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Beryllium Metal II. A Review of the Available Toxicity Data

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Beryllium metal was classified in Europe collectively with beryllium compounds, e.g. soluble salts. Toxicological equivalence was assumed despite greatly differing physicochemical properties. Following introduction of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, beryllium metal was classified as individual substance and more investigational efforts to appropriately characterize beryllium metal as a specific substance apart from soluble beryllium compounds was required. A literature search on toxicity of beryllium metal was conducted, and the resulting literature compiled together with the results of a recently performed study package into a comprehensive data set. Testing performed under Organisation for Economic Co-Operation and Development guidelines and Good Laboratory Practice concluded that beryllium metal was neither a skin irritant, an eye irritant, a skin sensitizer nor evoked any clinical signs of acute oral toxicity; discrepancies between the current legal classification of beryllium metal in the European Union (EU) and the experimental results were identified. Furthermore, genotoxicity and carcinogenicity were discussed in the context of the literature data and the new experimental data. It was concluded that beryllium metal is unlikely to be a classical nonthreshold mutagen. Effects on DNA repair and morphological cell transformation were observed but need further investigation to evaluate their relevance *in vivo*. Animal carcinogenicity studies deliver evidence of carcinogenicity in the rat; however, lung overload may be a species-specific confounding factor in the existing studies, and studies in other species do not give convincing evidence of carcinogenicity. Epidemiology has been intensively discussed over the last years and has the problem that the studies base on the same US beryllium production population and do not distinguish between metal and soluble compounds. It is noted that the correlation between beryllium exposure and carcinogenicity, even including the soluble compounds, remains under discussion in the scientific community and active research is continuing.

Keywords: acute toxicity; beryllium; carcinogenicity; classification; epidemiology; genotoxicity; inhalation; sensitization

INTRODUCTION

Beryllium metal has physical properties that make its use essential for certain operations, but beryllium is known to cause adverse health effects in humans upon inhalation. Furthermore, the carcinogenicity of beryllium and its compounds is under scientific discussion and has been the subject of numerous reviews and evaluations. To assess the risk of beryllium for human health and focus the scientific efforts, it is necessary to understand the exposure pattern of humans to beryllium.

Exposure

The general population is continuously exposed to low levels of naturally occurring beryllium (for example in coal, wood, foodstuffs, gemstones) via ambient air, drinking water, and diet. Aside from the naturally occurring sources, exposure of humans

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to beryllium is limited to inhalation and dermal contact during occupational processes. Most of the exposures occur in the manufacturing operations during production of beryllium metal and beryllium-containing alloys.

It should be noted that the overall market volume of beryllium is relatively small; ~146 metric tons are sold worldwide per year.

1. Soluble beryllium compounds are extremely rare in commerce with only small laboratory quantities being occasionally used. Aside from these deminimus quantities, exposure of humans to the soluble species is restricted to the primary extraction and concentration facilities, which is limited to four producing locations worldwide.
2. Approximately 20 tons per year of pure beryllium metal is used in very specific applications, such as X-ray windows, nuclear/fusion reactors, and aerospace applications, and is not used in consumer goods except for the small quantity (grams) used to manufacture high end audio speakers. The beryllium metal parts are typically supplied by specialty manufacturers as articles in solid form, i.e. machined parts ready for assembly.
3. Alloys containing beryllium represent by far the biggest percentage (75%) of the 120 ton per year beryllium-containing material market (Jaskula, 2010). They are used in electronics, energy, automotive, and aeronautic applications because of their high strength, elasticity, electrical and thermal conductivity, resistance to oxidation, and high melting point. Parts containing these alloys are typically sold in a finished or semifinished solid form requiring no further processing and are used as internal small components in various industrial and consumer products.

In the European Union (EU), Japan and the United States of America (USA), effective workplace and environmental control measures are in place to minimize exposures of workers and the general public during production and recycling operations. Recent studies on the downstream processing of alloys and recycling facilities indicate airborne concentrations well below 0.2 μg m^{-3} [Kent *et al.*, 2007; Brush-Wellman Inc. (unpublished data)].

Taking the advances made in controlling exposures to beryllium in production operations, the form on the market (solid article) and the low solubility of metallic beryllium in aqueous media into account, the environmental or human exposure potential outside the processing facilities is considered to be very limited.

Current classification

Insoluble beryllium metal has historically been classified together with its salts and soluble compounds in the EU in the appendix to Directive 67/548/EEC due to lack of data on individual beryllium compounds. Full read across from individual substances was applied, despite the great differences in physicochemical properties of the individual substances (Table 1), which strongly influences systemic bioavailability of the individual compounds and thus have significant effects on the toxicity.

According to the European Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), classification of beryllium metal as an individual substance is required. The current harmonized classification in the new Commission Regulation 1272/2008 for beryllium is a translation of the harmonized classification of beryllium and its soluble compounds into the new Globally Harmonized System-language, plus addition of the H411 for long-lasting toxicity toward aquatic organisms (Table 2).

