Concerted Uranium Research in Europe (CURE): toward a collaborative project integrating dosimetry, epidemiology and radiobiology to study the effects of occupational uranium exposure

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Concerted Uranium Research in Europe (CURE): toward a collaborative project integrating dosimetry, epidemiology and radiobiology to study the effects of occupational uranium exposure

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Abstract
The potential health impacts of chronic exposures to uranium, as they occur in occupational settings, are not well characterized. Most epidemiological studies have been limited by small sample sizes, and a lack of harmonization of methods used to quantify radiation doses resulting from uranium exposure. Experimental studies have shown that uranium has biological effects, but their implications for human health are not clear. New studies that would combine the strengths of large, well-designed epidemiological datasets with those of state-of-the-art biological methods would help improve the characterization of the biological and health effects of occupational uranium exposure. The aim of the European Commission concerted action CURE (Concerted Uranium Research in Europe) was to develop protocols for such a future collaborative research project, in which dosimetry, epidemiology and biology would be integrated to better characterize the effects of occupational uranium exposure. These protocols were developed from existing European cohorts of workers exposed to uranium together with expertise in epidemiology, biology and dosimetry of CURE partner institutions. The preparatory work of CURE should allow a large scale collaborative project to be launched, in order to better characterize the effects of uranium exposure and more generally of alpha particles and low doses of ionizing radiation.

Keywords: uranium, radionuclides, dosimetry, biomarkers, molecular epidemiology, epidemiology, occupational exposure
primarily the fact they are characterized by a heterogeneous distribution of dose in the body from much more densely ionizing radiation at the cellular level.

Uranium is an element with alpha-emitting isotopes, naturally occurring in the environment as a mixture of three of them: by mass, $^{238}\text{U}$ is the most abundant (99.275%), while $^{235}\text{U}$ and $^{234}\text{U}$ represent 0.72% and 0.005%, respectively (UNSCEAR 2008). All three isotopes exhibit the same chemical properties, but have different radioactive characteristics: $^{238}\text{U}$ has low specific activity, with a half-life of $4.5 \times 10^9$ years, whereas $^{235}\text{U}$ and especially $^{234}\text{U}$ are more radioactive, with half-lives of $7.0 \times 10^8$ and $2.5 \times 10^5$ years, respectively (ATSDR 2013). The general population is routinely exposed to natural uranium through ingesting it in food and drinking water (ATSDR 2013, Ansoborlo et al. 2015).

Uranium is the main raw material used in the production of nuclear fuel. Most civil reactors use enriched uranium, characterized by a higher proportion of $^{235}\text{U}$ (2 to 5%) and $^{234}\text{U}$, with respect to the natural composition. Enriched uranium is therefore more radioactive than natural uranium (UNSCEAR 2008). The industrial process of enrichment also generates depleted uranium, a by-product which is less radioactive (containing less $^{235}\text{U}$ and $^{234}\text{U}$) than natural uranium and which has been used as counterweights or ballast in aircraft, for shielding and in military applications. Other, human-made (artificial), isotopes of uranium are also encountered at certain stages of the fuel cycle, for instance $^{232}\text{U}$ in reprocessed uranium (Guseva Canu et al. 2011). Since uranium is present, in various isotopic compositions, at all stages of the nuclear fuel cycle (see figure 1), hundreds of thousands of workers involved in this cycle have been occupationally exposed to uranium worldwide (probably more than any other radio-element). These workers have generally been exposed at low levels, but potentially over protracted periods, primarily through inhalation of particulate aerosols.

Because uranium is radioactive and a heavy metal, it is potentially both radiologically and chemically toxic (Dublineau et al. 2014). Experimental studies on natural or depleted uranium (both being only weakly radioactive i.e. showing low specific activity) have reported significant biological effects including genotoxicity (Stearns et al. 2005, Bal et al. 2011), renal toxicity (Gueguen and Rouas 2012), lung tumor formation (Mitchel et al. 1999) and other lung damage (Leach et al. 1973) as well as impairment of cerebral function (Lestaevlel et al. 2005), increased oxidative stress, damage to the skin, impaired bone formation or reproductive function (ATSDR 2013) and modified metabolism (Grison et al. 2013). Radiotoxicity has been shown to occur in addition to chemical toxicity, especially for exposures to enriched or reprocessed uranium (Houpert et al. 2005, Grignard et al. 2008). However, the implications of these experimental findings for the quantification of risk to human health are not clear. This is especially the case for chronic inhalation of uranium compounds with different chemical forms and isotopic compositions. Experimental data relevant to such exposure situations, which generally occur in occupational settings, remain limited (Leach et al. 1973, Mitchel et al. 1999, Monleau et al. 2005, Ibanez et al. 2014). It is therefore important to directly investigate the potential biological and health effects associated with occupational exposure to uranium.

Cohorts of uranium miners and other workers employed at later stages of the uranium cycle are of major interest for the study of the health effects of uranium, and more generally of internally deposited radionuclides (Laurier et al. 2012). The strengths of these cohorts include regular collection of socio-demographic and dosimetric data (Blanchardon et al. 2007, Marsh et al. 2012), and of health surveillance data from medical check-ups conducted by the occupational medicine service (Garsi et al. 2014, Drubay et al. 2015). In these cohorts, subjects have been followed up for several decades at the individual level for mortality and, in some cases, for cancer incidence (Gillies and Haylock 2014). Although uranium miners have been exposed to uranium ore dust, previous epidemiological studies in these miners have predominantly aimed to investigate the health effects of exposure to radon (a decay product
of uranium) which contributes the majority of the radiation dose in many mines (NRC 1999). Potential associations between uranium exposure and health outcomes in these miners warrant additional studies (Kreuzer et al 2010a, Rage et al 2015, Tomasek and Malatova 2006). Epidemiological studies focusing on the health effects of uranium exposure at later stages of the nuclear fuel cycle have also been performed (e.g.: Dupree et al 1995, Chan et al 2010, Guseva Canu et al 2011, Silver et al 2013, Zablotska et al 2013, Gillies and Haylock 2014, Kreuzer et al 2015b, Zhivin et al 2015, Samson et al 2016) but were generally limited by low statistical power for characterizing exposure-risk relationships. In addition, many of them did not include any quantification of radiation dose resulting from uranium exposure or did not use harmonized dosimetric approaches, which limits the comparability of their results (Canu et al 2008, Zhivin et al 2014). Therefore, individual epidemiological studies conducted so far do not provide reliable quantification of the potential health risks associated with uranium exposure (Zhivin et al 2014). In spite of these limitations, some studies have reported biologically plausible positive associations between uranium exposure and lung cancer (Ritz 1999,}

**Figure 1.** The nuclear fuel cycle. © Institut de Radioprotection et de Sûreté Nucléaire (IRSN).
Guseva Canu et al (2011), lymphatic and hematopoietic tumours (Yiin et al 2009, Guseva Canu et al 2011). In addition, excesses have been observed for circulatory diseases and intestinal cancer in single studies (Guseva Canu et al 2012, Silver et al 2013), however up to date few studies examined these outcomes and provided inconsistent results (Zablotska et al 2013, Kreuzer et al 2015b). In order to provide improved estimates of uranium exposure risks, large-scale epidemiological studies which address the limitations of previous studies are needed. Existing cohorts should be pooled, after verification of their compatibility, in order to increase the statistical power of analyses. Individual organ doses resulting from uranium exposures should be assessed using harmonized state-of-the-art methodology in all study cohorts.

