protecting workers, or reducing their exposure, take these kinds of properties into account.

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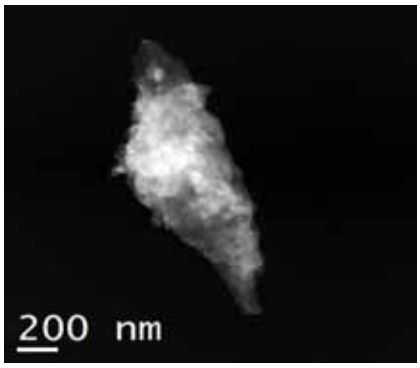
APPENDIX I

a) 

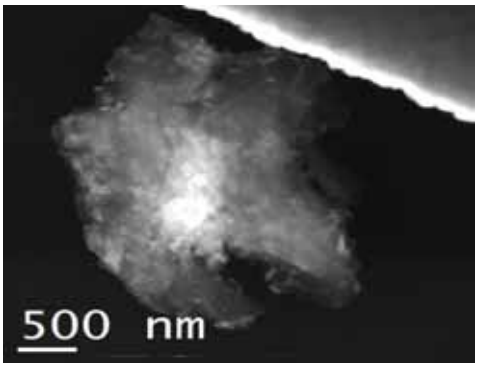
b) 

Figure a – TEM (transmission electron microscopy) micrographs (Z contrast) showing particles of Be: a) Be-F and b) Be-T.

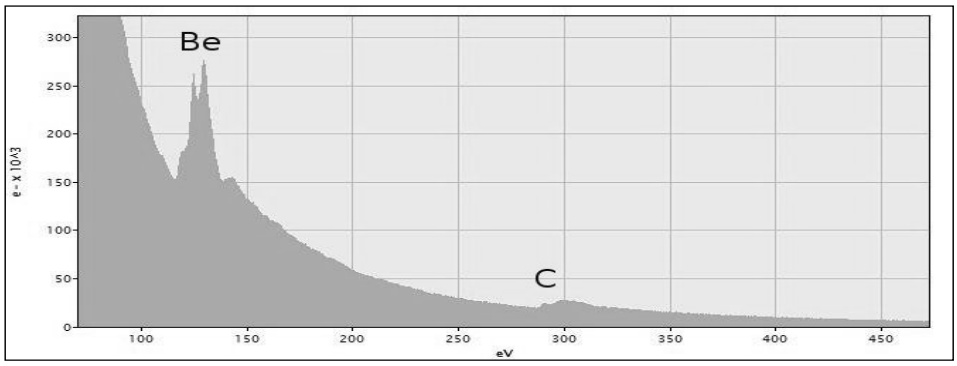


Figure b - PEELS spectrum (electron energy-loss spectroscopy) acquired on a powder particle showing the detection of Be. The carbon edge (discontinuity) comes from the film holding the powder particles.

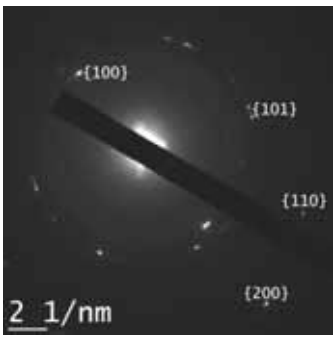


Figure c – Plate showing the electron diffraction pattern acquired on a single Be-containing powder particle.

The presence of rings indicates that the particle is made up of several crystals. The interplanar spacing measured matches that of pure Be and the families of (atomic) planes are identified in the figure.