TOXICITY

Acute toxicity and local effects

Acute oral toxicity of beryllium metal has recently been tested in rats, and results are reported in this issue of *Annals of Occupational Hygiene* (Strupp, 2010). The dose that was lethal to 50% of the test animals (LD_{50}) was found to be >2000 mg kg^{-1} body weight. This result was not surprising as oral bioavailability of beryllium is known to be very low. Although no direct toxicokinetic data exists to support this for the metal itself, studies on soluble beryllium salts give indirect evidence that oral bioavailability from solutions is <1% across different animal species (Richmond *et al.*, 1964; Furchner *et al.*, 1973), and oral bioavailability of particulate matter was found to be even by orders of magnitude lower

Table 1. Physicochemical properties of beryllium compounds

Compound	Melting point (°C)	Solubility in water (g per 100 g water)
Beryllium metal	1287[a]	<0.00005[b]
Beryllium chloride	415[a]	71.5[a]
Beryllium sulphate	1127[a]	41.3[a]
Beryllium oxide	2578[a]	<0.00005[b]

[a]From CRC Handbook of Chemistry and Physics, 89th edition, Taylor and Francis, Boca Raton, USA.
[b]From beryllium consortium owned data.

Table 2. Classification of beryllium metal/beryllium and beryllium compounds

	Harmonized classification in the			
	Dangerous substance directive (Annex I of Directive 67/548/EEC; Index No 004-001-00-7)		Classification, labeling, and packaging regulation (Annex VI of Regulation (EC) No 1272/2008; Index No 004-001-007)	
Classification	R-phrase	Meaning	H-phrase	Meaning
	R49 (Carc. Cat. 2)	May cause cancer by inhalation.	H350i (Carc. Cat 1B)	May Cause cancer (inhalation).
	R26	Also very toxic by inhalation.	H330	Fatal if inhaled.
	R25–R48/23	Also toxic if swallowed. Also toxic: danger of serious damage to health by prolonged exposure through inhalation.	H301 H372	Toxic if swallowed Causes damage to organs through prolonged or repeated exposure.
	R36/37/38	Irritating to eyes, respiratory system, and skin.	H319 H335 H315	Causes serious eye irritation. May cause respiratory irritation. Causes skin irritation.
	R43	May cause sensitization by skin contact.	H411	Toxic to aquatic life with long-lasting effects (only for beryllium compounds, not for beryllium).

Carc. Cat. = Carcinogen Category.

(LeFevre and Joel, 1986). Based on this result, no classification of beryllium metal for acute oral toxicity according to Regulation (EC) No 1272/2008 is considered required. The risk phrase R25-'Toxic if swallowed' currently assigned to beryllium and its compounds is not considered to be suitable for beryllium metal as an individual substance.

Acute inhalation toxicity tests in rats were not conducted as part of this study program, even though the available literature on inhalation toxicity of beryllium metal indicates that acute short-term (4 h) exposure to extensive levels might well be tolerated by rats without leading to mortality (Haley *et al.*, 1990). Delayed toxic effects in rats after short (acute to subacute) inhalation exposure have been reported in the public literature (Haley *et al.*, 1990; Nikula *et al.*, 1997a; Finch *et al.*, 1998b). From a technical perspective, the current labeling for acute inhalation (T^+, R26-'Very toxic by inhalation') might not be correct but is considered by the industry to be appropriate for warnings to beryllium workers to prevent inhalation of beryllium metal dusts, and conducting animal tests is not considered ethically justified when scientific and regulatory consensus exists that inhalation exposures to airborne beryllium should be well controlled.

Local skin and eye effects of beryllium metal have been tested in rabbits (Strupp, 2010). No irritative or corrosive actions towards the skin have been observed at any observation time point. Ocular applications resulted in minimal initial chemosis (likely due to reaction of the eye to the powder as a foreign body) that was completely reversible within 24 h and slight initial redness that decreased within 24 h to minimal reactions and was fully reversible within 7 days (Grade 0). Thus, the mean over 24, 48, and 72 h does not meet or exceed the Grade 2 for redness or chemosis, and the observed effects are fully reversible within 21 days. Based on these results, no eye or skin irritation classification of beryllium metal according to Regulation (EC) No 1272/2008 is considered required. The risk phrases R36/38-'Irritating to Eyes and Skin' currently assigned to beryllium and its compounds are not considered to be suitable for beryllium metal.

Unlike insoluble forms, soluble beryllium compounds are known to have a risk of a dermal sensitization reaction. Data in the public literature (Curtis, 1951; Zissu *et al.*, 1996) does not give a satisfactory answer for the metal. The single human study (Curtis, 1995) investigated beryllium metal powder and beryllium in solid forms (discs) in a patch test on 13 patients who had a history of sensitization reactions in a beryllium plant and 16 control persons who had no history of beryllium exposure. None of the subjects of the control group reacted to either form of the metal by spontaneous reactions and none of the patients reacted to the metal discs. Three of the

13 patients reacted in the patch test with the metal powder, but the author determined that this reaction was likely caused by the impurity of a soluble beryllium fluoride salt, which was present at 0.025–0.2% in the metal powder. Beryllium metal production processes were changed in the 1950s, resulting in the elimination of salt impurities. In a guinea pig study (Zissu *et al.*, 1996), soluble beryllium sulphate was used to induce sensitivity and beryllium-containing alloys for challenge. As the quantitative systemic availability of metal ions is an important factor in the development of a sensitization reaction, the study is not considered suitable for assessment of the metal. To investigate the skin sensitizing potential of beryllium metal, a guinea pig maximization test was conducted with induction and challenge using beryllium metal (Strupp, 2010). None of the test animals elicited positive skin reactions at challenge. Based on these results, no classification of beryllium metal according to Regulation (EC) No 1272/2008 is considered required. The risk phrase R43-'May cause sensitization by skin contact' currently assigned to beryllium and its compounds is not considered to be suitable for beryllium metal as an individual substance.