In addition, further improvements to the understanding and quantification of biological and health effects of uranium exposure can be gained through integrated epidemiological and radiobiological research (Laurier et al 2012, Pernot et al 2012, Kreuzer et al 2015a). One way to do this would be to conduct molecular epidemiological studies, by integrating the use of biomarkers in epidemiological studies (Palmer 2007, Zins et al 2010, Kreuzer et al 2015a). Various types of biomarkers could be used in epidemiological studies as a means to improve the risk assessment process, as discussed in (Pernot et al 2012). Firstly, new biomarkers of exposure might help refine the assessment of uranium intake and resulting dose. For instance, non-targeted techniques (OMICS) open new perspectives for that purpose (Grison et al 2013). This might be helpful to overcome some limitations of currently used bioassays (e.g.: short persistence, lack of information on the physicochemical form of incorporated uranium compounds). Secondly, biomarkers of early and late biological effects (e.g.: markers of biological changes in putative target organs or tissues for uranium) may help to elucidate biological response mechanisms induced by uranium exposure in human populations. Subsequently, if a sufficiently large group of workers can be followed-up in the long-term, associations between these biomarkers and risks of disease could be quantified. This would be useful (1) to determine their predictive value for risk quantification and (2) to help translate biological responses observed in many experimental studies (Brugge and Buchner 2011) into health risk estimates. Molecular epidemiology might thus lead to the identification or validation of new biomarkers useful for the surveillance of workers occupationally exposed to uranium.

Pioneering molecular epidemiology studies have attempted to characterize the biological effects of uranium exposure in the general population from consuming water naturally contaminated with uranium (Mao et al 1995, Kurttio et al 2002, 2005, 2006, Selden et al 2009, Zamora et al 2009). Such studies have also be carried out in occupationally exposed groups, including miners, other uranium workers and the military (Dounce et al 1949, Clarkson and Kench 1952, Eisenbud and Quigley 1956, Luessenhop et al 1958, Brandom et al 1972, Thun et al 1985, Martin et al 1991, Milacic 2008, Milacic and Simic 2009, McDiarmid et al 2011a, 2011b, Prat et al 2011, Zolzer et al 2012a, 2012b, McDiarmid et al 2013, Li et al 2014, Leng et al 2016). However, molecular epidemiology studies conducted so far have often lacked organ doses, and the cross sectional design used in the majority of studies is not appropriate for a detailed characterization of the chronic effects of protracted, cumulative, uranium exposure (Canu et al 2011). An efficient solution that would address these limitations would be to nest molecular epidemiology studies within long-standing established cohorts for which data on dosimetry and covariates have already been, or are planned to be, collected (Gomolka et al 2012).

The objective of the CURE (Concerted Uranium Research in Europe) project was to develop a multidisciplinary and collaborative research protocol, integrating epidemiology, biology/toxicology and dosimetry to improve both the understanding and quantification of biological and health effects associated with occupational uranium exposure in Europe.
This general objective included two specific aims:

- To prepare a common protocol for pooled epidemiological analyses of cohorts of uranium miners and workers, that would overcome the limitations of previous studies, in order to directly estimate the potential health risks associated with uranium exposure.
- To verify the feasibility of a state-of-the-art molecular epidemiology approach in established cohorts of uranium miners or workers and, if feasible, to produce a common protocol for the development of molecular epidemiological studies.

The importance of the production and use of the best possible dose estimates and the consideration of sources of uncertainties at each step of the research, was recognised.

The full protocol produced by the CURE project has been described in a comprehensive, publicly available report (Laurent et al 2015). The present paper provides an overview of the collaborative and multidisciplinary approach of the project, its main outputs and conclusions.

2. Material and methods

CURE was a concerted action supported by the European Commission 7th Framework Program network of excellence DoReMi (http://doremi-noe.net/). The project duration was 18 months, from July 1, 2013 to December 31, 2014. CURE gathered together the main organizations conducting or analyzing cohort studies of miners and other workers occupationally exposed to uranium in Europe, as well as organizations with recognized expertise in internal dosimetry or in radiobiology. In total 9 partners from 6 Countries participated in the CURE project: Institut de Radioprotection et de Sûreté Nucléaire (IRSN, France), Bundesamt für Strahlenschutz (BfS, Germany), Public Health England (PHE, United Kingdom), Nuvia Limited (United Kingdom), Atomic Weapons Establishment (AWE, United Kingdom), StudieCentrum voor Kernenergie • Centre d’étude de l’Energie Nucléaire (SCK•CEN, Belgium), Státní Ustav Radiační Ochrany (SURO, Czech Republic), Centre for Research in Environmental Epidemiology (CREAL, Spain) and Institut Curie (IC, France). IRSN was the project coordinator.

The CURE project initially had four main work packages (WPs): the first was dedicated to epidemiology, the second to dosimetry, the third to biology and the fourth to the management and general coordination of the project. Subsequently, an Uncertainty Working Group (UWG) was added, which included members from the three main WPs (epidemiology, dosimetry and biology). The UWG aimed to identify the sources of uncertainties at different steps of the project and methods for addressing them. The main tasks of each WP and interactions between them are briefly summarized in figure 2.

3. Results: integrated protocol

The final integrated protocol lists European cohorts and cohort subsets of potential interest to future pooled epidemiological analyses and molecular epidemiology studies. It describes the biological protocol developed to enable a state-of-the-art centralized joint biobank to be set up for uranium workers, in order to conduct molecular epidemiology studies. A harmonized dosimetry protocol, applicable to both conventional and molecular epidemiological studies, is presented. Methods to be used for statistical analyses are summarized. The main sources of uncertainties identified by the CURE project, as well as proposed methods to address them, are also listed.