Repeated dose toxicity

While some repeated dose studies with soluble compounds exist, no repeated dose studies with beryllium metal are available. The only studies that partly match this study type are studies with an initial acute or subacute inhalation exposure period (often targeting at in initial lung burden) and an extended follow-up period (Haley *et al.*, 1990; Finch *et al.*, 1991a; Nikula *et al.*, 1997; Finch *et al.*, 1998b).

Although no animal experimental data are available, it is clear from epidemiology studies that beryllium is hazardous to human health when inhaled. Inhalation of high doses of soluble compounds is known to cause acute beryllium disease, which is an inflammatory obstructive lung disease after high short-term exposure (Eisenbud, 1955; Cummings *et al.*, 2009) typically not or very rarely observed anymore due to application of modern worker safety measures. Chronic beryllium disease (CBD) can occur after exposure to comparatively low doses of beryllium but requires involvement of an immune response and there is some evidence that it might predominantly or even exclusively occur in sensitized workers believed to have a genetic predisposition (Deubner *et al.*, 2001). CBD is a granulomatous lung disorder with clinical symptoms similar to sarcoidosis, currently diagnosed by the parallel occurrence of noncaseating granulomas and a positive beryllium lymphocyte proliferation test. It is clearly the most important toxicological aspect of beryllium, and thus, classification for specific target organ (respiratory system) toxicity at low inhalation doses is considered appropriate for beryllium metal. As classification for this endpoint is considered appropriate, and excellent scientific reviews on CBD exist (Samuel and Maier, 2008; McCleskey *et al.*, 2009), it is not further discussed here but reference is made to the mentioned reviews.

Genotoxicity and carcinogenicity

Carcinogenesis has been intensively studied in animals. Furthermore, cancer rates have been studied in US beryllium production workers. 'Beryllium and its compounds' are classified by International Agency for Research on Cancer and the German Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe der Deutschen Forschungsgemeinschaft (MAK commission) as Class 1 carcinogens ('known carcinogen to humans'). In the European community, beryllium and its compounds are classified as Class 2 carcinogens ('known animal carcinogens that are suspected to be human carcinogens'). The key literature on which the EU classification was based could not be identified as the documentation at the respective European authorities is restricted to meeting minutes. As a result, an extensive literature search was conducted to identify the key studies.

The following databases were screened for publications relating to beryllium-induced carcinogenicity in 2007: PubMed, MedPilot, ToxNET, and Toxline Special. A total of 1531 publications were identified dealing directly with toxicity of beryllium and its compounds, of which 38 addressed carcinogenicity and 21 genotoxicity.

Genotoxicity. When discussing potential carcinogenic properties of a substance, information about genotoxicity is required as a basis for assessment, especially with regard to potential threshold/nonthreshold modes of action.

Genotoxicity of beryllium metal was never investigated, while genotoxicity of soluble beryllium compounds has been intensively studied. The likely reason for this is the low solubility of the metal in the cell culture media used in the *in vitro* tests and the nontrivial inhalation exposure necessary for adequate *in vivo* genotoxicity tests. Results obtained with soluble beryllium compounds led to surprisingly inconsistent results between independent

laboratories. No clear picture on genotoxicity could be achieved, mainly due to limited experimental designs and reporting. Data on exposure-relevant beryllium species as they are found in an occupational environment does not exist (Gordon and Bowser, 2003).

To fill this data gap, the typical genotoxicity testing program *in vitro* as recommended during safety evaluation of substances for official registration and as recommended in the literature (Müller *et al.*, 1999; Kirkland *et al.*, 2005) was conducted. Internationally Organisation for Economic Co-Operation and Development validated *in vitro* genotoxicity test methods were used, and detailed results are reported in this issue of *Annals of Occupational Hygiene* (Strupp, 2010). Genotoxicity of beryllium metal extracts was investigated by a reverse mutation assay in *Salmonella typhimurium/Escherichia coli* (Ames test), a mammalian cell chromosome aberration test in human lymphocytes and a mammalian cell gene mutation test at the hypoxanthine-guanine phosphoribosyltransferase locus (HPRT test) in V79 cells. DNA repair synthesis (as a marker of DNA damage) was addressed in an unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes. Furthermore, morphological cell transformation was addressed by a cell transformation test in Syrian Hamster embryo cells (SHE assay) (LeBoef *et al.*, 1996) and potential effects on DNA repair enzymes by a modification of the UDS assay.

It is recognized that there are limitations in the ability of the assay systems used to predict complex or secondary mechanisms of genotoxicity (which is true for all *in vitro* systems), but primary genotoxicants can be identified, which is important information for risk assessment. The beryllium ion may play a key role in biological processes as relative cytotoxicity increases when applying soluble beryllium compounds instead of metallic beryllium or metallic particles with smaller diameter (thereby increasing the surface) (Finch *et al.*, 1988, 1991). However, toxicity occurs only at high concentrations (high micromolar range) and is obviously not-as may be postulated for a metal-mediated by reactive oxygen species production with subsequent initiation of apoptosis (Lavastre *et al.*, 2002). Testing the soluble metal salts and applying full read across to beryllium metal are considered oversimplified as the solubility (and thus the local exposure of cells coming in contact with the metal ion) is *in vivo* unlikely to be greater than *in vitro* in the chosen experimental setup. To test barely water-soluble materials for genotoxicity *in vitro* is a difficult task and has limitations. When medical devices are tested, typically extracts of the device are prepared in accordance with ISO 10993 and applied in the genotoxicity tests and not a mixture of soluble salts of all ingredients in the devices. While primary isolated epithelial cell might be incubated with particulate matter as they are morphologically adapted to contact with particulate material, it is not recommendable to expose all cell types used in genotoxicity testing to the physical stress that the presence of particles will cause. In metal toxicity, the ion is typically made responsible for genotoxic actions. As a basic approximation, an ion formation test was conducted with the intention to compare the dissolution behavior of beryllium metal and beryllium chloride (as a representative of soluble beryllium compounds) under conditions simulating inhaled beryllium metal in the human lung [nonabrasive shaking for up to 28 days in the dark under normal lung (pH 7.4) and lysosomal (pH 4.5) conditions]. It is recognized that this assay cannot reflect the complex *in vivo* situation of the lung, but it can at least define upper borders of exposure. The result of this investigation (Strupp, 2010) was that beryllium chloride obviously dissolved immediately up to the limit of solubility, which was ~4 to 7% of the beryllium theoretically available for dissolution (=mass of the beryllium−part of the loaded beryllium chloride) in the normal lung medium and ~90% in the lysosomal fluid with the lower pH. The amount of dissolved beryllium did not significantly increase over the test period of 28 days and just varied slightly in the range of normal variation. Beryllium metal behaved differently. In both fluids, the amount of dissolved beryllium increased over time but stayed largely below the amounts dissolved from the chloride (Fig. 1).

The data indicates that dissolution kinetics of the soluble forms and the metal are largely different. Although considerable amounts of beryllium (up to ~30%) can dissolve in the artificial lysosomal fluids within 28 days, the local concentration achieved in the lung is still considered to be by orders of magnitude lower than from soluble compounds as ions are mobile and likely to be distributed/excreted more quickly than dissolution of the metal particles occurs. Furthermore, there is indirect evidence from the toxicology studies that unlimited dissolution in the lysosomes (with low pH) does not take place: most of the beryllium metal, once inhaled, remains in the lung of the experimental animals for an extended period. Clearance halftimes have been reported to be 180–260 days in rats (Finch *et al.*, 1990), so unlimited immediate dissolution in the lysosomes clearly does not take place. Beryllium can be recovered from the lungs of workers with CBD

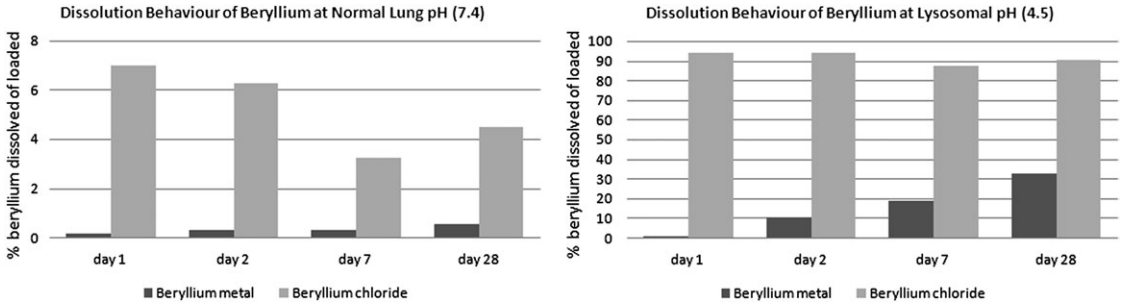

Fig. 1. Dissolution behaviour of beryllium metal and beryllium chloride in artificial biological fluids.

at autopsy, often years after exposure ceased (Verma *et al.*, 2003). Based on the results obtained, it is considered more relevant to test the metal extracts in the genotoxicity assays *in vitro* rather than using soluble compounds.

With regards to gene mutation, published literature with soluble beryllium compounds revealed no genotoxic potential when applying the standard Ames test (Arlauskas *et al.*, 1985; Ashby *et al.*, 1990; Kuroda *et al.*, 1991) and a weakly positive response when applying nonstandard assays (Zakour and Glickman, 1984; Arlauskas *et al.*, 1985). In contrast to these results obtained in bacteria, published literature on soluble beryllium compounds demonstrated a weak positive response in mammalian cells (Hsie, 1978, 1979; Miyaki *et al.*, 1979).