3.1. Epidemiologic cohorts and subsets for analyses

This section presents cohorts and cohort subsets identified as suitable for inclusion in the three different aspects of the proposed study. The first aspect would be the characterization
of a general risk profile, by an analysis of mortality and morbidity (where available) rates in cohorts of uranium workers as compared to the rates in the general population (from their respective countries). This could be done for the entire population of pooled cohorts, regardless of whether detailed dosimetry data are available or not. The second aspect would be an analysis of the associations between uranium exposure and health risks (‘dose-response analyses’) among subgroups of workers for which uranium exposure and resulting doses could be estimated with the highest confidence. The third aspect would be the collection of biological samples for future molecular epidemiology studies.

3.1.1. Cohorts for general mortality or morbidity analyses. Six groups of miners and nuclear fuel cycle workers exposed to uranium in Europe are suitable for inclusion in pooled epidemiological analyses. The main characteristics of each of these cohorts are presented in tables 1 and 2. Cohorts of uranium miners and millers (who handle uranium ore but have very much lower radon exposures than underground miners) will be presented separately from other cohorts of uranium workers because of the markedly different exposure conditions in these groups. Henceforth, for simplicity, the term ‘nuclear worker’ will exclude miners and millers.

Cohorts of uranium miners have been established in Germany (\(N = 58982\) miners) (Kreuzer et al. 2010b), France (\(N = 5086\)) (Rage et al. 2015) and in the Czech Republic (\(N = 7513\)) (Tomasek 2012) (see table 1). These populations (total \(N > 70000\)) have been followed-up for several decades. As mentioned above, uranium miners were not only exposed to uranium ore dust but also to relatively high levels of radon, especially in the early years (1940s and 1950s) of underground mining due to bad ventilation conditions. At that time, absorbed lung doses from inhalation of radon progeny were appreciably higher than lung doses from inhalation of uranium dust. For this reason, CURE identified subgroups of miners exposed to low radon concentrations, but still relatively high uranium concentrations, so that radon exposure is less likely to obscure potential effects of uranium exposure. In some settings, non-trivial proportions of doses to organs other than lung may be due to uranium dust, for instance about half of the equivalent dose to the red bone marrow in Czech miners has been estimated to be due to long-lived radionuclides present in the uranium ore (Tomasek and Malatova 2006). Miners who could be included are those employed from 1955 onwards. The identified potential study
population includes 40,000 uranium miners with low radon exposures and millers (altogether, about 29,400 subjects from Germany, 3,400 from France and 7,500 from the Czech Republic). In addition, sensitivity analyses could be conducted in subgroups of millers and open pit miners, who never worked underground and therefore had very low radon exposures ($N = 6,227$). A specific cohort of uranium millers has already been set up in Germany (Kreuzer et al. 2015b) ($N = 4,054$). Others are being set up in France ($N = 1,291$) and in the Czech Republic ($N \sim 980$) (see table 1).

At later stages of the nuclear fuel cycle, nuclear workers are exposed to uranium as part of various activities: purification, conversion and enrichment of uranium, fuel manufacturing and reprocessing, decommissioning, recovery and decontamination of effluents and waste, weapons production and research (see table 2). These workers have been included in several existing cohorts. These include three cohorts in the United Kingdom: the British Nuclear Fuels Limited (BNFL) (Gillies and Haylock 2014), United Kingdom Atomic Energy Authority (UKAEA) (Atkinson et al. 2007) and AWE (Carpenter et al. 1994, Johnson et al. 1999) cohorts (these latter two are both contained within the SHIELD database). In France, the TRACY cohort including workers from AREVA and former subsidiary companies has also been set up (Samson et al. 2016). A cohort of nuclear workers has been set up in Belgium (Engels et al. 2005), but it does not include all uranium exposed workers at present. However, a cohort of Belgian uranium workers employed at Franco Belge de Fabrication du Combustible (FBFC) could be set-up using available computerized information. For the first time, a large joint cohort of about 40,000 European nuclear workers potentially exposed to uranium could be assembled by pooling data from the UK ($N \sim 24,500$), France ($N \sim 12,700$) and Belgium ($N \sim 1650$). Such a joint cohort would cover all the major stages of the uranium cycle (see figure 1).

3.1.2. Cohorts and cohort subsets for dose response analyses. For the groups of miners with low radon exposure, individual information on exposure to uranium, radon and external gamma radiation is already available, mostly from ambient measurements and based on detailed job-exposure matrices, but also from individual monitoring of exposure. Internal doses to several organs (lung, kidney, liver, red bone marrow) were calculated within the framework of the European Commission 6th Framework Program for research, Alpha risk project (Marsh et al. 2012) and the contribution of each exposure component (uranium, radon and external gamma radiation) was quantified. The population considered for dose-response analyses could therefore be as large as that which could be included in the general mortality analyses.

For nuclear workers involved in subsequent stages of the nuclear fuel cycle, the evaluation of occupational internal exposure to uranium is usually based on individual bioassay monitoring results. Routine bioassay measurement of uranium and other radionuclides, primarily in urine but also faeces, whole-body or lungs, were conducted on a regular basis, and additional follow-up measurements were performed in the event of incidents (see section 3.3). The availability and quality of such data are the key criteria for inclusion in analyses of dose-response relationships. CURE identified subgroups for which both internal doses resulting from uranium exposure and external doses could be estimated. Such data could be obtained for 4,500 workers from France, 24,700 from the UK and 800 from Belgium. In total, a cohort of 30,000 workers could be available for the purpose of studying dose-response relationships.

3.1.3. Cohorts proposed for pilot molecular epidemiology studies. CURE thoroughly examined options to launch pilot, prospective molecular epidemiology studies in currently active uranium workers. Regular medical check-ups conducted routinely as part of occupational medical surveillance over the workers’ careers constitute an unique potential framework for the prospective and repeated collection of biological samples (Laurier et al. 2012).
Table 1. Main characteristics of the European cohorts of uranium miners and millers in Europe.

<table>
<thead>
<tr>
<th>Site and country (1)</th>
<th>Cycle stage</th>
<th>Companies involved</th>
<th>Uranium compounds</th>
<th>Workers (≈N)</th>
<th>Job exposure matrix</th>
<th>Medical files information</th>
<th>Technical feasibility to collect biosamples (urine, blood…)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole miners cohort (CZ S+N)</td>
<td>Mine</td>
<td>JD/CSUP/ DIAMO</td>
<td>Ore dust</td>
<td>9978</td>
<td>No</td>
<td>Smoking in a lung cancer case-control set (1029+2648)</td>
<td>No</td>
</tr>
<tr>
<td>Rozna (CZ R) Mine/mill</td>
<td>CSUP/ DIAMO</td>
<td>U₂O₈, UO₂, U(SiO₄)₁₋ₓ(OH)ₓ</td>
<td>1300</td>
<td>Personal dosimeters</td>
<td>Can be collected</td>
<td>Confirmed</td>
<td></td>
</tr>
<tr>
<td>Whole miners cohort (FR) Mine</td>
<td>CEA—COGEMA (AREVA)</td>
<td>Ore dust</td>
<td>5086</td>
<td>No</td>
<td>Only for sub-samples: case control studies of lung cancer (n = 600) and cardiovascular diseases (n = 300)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Lodève, les Bois Noirs, Bessines, L’Escarpière, Jouac (FR) Mills</td>
<td>SIMO-SMJ</td>
<td>Ore dust, UO₄, MgU₂O₇, (NH₄)₂U₂O₇</td>
<td>1291</td>
<td>No</td>
<td>To be determined</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Wismut (cohort, GE) Underground miner</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>40443</td>
<td>Yes</td>
<td>Only smoking from a Case control study of lung cancer (10%)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Wismut (cohort, GE) Open pit miner</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>1299</td>
<td>Yes</td>
<td>Only smoking from a Case control study of lung cancer (10%)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Wismut (cohort, GE) Surface workers</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>9223</td>
<td>Yes</td>
<td>Only smoking from a Case control study of lung cancer (10%)</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
| Site and country (1) | Cycle stage | Companies involved | Uranium compounds | Workers (≈N) | Job exposure matrix | Medical files information | Technical feasibility to collect biosamples (urine, blood…)
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wismut (cohort, GE)</td>
<td>Mixed mining activity</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>3856</td>
<td>Yes</td>
<td>Only smoking from a Case control study of lung cancer (10%)</td>
<td>No</td>
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<tr>
<td>Wismut (cohort, GE)</td>
<td>Millers</td>
<td>Wismut</td>
<td>Ore dust and yellow cake</td>
<td>4161</td>
<td>Yes</td>
<td>Only smoking from a Case control study of lung cancer (10%)</td>
<td>No</td>
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<tr>
<td>Wismut (biobank, GE)</td>
<td>Mixed mining activity</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>400</td>
<td>Yes</td>
<td>Smoking and blood pressure available, maybe more (weight/height, blood cell counts…)</td>
<td>Blood already collected no other sampling possible.</td>
</tr>
<tr>
<td>Wismut (biobank, GE)</td>
<td>Millers</td>
<td>Wismut</td>
<td>Ore dust and yellow cake</td>
<td>39</td>
<td>Yes</td>
<td>Smoking and blood pressure available, maybe more (weight/height, blood cell counts…)</td>
<td>Blood already collected, no other sampling possible.</td>
</tr>
<tr>
<td>Wismut (archives, GE)</td>
<td>Mixed mining activity</td>
<td>Wismut</td>
<td>Ore dust</td>
<td>230</td>
<td>Yes</td>
<td>No</td>
<td>DNA and RNA from lung cancer tissue and normal lung tissue</td>
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<tr>
<td>Wismut (archives, GE)</td>
<td>Millers</td>
<td>Wismut</td>
<td>Ore dust and yellow cake</td>
<td>20</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
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</table>

(1) CZ R: Czech Republic; FR: France; GE: Germany.
<table>
<thead>
<tr>
<th>Site and country (1)</th>
<th>Cycle stages (2)</th>
<th>Companies involved</th>
<th>Uranium compounds</th>
<th>Workers (≈ N)</th>
<th>Radiotoxicological data</th>
<th>Job exposure matrix</th>
<th>Medical files information</th>
<th>Technical feasibility to collect biosamples (urine, blood…)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvési (FR)</td>
<td>Purification/</td>
<td>AREVA NC</td>
<td>U₃O₈, UO₂(NO₃)₂, (NH₄)₂U₂O₇, UO₃, UO₂, UF₄, U metal</td>
<td>750</td>
<td>Computerized</td>
<td>Available in 2017</td>
<td>Computerized</td>
<td>Confirmed</td>
</tr>
<tr>
<td></td>
<td>CO UF₆</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pierrelatte (FR)</td>
<td>CO UF₆</td>
<td>AREVA NC</td>
<td>UF₆, UF₅</td>
<td>1000</td>
<td>Computerized</td>
<td>No</td>
<td>Computerized</td>
<td>Unknown</td>
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<td>Pierrelatte (FR)</td>
<td>EN (civil)</td>
<td>EURODIF</td>
<td>UF₆</td>
<td>2100</td>
<td>Available but not computerized</td>
<td>Available</td>
<td>Available but not computerized</td>
<td>Unknown</td>
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<tr>
<td>Pierrelatte (FR)</td>
<td>EN (military)</td>
<td>CEA, AREVA NC</td>
<td>UF₆</td>
<td>3100 Overlap with CEA: about 1700 workers</td>
<td>Available but not computerized</td>
<td>Available</td>
<td>Available but not computerized</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pierrelatte (FR)</td>
<td>PDU</td>
<td>AREVA NC</td>
<td>UF₆, UO₂, U₃O₈</td>
<td></td>
<td>Available but not computerized</td>
<td>Available</td>
<td>Available but not computerized</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pierrelatte (FR)</td>
<td>PRU</td>
<td>AREVA NC</td>
<td>UO₂(NO₃)₂, UO₂, U₃O₈</td>
<td></td>
<td>Computerized only for a nested case control study of cardiovascular diseases (n ~ 395)</td>
<td>Available</td>
<td>Available but not computerized</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Site and country (1)</th>
<th>Cycle stages (2)</th>
<th>Companies involved</th>
<th>Uranium compounds</th>
<th>Workers (≈N)</th>
<th>Radiotoxicological data</th>
<th>Job exposure matrix</th>
<th>Medical files information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pierrelatte (FR)</td>
<td>RA</td>
<td>CEA</td>
<td>UF₆, UF₆</td>
<td>5000</td>
<td>Available but not computerized</td>
<td>Partly available</td>
<td>Available but not computerized</td>
</tr>
<tr>
<td>Pierrelatte (FR)</td>
<td>RW</td>
<td>SOCATRI</td>
<td>UF₆, UF₆, UO₂F₅, UO₄, U₃O₈, U₃O₈, (NH₄)₂U₂O₇, UO₂(NO₃)₂</td>
<td>800</td>
<td>Available</td>
<td>Computerized</td>
<td>Computerized</td>
</tr>
<tr>
<td>Romans/</td>
<td>FO</td>
<td>FBFC, CERCA</td>
<td>UO₂, UO₂F₂, UO₂, U₃Al₆-Al, U₃Si₂-Al, UO₂(NO₃)₂, UO₂, UF₆</td>
<td>2100</td>
<td>Computerized</td>
<td>No</td>
<td>Computerized</td>
</tr>
<tr>
<td>Pierrelatte (FR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marcoule (FR)</td>
<td>FX</td>
<td>MELOX</td>
<td>UF₆, UO₂, UF₆, UF₄</td>
<td>850</td>
<td>Computerized</td>
<td>No</td>
<td>Partly known</td>
</tr>
<tr>
<td>Aldermaston (UK)</td>
<td>WP</td>
<td>AWE</td>
<td>Predominantly insoluble oxides</td>
<td>3694</td>
<td>Available</td>
<td>No</td>
<td>Available</td>
</tr>
<tr>
<td>Dounreay (UK)</td>
<td>RO, EP, RP, RA</td>
<td>UKAEA</td>
<td>UO₂ fuel, various metal alloys, UO₂(NO₃)₂</td>
<td>4203</td>
<td>Computerized</td>
<td>No</td>
<td>Available</td>
</tr>
<tr>
<td>Harwell (UK)</td>
<td>RO, RA</td>
<td>UKAEA</td>
<td>U metal, U oxides, UO₂(NO₃)₂</td>
<td>1047</td>
<td>Computerized</td>
<td>No</td>
<td>Available</td>
</tr>
<tr>
<td>Winfrith (UK)</td>
<td>RO, EP, RA</td>
<td>UKAEA</td>
<td>UO₃, uranium-thorium carbide fuel elements</td>
<td>582</td>
<td>Computerized</td>
<td>No</td>
<td>Available</td>
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</tbody>
</table>