In a recent bacterial gene mutation (Ames) assay recently performed with beryllium metal extracts, no mutagenic potential was observed. The same is true for a mammalian cell gene mutation (HPRT) assay (Strupp, 2010).

With regard to cytogenicity, published literature with soluble beryllium compounds did not, in general, show a potential for cytogenetic effects (Brooks *et al.*, 1989; Ashby *et al.*, 1990), while in one publication a strong increase in structural chromosome aberrations was observed in human lymphocytes after treatment with $BeSO_4$ (Larramendy *et al.*, 1981). A potential for sister chromatid exchange (which could not be verified for hardly soluble beryllium compounds) was observed in one study (Kuroda *et al.*, 1991), while no potential for sister chromatid exchanges was identified in another study (Anderson, 1983). *In vitro* micronucleus testing in SHE cells was positive for soluble compounds (Fritzenschaf *et al.*, 1993), while oral exposure to beryllium sulphate did not increase the incidence of micronucleated bone marrow cells *in vivo* (Ashby *et al.*, 1990). A chromosome aberration assay conducted with beryllium metal extracts (Strupp, 2010) did not demonstrate a cytogenetic activity of the metal extract.

Morphological cell transformation upon exposure of cells to soluble beryllium compounds was reported to occur (Kommitowski, 1973; DiPaolo and Casto, 1979; Dunkel *et al.*, 1981; Zhou *et al.*, 1999; Joseph *et al.*, 2001; Keshava *et al.*, 2001). This was verified for beryllium metal by a SHE cell transformation assay conducted with beryllium metal extracts (Strupp, 2010). This finding is indicative that the concentration of beryllium ions dissolved in the culture media was obviously high enough to trigger effects in cell culture systems during *in vitro* testing and thus was adequate in all tests conducted for this publication. The beryllium ion may interfere with cell growth or metabolic regulation, but further conclusions are difficult due to the general character of the assay and the relatively small database on reference substances with elucidated mode of action. It should be noted that the SHE cell transformation assay has a high specificity in prediction of rodent carcinogenesis, but predictive power for distinguishing rodent and human carcinogens is lower, which may be a result of the rodent source of cells. In a systematic approach to correlate known rat and human carcinogens with the results of the SHE assay, high concordance (89%) in case of known rodent carcinogens was found, while concordance was lower (37%) between SHE assay results and known human carcinogens (Mauthe *et al.*, 2001). Thus, residual uncertainty on the relevance for man remains, especially in absence of classical genotoxicity findings.

DNA repair synthesis, as an indirect marker of DNA damage, was investigated with beryllium metal extracts in primary rat hepatocytes in an UDS assay. No DNA repair synthesis (and thus no hints of preceding DNA damage) was observed upon beryllium metal exposure (Strupp, 2010). A reduction of the expression of messenger RNA (mRNA) coding for DNA repair proteins was observed upon incubation

of a continuous human cell line with a soluble beryllium compound (Joseph *et al.*, 2001) and was suspected to be a relevant mechanism for potential carcinogenicity of beryllium (Beyersmann and Hartwig, 2008). To investigate if this effect on the mRNA level translates into a final functional response of the cells and might be relevant for beryllium metal, the UDS assay was slightly modified. The DNA of rat primary hepatocytes was intentionally damaged by incubation with 2-acetylaminofluorene, a known DNA damaging agent, and coincubated with beryllium metal extracts (Strupp, 2010). DNA repair synthesis was reduced by coincubation with beryllium metal extract. However, it should be noted that this effect was only observed when the concurrent damage of the DNA was massive (>80% cells in repair), while no effects was observed in cells with lower damage to the DNA (Fig. 2).

Taken together, the investigations on mutagenicity of beryllium metal lead to the following conclusion: beryllium metal is no classical mutagen as no hints for gene mutation or cytogenicity were observed. The findings for the metal extracts are not in contrast to what was found with soluble compounds: although some older studies reported positive results with soluble compounds, there was no strong and conclusive evidence of a mutagenic or cytogenic effect of soluble beryllium compounds in a weight-of-evidence approach.

Beryllium metal does not directly damage the DNA of the cells. There is evidence that beryllium metal can lead to morphological cell transformation and inhibition of DNA repair synthesis. The relevance of these findings for humans is difficult to evaluate as the assays are relatively new and adequate *in vivo* genotoxicity tests to put the observed events into a quantitative perspective (e.g. are they observed at realistic exposures *in vivo* or a high-dose artifact *in vitro*?) are not available. The effects observed on DNA repair do not seem to be restricted to beryllium, similar findings are reported for soluble nickel compounds (Hartwig *et al.*, 1994), and competition with the Mg^{2+} required for DNA repair was postulated as a potential reason. One conclusion that can be drawn from the data is that the mechanism of genotoxicity, if relevant *in vivo*, is unlikely to be a nonthreshold mechanism. A practical threshold can be postulated for beryllium metal since both direct DNA repair enzyme inhibition or DNA/protein expression-mediated effects do definitely require more than one molecule (or in this case ion) to inhibit all DNA repair enzyme molecules or to bind to enough promoter regions to trigger transformation effects in a cell. The effects observed in the SHE assay and the modified UDS assay indicate that the concentrations in the extracts were high enough to lead to biological effects, so despite of the only moderate achieved concentrations of beryllium ions in the extracts, the cells were exposed sufficiently, and exposure is more realistic than when testing soluble compounds. The conclusion is that beryllium metal is obviously not a classical mutagen and has no cytogenetic effects at realistic exposure conditions. The findings on DNA repair and cell transformation deserve attention and further investigations on their relevance *in vivo*; however, a nonthreshold genotoxic effect of beryllium metal is unlikely.