(Continued)
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<tr>
<th>Site and country (1)</th>
<th>Cycle stages (2)</th>
<th>Companies involved</th>
<th>Uranium compounds</th>
<th>Workers (≈N)</th>
<th>Radiotoxicological data</th>
<th>Job exposure matrix</th>
<th>Medical files information</th>
<th>Technical feasibility to collect biosamples (urine, blood…)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Springfields (UK)</td>
<td>FM, FO, RA</td>
<td>BNFL (NDA)</td>
<td>U, U₂O₃, UO₂, (NH₄)₂U₂O₇, UO₂(NO₃)₂, UO₆, UO₂F₃, UF₄, UF₆</td>
<td>9422</td>
<td>Computerized</td>
<td>No</td>
<td>Occupation health records exist for some workers but their retention and any access to them is currently under review</td>
<td>Some samples have been taken previously but not currently possible</td>
</tr>
<tr>
<td>Sellafield (UK)</td>
<td>RP, PR, RW, RA, FX</td>
<td>BNFL (NDA)</td>
<td>U, U₂O₂(NO₃)₂, UO₂, UO₂, UH₄</td>
<td>2150</td>
<td>Computerized</td>
<td>In progress</td>
<td>Not currently available</td>
<td>Unknown</td>
</tr>
<tr>
<td>Capenhurst (UK)</td>
<td>EN, RA</td>
<td>BNFL (NDA)</td>
<td>UF₆, UF₆, UO₂(NO₃)₂, UO₂F₂, UO₃</td>
<td>3580</td>
<td>Computerized</td>
<td>No</td>
<td>Partly computerized</td>
<td>Confirmed</td>
</tr>
<tr>
<td>(BE) Dessel</td>
<td>FO</td>
<td>FBFC</td>
<td>UO₂, U₂O₈ (UF₆ before 1985?)</td>
<td>1650</td>
<td>Partly computerized</td>
<td>No</td>
<td>Partly computerized</td>
<td>Confirmed</td>
</tr>
</tbody>
</table>

(1) FR: France; UK: United Kingdom; BE: Belgium.
A state-of-the-art biobank centralizing such samples from a fixed cohort of individuals over many years would be a valuable resource for a detailed assessment of the long-term effects of protracted exposure to uranium, as is the case for other types of occupational or environmental exposures (Palmer 2007, Zins et al 2010).

The criteria developed to identify suitable prospective cohorts for pilot molecular epidemiology studies were as follows: (1) population of active nuclear workers, millers or miners (2) regularly undergoing medical check-ups by occupational medicine services (3) regularly monitored for uranium exposure and (4) with the highest levels of exposure among existing situations. In addition, although this was not an absolute criterion, during the course of the CURE project it was considered that conducting investigations on industrial sites already included in established cohorts would be an advantage because of preexisting collection and validation of data but also of knowledge of interlocutors for feasibility studies. Therefore, the possibilities on such sites were examined with priority. Several possible prospective cohorts matching these conditions have been proposed by CURE partners: uranium workers employed at an AREVA uranium conversion plant in Malvesi (France, N ~ 250), uranium millers and miners employed at the Rozna mine (Czech Republic, N ~ 100) and workers involved in decommissioning activities in Dessel (Belgium, N ~ 40). The feasibility of collecting biological materials in these cohorts was investigated in detail, which helped when developing a biological protocol for a molecular epidemiology study (see section 3.2 below).

In parallel, the possibility of examining biological samples collected years to decades after exposure was also considered. This approach is of immediate interest to the ongoing search for persistent biomarkers of exposure or biomarkers of late biological effects, such as a specific biological signature of radiation-induced disease (e.g. uranium specific ‘fingerprints’ in tumor tissue, as recently identified for 131I (Hess et al 2011)), as well as to the study of individual susceptibility. A state-of-the-art biobank of biological samples collected in former uranium miners retired from the Wismut company (Germany) has been set up at BfS (Gomolka et al 2012) and may provide interesting material for such analyses.

3.2. Biological protocol for a molecular epidemiology study

The aim of the biological protocol is to provide a strategy as well as study methods and tools to perform relevant biological sampling, biobanking (including the collection of associated data on potential confounders and effect modifiers), biomarker measurements and analyses in cohorts of uranium exposed workers. The extensive biological protocol developed (Laurent et al 2015) is briefly summarized below. It defines all the aspects of the proposed study. The first is a list of biomarkers of interest in relation to uranium exposure and possible health outcomes, as well as a list of biospecimens that should be collected for the measurement of these biomarkers. Standard operating procedures (SOPs) for biospecimen collection, processing, transportation, storage and for biomarker measurement were developed. SOPs for functional tests (e.g.: to assess the cerebral or circulatory functions of workers) were also proposed. Specific regulations in force in each country were reviewed. An information sheet for workers and a related consent form were developed, as well as a questionnaire to collect the information needed for the interpretation of biomarkers and to perform appropriate statistical analyses.