Carcinogenicity. Animal studies: As mentioned previously, an extensive literature search was conducted and screened for beryllium metal as the substance of interest and inhalation as the relevant route of exposure. Five studies addressing carcinogenicity after lung exposure to beryllium metal were identified (one of these being reported or discussed in seven publications; see Table 3). One additional study with beryllium metal was identified (Hueper, 1954) but not considered to add relevant information for risk characterization under human exposure

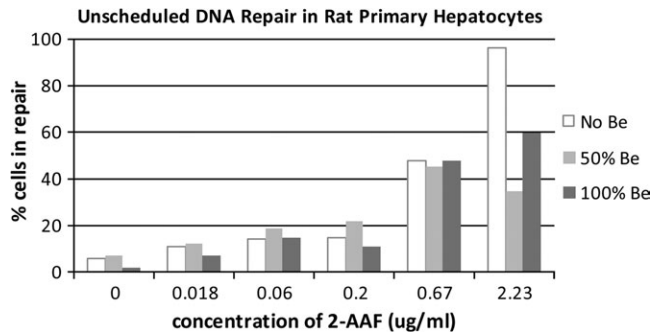

Fig. 2. UDS in rat primary hepatocytes upon coincubation with 2-acetylaminofluorene and beryllium metal.

Table 3. Animal carcinogenicity studies with beryllium metal

Test animal, route of exposure	Carcinogenic response identified	Data quality (Klimisch 1–4)	Reference
Rat inhalation	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 judgment 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.* (1994a/b), Finch *et al.* (1995, 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 per time point).	Groth *et al.* (1980)
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, 1998b)
Guinea pig intratracheal instillation	No	4 (No details on experiment).	Schepers (1961)

conditions due to the unphysiological routes of exposure in this study (intramuscular or intrapleural injection) and thus not included. No carcinogenicity study with oral or dermal exposure to beryllium metal was identified.

The studies were evaluated for quality and reporting by the system proposed by Klimisch *et al.* (1997). None of the identified studies were conducted under Good Laboratory Practice and of the 1531 studies screened, only one of the individual studies was designed and reported in a way that could be evaluated as 'reliable with restrictions (2)' according to the system proposed by Klimisch. Another study was published in smaller fragments in the scientific literature in form of abstracts of oral presentations or posters, experimental, and review articles. Although the individual publications do not report sufficient details to allow evaluation of the data quality, the overall information provided in the publications provides enough details to convince that the study was performed under well-controlled conditions and that the results are reliable. The other publications were 'not assignable (4)' under the Klimisch system as methods and results were only reported in abstract form without description of any experimental details or tabulation of individual data.

However, despite the overall low quality of reporting, application of a weight-of-evidence approach leads to the conclusion that the rat shows a robust carcinogenic response to inhaled beryllium metal even after single exposure. Experimental attempts were made in the study program to reproduce this effect in other species. No carcinogenic responses in mice and guinea pigs could be demonstrated (Schepers, 1961; Finch *et al.*, 1995; Nikula *et al.*, 1995; Finch *et al.*, 1998a,b) and only challenging the situation by using a sensitive animal model (p53 knockout mice or A/J mice with a massive lung tumor background) led to a weak positive response to inhaled beryllium metal.

Differences between rats and other rodent/nonrodent species in response to inhalation exposure to poorly soluble particulate substances have been observed and extensively discussed. Especially, the relevance of tumors observed in rats at conditions overloading the lung's clearance capacity for human health risk assessment has been discussed (Oberdörster, 1995a,b; ILSI Risk Science Institute Workshop Participants, 2000; Pott and Roller, 2005; Sivulka, 2006; Greim and Ziegler-Skylakakis, 2007; Valberg *et al.*, 2009). The particle deposition, clearance, and retention patterns have been demonstrated to be different in rats compared to other species (Muhle *et al.*, 1990; Goodman, 1995; Snipes, 1996; Mauderly, 1997; Nikula *et al.*, 1997; Nikula *et al.*, 2001; Elder *et al.*, 2005; Hext *et al.*, 2005), mainly due to airway geometry. Rats retain more of the particles in the lumen of the alveolar ducts and the alveoli and react at lower levels with

inflammation and fibrosis, while monkeys retain more material in the interstitium and do not demonstrate the strong reactions observed in rats at the same dose (Nikula *et al.*, 1997). The human dose equivalent at which such particle-related effects could occur is significantly higher than that in rat due to the anatomical and physiological differences between rats and humans with regard to particle deposition and retention patterns as well as breathings rates. Monkey and human macrophages have two/five times the volume of rat macrophages, respectively (Krombach, 1997). Thus, rat macrophages may be more sensitive to overload. In addition, differences in location of particles or macrophage-containing particles may partially account for the tendency of rats to respond to poorly soluble particles.