3.2.1. Proposed biomarkers. A list of biomarkers of interest to studies of the biological effects of uranium has been proposed (Guéguen et al 2016). This list was based on the knowledge of the biokinetics and biological effects of uranium gained from experimental studies and also on preliminary evidence from available epidemiological studies. Biomarkers of lung
cancer and other lung damage were proposed, since dust inhalation is the primary route of occupational exposure to uranium and results from experimental (Leach et al. 1973, Mitchel et al. 1999) and epidemiological (Ritz 1999, Guseva Canu et al. 2011) studies suggest this is potentially a key issue. Biomarkers of renal and bone damage were also proposed, because uranium accumulates in these organs and tissues and evidence from animal and epidemiological studies indicates uranium related effects in kidney (Prat et al. 2011, Gueguen and Rouas 2012, McDiarmid et al. 2013) and bone (Kurttio et al. 2005). Biomarkers of lympho-hematopoietic system impairment were proposed, because of possible irradiation of pulmonary macrophages, lymph nodes and bones (Guseva Canu et al. 2011). Biomarkers and functional tests of cardio-vascular damage were also considered, because of (1) possible effects due to uranium induced kidney impairment, (2) the known effect of particulate matter inhalation on the cardio-vascular system (Araujo and Nel 2009), (3) possible effects of low dose radiation on cardiovascular diseases (Little et al. 2012) and (4) the results from available epidemiological studies in uranium exposed subjects (Kurttio et al. 2006, Wagner et al. 2010, Guseva Canu et al. 2012), although confounding is a potential issue in these studies. Biomarkers of brain damage and related functional tests were proposed, since changes in cognitive functions have been observed in animal experiments (Lestaevel et al. 2005) but only limited data are available in humans (McDiarmid et al. 2013). Looking at DNA damage using cytogenetic biomarkers to evaluate the radiotoxic and chemotoxic effects of uranium on genetic material (Martin et al. 1991, Darolles et al. 2010), was also proposed. Metabolite profiling was proposed because of previous identification of urinary metabolomic signatures of uranium exposure in rats (Grisson et al. 2013). Metabolomics and other types of non-targeted analyses (’-OMICs’) (Pernot et al. 2012) might help identify new biomarkers of uranium exposure in humans, and provide information about the biological pathways potentially affected. Finally, monitoring transcriptional biomarkers (of radiation exposure and resulting long term effects) by analyzing gene expression and specific epigenetic modifications was also found to be of particular interest.

All these biomarkers do not need to be investigated immediately. Modern biobanking methods allow for the long term conservation of biological material in suitable conditions for later analyses, years to decades after biospecimen collection (Palmer 2007, Zins et al. 2010). The SOPs produced will ensure that the quality of the collected and stored material will facilitate future research, including analyses of potential new biomarkers as they are identified through experimental and molecular epidemiological research. The list of biomarkers identified by CURE is therefore still open to the addition of emerging biomarkers.

3.2.2. Biospecimens to be sampled. In order to investigate the proposed biomarkers, it would be necessary to collect the following biospecimens from study participants: blood, urine, sputum or nasal swabs, and saliva (Pernot et al. 2014). Again, ad hoc SOPs were developed for the purpose of optimal sampling and processing of biospecimens to facilitate later analyses of biomarkers. Additional biospecimens such as hair and nails were proposed shortly after the end of the CURE project (Sahoo et al. 2015) and SOPs still need to be developed for their collection and analysis.

3.2.3. Questionnaire. A questionnaire was designed for completion by all voluntary participants in the study at the time of biological sampling. As a complement to the retrospective information available in medical and administrative records from the source cohorts, data collected through this questionnaire would allow potential confounding factors and effect modifiers to be identified and taken into account in the subsequent statistical analysis of biological data. Specific and detailed questions are included in the proposed questionnaire, relating to current and previous diseases, medications, dietary and hormonal supplements, previous
exposure to radiation for medical reasons (i.e.: type of procedure, part of the body irradiated, number of times and corresponding years), alcohol and tobacco consumption (age at start, age at quitting, type of products consumed and amount consumed on typical days), physical activity and sleeping habits. Declarative information on occupational exposures will also be collected, which will complement job exposure matrices already available for retrospective cohorts or cohort subsets (e.g.: Dahmann et al 2008, Guseva Canu et al 2009).

3.2.4. Logistics strategy for the collection of biospecimens in pilot cohorts. Feasibility studies were conducted to set up a logistics strategy integrating the collection, processing, transportation and long-term storage of biospecimens from active and voluntary uranium workers. These feasibility studies focused on the possibility of conducting biological sampling and administrating the related questionnaire at the time of routine medical check-ups organized by the occupational medicine service, generally on a yearly basis. Logistics strategies for biological sampling of active workers were developed, assessed and refined, using the proposed pilot cohorts from the uranium conversion plant in Malvesi (France) and Rozna uranium mine and milling plant (Czech Republic).

The proposed logistic strategy had to be compatible with conditions in the field e.g. timing of medical check-ups, the standard practices and specific considerations of occupational medical staff, the need for subjects to fast prior to collection of samples and the availability of local resources. This single logistic framework also had to facilitate the collection of the biospecimens needed for subsequent measurement of a wide array of biomarkers, for which processing or storage conditions could markedly differ, and to do this in a resource-efficient way. Despite some specific differences, both feasibility studies reached similar conclusions regarding the technical feasibility of collecting biospecimens of interest from workers during occupational health check-ups on a voluntary basis. While the pilot project’s scientific aims and general procedures were very well received by occupational medical staff, in both locations doctors requested help from a clinical research associate to conduct the study (e.g. to administer questionnaires, complete quality assurance documentation, label sample containers and so on) and stated their preference for processing and temporary storage of samples to take place at an external laboratory (typically the subcontractor laboratory which usually processes the samples routinely collected as part of medical check-ups). After these steps, long-term storage of samples is intended to take place in a centralized joint biobank, before distribution to researchers for analyses.