Lung overload is likely to have been present in the rat carcinogenicity studies on beryllium metal. The carcinogenicity study was performed with lung burdens of 33, 84, and 420 µg (Finch *et al.*, 1994a, 1996). Greater than 50% mortality in the high-dose group was observed within 3 weeks after exposure; in a parallel study on lung pathology, chronic inflammation of the rat lung was observed at 10–100 µg, and late-occurring minimal fibrosis at 1.8 µg (Finch *et al.*, 1996). Furthermore, significantly reduced clearance of a tracer particle was described at lung burdens of $\geq$2 µg (Finch *et al.*, 1991b). It should be noted that the positive carcinogenicity study in rats was announced to be repeated at lower doses not overloading the lung (Finch *et al.*, 1994a, 1996), but no results on neoplastic parameters were published, while general toxicity findings on this study repeat were published (Finch *et al.*, 1994b,c, 1996).

The general predictivity of rodent carcinogenicity testing for the human situation is not satisfactory. Alternative methods are currently under evaluation but have not been validated or accepted as standard methods. Systematic review of the carcinogenicity potency database (Gold *et al.*, 1998) revealed that ~50% of the chemicals tested for carcinogenicity in rodents gave positive results in at least one species. Compared to the data obtained by epidemiology and occupational health monitoring, there is a clear indication that there is a high amount of false-positive results. Alternative methods (e.g. carcinogenomics, *in vitro* methods) are currently being evaluated (Vinken *et al.*, 2008) and are promising to show a better correlation to the human situation than the rodent carcinogenicity testing, but until the validations are finished, it remains unclear which tests give better predictions for the human situation. Data from alternative methods may add valuable information to the available database, especially for the challenging human health risk assessment for inhalation exposure to poorly soluble particles.

Epidemiology: Several epidemiological studies on lung cancer risk in beryllium workers have been performed. The early epidemiological work had shortcomings in exposure reporting and confounding factors were not adequately addressed (Mancuso and El Attar, 1969; Mancuso *et al.*, 1970, Mancuso, 1979, 1980; Infante *et al.*, 1980; Wagoner *et al.*, 1980; Steenland and Ward, 1991). They were recently reviewed in detail (Hollins *et al.*, 2009).

Three more recent studies, performed according to modern methods, are available: a larger cohort mortality study (Ward *et al.*, 1992), a nested case–control study basing on the same cohort in USA (Sanderson *et al.*, 2001) and a small mortality study based on the Beryllium Case Registry in UK (Williams, 1996). The difficulty in interpreting epidemiological data with regard to beryllium metal is that the large cohort mortality study does not distinguish between workers exposure to the metal or soluble compounds. Referring to the description on exposure given in the introduction of this article, exposure to soluble beryllium compounds is restricted to the extraction facilities, while exposure of downstream industry workers is toward the metal (mainly in alloys), and general public is more or less only exposed to the naturally occurring background levels.

The only study that deals with exposure to the metal only, although not explicitly stated in the study, is the Beryllium Case Registry study in UK (Williams, 1996). It can be concluded that exposure in this study was to the metal only as during the period reported in the study no soluble beryllium compounds were in commerce in UK. The study is based on autopsy of all beryllium-exposed workers who died from CBD (30 cases). No case of lung cancer was identified among those deaths.

The large cohort study dealing with exposure to metal and soluble compounds is the culmination of a series of overlapping cohort mortality studies of lung cancer in beryllium workers, funded, and performed by the US National Institute for Occupational Safety and Health (NIOSH) in cooperation with the US beryllium industry. It is the most comprehensive cohort mortality study to date, investigating 9332 workers at eight facilities (Ward *et al.*, 1992). The study recommended the performance of a nested control study of the relationship of estimated beryllium exposure to lung cancer, which was subsequently carried out and published in 2001 (Sanderson *et al.*, 2001).

In the cohort mortality study (Ward *et al.*, 1992), the overall standardized mortality ratio (SMR) was 1.26 (95% confidence limits 1.12–1.42) which when corrected for smoking was 1.09 (95% confidence limits include 1.0). Two of the eight study cohorts had significantly elevated lung cancer SMRs (1.69 and 1.24, respectively). One of these was reduced to 1.49 after correction for smoking, the others corrected to 1.09, 0.96, or 1.02 (95% confidence limits overlapping unity), depending on the smoking correction method and reference used (Ward *et al.*, 1992; Levy *et al.*, 2002). Despite the paucity of statistically significant elevated SMRs after correction for smoking, the study presented a series of analyses and *ad hoc* observations on the smoking uncorrected SMRs. No analyses or *ad hoc* comparisons were performed on smoking corrected SMRs. The single plant where statistically significant evidence was identified led the authors to conclude that 'Occupational exposure to beryllium is the most plausible explanation for the increased risk of lung cancer observed in this study'. Another study (Levy *et al.*, 2009) reanalyzed the data of the cohort mortality study using proportional hazards analysis and reexamined patterns without conditioning on statistical significance. This study concluded 'The patterns observed provide little support for an association of lung cancer with beryllium work factors. This result is likely due to the absence in the original study of a significant overall excess of lung cancer after smoking adjustment'. The available historical smoking information is a significant limitation and a source of scientific discussion (Richardson, 2010).

The nested control study (Sanderson *et al.*, 2001) performed as follow-up to the previously discussed study used the largest single plant cohort (N = 3569) in the cohort mortality study. With follow-up through 1992, 142 subjects were identified who died from lung cancer and these were compared to 710 control subjects, five selected for each case (for time worked, cumulative, average, and maximum exposure). Cases did not have significantly higher values for any of the exposure metrics until exposure was lagged with latency assumptions of 10 and 20 years, resulting in significant case–control differences being observed. The study was criticized with the argument that cases and controls had different mean ages of hire and that the lagging results were severely attenuated when these differences were narrowed by closer case–control matching on age at hire (Levy *et al.*, 2007). Also, in simulations in which cohort members were selected at random to serve as 'cases', large differences between these randomly selected subjects and their controls were observed when exposure was lagged (Deubner *et al.*, 2007).

In response to these criticisms, the study data were reanalyzed (Schubauer-Berigan *et al.*, 2008) using date of birth or age at hire as covariates, with the result that the relationship of lagged exposure to lung cancer was attenuated. Most importantly in this reanalysis, neither time worked with beryllium nor cumulative beryllium exposure were significantly associated with lung cancer whether lagged or unlagged, agreeing with Levy *et al.* (2007). This analysis showed that while average and maximum exposure were not significantly associated with lung cancer unlagged or lagged 20 years, an association persisted at lag 10 years.

There has been extensive commentary (Deubner and Roth, 2009; Hein *et al.*, 2009; Langholz and Richardson, 2009; Wacholder, 2009) on the two studies and their reanalysis. This commentary has suggested that the results in Levy *et al.* (2007) may be affected by negative bias due to the method of control selection and that residual date of birth confounding may be present in the results in Schubauer-Berigan *et al.* (2008).

Thus, considerable uncertainty remains whether rates of lung cancer are significantly increased in beryllium workers after smoking correction and whether the degree of beryllium exposure in beryllium workers (exposure to different species of beryllium) is related to the development of lung cancer. This uncertainty was recently articulated in a review (Hollins *et al.*, 2009) and also in the EU Commission manuscript 'Information Notices on occupational Diseases: A guide to Diagnosis' (European Commission, 2009).

Taken together, the epidemiological studies deliver no clear evidence of whether a correlation exists or not between beryllium (and compounds) inhalation exposure and lung carcinogenicity. The same cohort is extensively discussed in the scientific community, and—depending on the reviewer—statistical significance is found or not. No initiatives to collect data to build up a new cohort (reflecting current exposures and with detailed investigation of smoking habits) have been taken so far.

CONCLUSIONS

Beryllium metal differs significantly from beryllium salts in regard to physicochemical properties and dissolution kinetics in body fluids; read across of toxicological properties is not considered appropriate for all endpoints. The current classification of beryllium and its compounds is not considered

adequate for beryllium metal as an individual substance as new studies do not confirm the risks for acute oral toxicity, skin and eye irritating effects as well as skin-sensitizing effects assigned to beryllium metal.

Beryllium metal was found not to be a classical mutagen but to have *in vitro* effects on cell transformation and DNA repair synthesis. From the results of the *in vitro* tests, it can be postulated that beryllium metal is unlikely to exhibit nonthreshold effects.

The animal carcinogenicity studies on beryllium metal are very much focused on carcinogenicity in the rat. Although there is an indication that the rat is not an ideal model for this endpoint for poorly soluble particulate substances due to special lung reactions, carcinogenicity data in other species are scarce. It is concluded that additional carcinogenicity studies in rats would not further elucidate the situation, while mechanistic studies in nonrodent species and evidence-based medicine have the potential to add valuable information for human health risk assessment.

Discussions on appropriate analysis of the existing epidemiological data are still ongoing, and the evidence for carcinogenicity is weak compared to typical known human carcinogens, despite very high exposure levels during the periods epidemiologically analyzed. In addition, as exposure in the epidemiological studies included mixed exposure to soluble and insoluble beryllium compounds, it is questionable if this evidence is appropriate for classification of the pure metal.

The studies on beryllium metal, coupled with the analysis of the literature, suggest that full read across from soluble beryllium compounds to pure beryllium metal may not be appropriate and demonstrates that care should be taken when attempting to classify metals and metal compounds by applying read across. Decisions regarding the toxicity of data rich substances should be based on high-quality studies. It is clear that the often maligned REACH regulation does provide a unique opportunity for toxicologists, authorities, and industry to truly assess the quality of past data and the opportunity to generate new high-quality data that can and should be used to guide our future risk management initiatives.

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