3.3. Dosimetry protocol

To permit the study of relationships between epidemiological and biological endpoints of interest and uranium exposure, the relevant annual absorbed doses resulting from uranium exposure need to be calculated. Hence, doses to the following organs and tissues, for each individual worker, from the first year of radiation work to the end of follow-up, will be required: extra-thoracic airways (mouth and nose), lung, lymph nodes, red bone marrow, endosteum (bone surfaces), kidney, liver, stomach, small intestine, colon, heart and brain.

Doses to the organs and tissues of interest due to exposures to radiation sources other than uranium will also need to be estimated. To investigate potential differences in effect due to radiation quality, total doses arising from the alpha radiation alone, as compared to the sum of alpha, beta and gamma radiation doses, need to be calculated. The mean annual mass of uranium (i.e. the ‘burden’) in the lung, kidney and brain, need to be calculated to investigate its potential chemical toxicity in addition to its potential radiological toxicity.

334
The procedures for dose assessment for miners, millers and other uranium exposed workers are significantly different, although they make use of the same biokinetic (ICRP 1994, 1995, 2015, 2016b) and dosimetric (ICRP 2008, 2009, 2016a) models, which have been developed by the International Commission on Radiological Protection (ICRP). The specifics of dose assessment in each occupational group are presented in detail in (Blanchardon et al. 2014a, 2014b) and are briefly summarized below.

3.3.1. Dose assessment for miners and millers. For miners, intakes of radionuclides are calculated as the product of an activity concentration of a radionuclide in the ambient air (measurements and records of long-lived radionuclides, radon gas and radon progeny), average breathing rate (based on the assumed level of physical activity for each type of work or job) and the duration of exposure. The dose is proportional to the intake and depends on the physicochemical parameters of the contaminant (e.g. particle size, solubility). Hence, annual absorbed doses to organs/tissues are calculated from intake estimates using the aforementioned ICRP biokinetic/dosimetric models and the physicochemical properties of the contaminant (Marsh et al. 2012).

For uranium and long-lived radionuclides, constant chronic inhalation over each year of exposure is assumed. Because of their longer radioactive half-lives and retention within the body, inhaled long-lived radionuclides in the ore dust continue to deliver doses to organs/tissues following cessation of exposure: this is also accounted for when calculating annual doses. For radon and its progeny, acute intakes are assumed because of their shorter half-lives and retention in the body.

Compared with uranium miners, the millers have low radon exposures but still have potentially significant uranium intakes from the ore. Milling operations and related information from German and French mills were reviewed to define specific parameter values for millers’ exposure (Blanchardon et al. 2014b).

3.3.2. Dose assessment for other uranium fuel cycle workers. At later stages of the nuclear fuel cycle, occupational intakes of radionuclides are estimated from bioassay measurement results (mainly urine samples, but also faecal samples and in vivo monitoring of whole-body or lung content) using biokinetic models and additional information on the exposure scenario including the type (acute/chronic) and time of intake as well as the physicochemical form of the contaminant (Bingham et al. 2016). By default, intakes would be assumed to take place by inhalation and, with regard to exposure scenarios, constant chronic intake regimes would be used for each period of routine exposure during employment and acute intakes would be assigned on the basis of records of incidents and special (e.g. in vivo, faecal) monitoring. In case of indication of a wound intake, the National Council on Radiation Protection & Measurements wound model (NCRP 2006) would be applied.

Nuclear fuel cycle workers have been employed in various processes involving uranium in different chemical forms (see table 2) and with different physical properties (e.g.: particle size and density). Particle size parameters have a relatively small effect on assessed intakes and doses, at least over the range of values typically found in the occupational environments for nuclear workers handling uranium (Riddell 2005). However, uranium compounds can have very different solubilities in biological fluids (e.g.: UF₄ is highly soluble whereas UO₂ is not). This solubility governs the extent and the kinetics of absorption from lung to blood, leading to different retention and excretion kinetics overall. Any knowledge and assumptions about the chemical form of incorporated uranium are therefore important to the correct interpretation of bioassay measurements and can also produce very large differences in estimated lung doses (Puncher et al. 2013), but generally not in systemic organ doses. The recent revision of the
ICRP Human Respiratory Tract Model (ICRP 2015), now has specific absorption parameter values for various forms of uranium, whereas previously all uranium compounds were simply assigned one of only three generic reference solubility ‘Types’ (ICRP 1994).

As part of CURE, any available information about the type of uranium compounds workers cohorts were exposed to was summarized (Blanchardon et al 2014a). If the uranium compounds to which a worker was exposed are known, then specific absorption parameter values will be applied. When less precise information is available, parameter values that provide a reasonable approximation of the expected biokinetics, based on mixtures of reference types that best reflect the likely chemical make-up, need to be used. A maximum likelihood approach is used to assess intakes: it derives intake value(s) that maximise the probability of observing the bioassay measurements, both real and censored values, under the hypothesis of the measurement error model specified. The estimate of intake is then translated into dose by a dosimetric model, using the same process outlined for miners and millers above.

Uranium analytical techniques are relatively sensitive and, in some instances, the detection limit (DL) could potentially be less than excretion from normal dietary intake of uranium in certain areas (Davesne et al 2014). In order to discriminate between non-occupational, i.e. dietary, excretion of uranium and that resulting from occupational exposures, a ‘reporting limit’ (RL) is sometimes employed by occupational physicians and laboratories. Results less than the RL are usually only recorded as being less than this limit, without indication of the raw measurement value (Anderson and Apostoaei 2015). When all bioassay data within a chronic exposure period are less than the DL or RL, two intake values will be calculated for that period: (1) a minimum value of zero and (2) a maximum value calculated by setting the last bioassay data as positive and equal to the value of the appropriate DL or RL, with all other bioassay data remaining censored.

3.4. Methods for statistical analyses

3.4.1. Epidemiological analyses. Standardised mortality ratios (SMRs) and associated 95% confidence intervals (Breslow and Day 1987) would be computed in order to compare the mortality rates, for various diseases of interest (both cancer and non-cancer) in the pooled cohorts with those in the general populations from which the cohort members are drawn (Gillies and Haylock 2014). The main focus of the pooled analyses would be on mortality, since mortality data (dates and causes of deaths) would be available for all cohorts. In addition, cancer incidence data are available in the UK cohorts from the beginning of 1971 and would also be available for the Belgian cohort from the beginning of 1999. This would allow for further sensitivity analyses of the impact of using mortality vs incidence data.

The aim of the dose-response analyses would be to study the relationships between absorbed dose from uranium and mortality from various diseases (again, both cancer and non-cancer), and, where available cancer incidence, while accounting for covariates, including internal exposure to other radionuclides and external gamma radiation exposure. Although exposure to uranium, radon and external gamma radiation have been found to be quite highly correlated in some cohorts of uranium miners (Vacquier et al 2011) this is not the case in specific subsets as e.g. in millers (Kreuzer et al 2015b). In addition, the large number of subjects to be included and the calculation of organ doses (Marsh et al 2012) should help avoid colinearity issues (Walsh et al 2010, Vacquier et al 2011).

Where ad hoc data are available, statistical analyses would be conducted to assess the impact of adjusting for specific potential confounders of the dose-response relationships between uranium exposure and disease risk (e.g. information on smoking status, body mass
index, blood pressure and other established cardiovascular risk factors already computerized for several thousands of workers or miners (see tables 1 and 2), occupational exposures other than uranium such as for instance trichloroethylene, fluorinated compounds, arsenic or silica documented using job exposure matrices in specific cohorts or cohort subsets (Dahmann et al 2008, Guseva Canu et al 2009)).

Cox regression (e.g.: using linear and log-linear Cox models) would preferentially be applied to datasets providing information at the individual level. Specific analyses would be conducted to characterize the shapes of the dose-responses relationships for both cancer and non-cancer diseases. The analyses would include separate estimation of the effects of external and internal (i.e. uranium) exposures and a comparison of the dose-response relationships. Excess relative risk per gray would be estimated for each type of exposure. This would make it possible to estimate a value of the relative biological effectiveness (RBE) of alpha particles for a given disease type, which could be compared with the value of the ICRP radiation weighting factor for alpha particles used for radiation protection purposes (ICRP 2007).

3.4.2. Exploratory analysis of biological information for molecular epidemiology. In the short term, the primary objective of pilot molecular epidemiology studies is to determine whether the standardized operating procedures (SOPs) produced as part of CURE can be implemented in an effective way in a prospective cohort. Therefore, in the first place biological data is intended to be available for a small cross-section of workers only. For most biomarkers, standard multivariate statistical analyses would be performed to determine potential differences in biomarker measurements between groups of workers exposed to uranium at different levels, after adjustment for potential confounding factors. Cross-validation procedures will be applied. If a sufficiently large pilot cohort can be assembled, it will be divided in a training sample for exploratory analyses and a validation sample. However if the pilot cohort is of modest size, it will be used as the training sample and validation would need to be conducted later in another, independent sample after extension of the study to a prospective cohort of larger scale. In the future, as repeated collection of biospecimens are conducted in the study cohorts, time-dependent mathematical models can be applied to model the dynamics of these biomarkers according to cumulative uranium exposure, and resulting absorbed doses (Smirnova et al 2014). As a last step, as the cohorts will further age and if their size is sufficient, the associations between cumulative uranium exposure, biomarkers and diseases could be studied through the use of joint models (Yang et al 2015).

3.5. Characterization and propagation of uncertainties

As part of CURE, sources of uncertainty and their potential impacts on the project outputs, have been identified at several stages of the process of dose estimation and risk evaluation (e.g. reconstruction of exposure conditions, dose calculation including biokinetic models and values of associated parameters, interpretation of bioassay, health outcome ascertainment, epidemiological and biomarker analyses) (Giussani et al 2016). Case studies conducted by UWG members, using their experience in previous research projects, have made it possible to identify the three priority sources of uncertainty to be addressed. The first is uncertainty on the lung solubility of uranium compounds encountered in some occupational settings. As previously stated, of all the parameters employed in the biokinetic models necessary for reconstruction of internal doses, those governing absorption of radionuclides from the lungs to blood have the greatest impact on estimates of lung dose derived from bioassay data. The identification of optimal methodologies to account for censored bioassay data (i.e. measurement below
DL or RL) when estimating intakes and resulting doses was identified as a second priority. The methods initially identified have already been described above, see section 3.3. The third priority is the identification of suitable statistical methodologies to account for the uncertainty inherent in estimates of dose when evaluating radiation-induced disease risk (Allodji et al 2012).

In order to address these issues, it is proposed that future research in this area should focus on characterizing the best estimates and probability distributions of model parameters, and on testing and comparing methodologies to account for the uncertainty inherent in estimates of dose when evaluating radiation-induced disease risk. To that end, it was concluded that the creation of a synthetic cohort, for which all inputs and boundary conditions are known and controllable, should be explored. These proposals are considered to be a partial road map for a middle-to-long-term strategic plan for work on uncertainties.

Other sources of uncertainty have been identified (Giussani et al 2016), including those related to the simplifying assumptions at the basis of the structures of the systemic models. However, the models chosen in the definition of the dosimetric protocols, corresponding to the most recent versions recommended by ICRP for the operational radiation protection of uranium workers, were considered to represent best current knowledge and only a moderate potential source of uncertainty.

4. Discussion

The multidisciplinary approach adopted for CURE, integrating dosimetry, epidemiology, biology and biostatistics, has allowed the production of a research protocol with a strong potential to improve both the understanding and the quantification of the biological and health effects of uranium exposure, and more generally of internal contamination with alpha emitters and low doses of ionising radiation. The extensive experience of the CURE partners facilitated the production of a comprehensive and workable research protocol.

The preparatory work conducted as part of CURE would make it possible to conduct the first large-scale pooled epidemiological analysis of uranium worker and miner cohorts in Europe. Using large cohorts with harmonized dosimetry and long high quality follow-up would optimize statistical power to detect increased risks. Such a pooled epidemiological analysis would address the main limitations of previous studies in the field and would have the potential to deliver results directly relevant to current priority questions in radiation protection (High Level and Expert Group 2009), e.g.: the shape of the dose response relationship at low-doses for cancer and non-cancer diseases, individual radio-sensitivity (as genomic DNA would be collected), the effects of internal emitters and radiation quality. In particular, information about the relative magnitude of risks, for internal alpha and external gamma radiation, would help to determine RBE values for different disease types and assess the validity of the current value for the radiation weighting factor for alpha particles (wR) (ICRP 2007).

Uncertainty is inherent to the dose calculation and risk analysis processes (UNSCEAR 2012). The assessment and treatment of uncertainties is a complex area and approaches to this are still developing as knowledge improves. For instance, new approaches have been proposed since the end of CURE to take into account both shared and unshared sources of uncertainties on dose estimates in epidemiological analyses (Land et al 2015, Simon et al 2015, Stram et al 2015, Kwon et al 2016). It was recognised from the start of CURE that, due to the limited time and resources available within the project it would not be possible to develop the definitive final methodology for the treatment of uncertainties as part of future analyses, but also that this should be a priority for future research. Significant progress toward the identification,
characterisation and potential reduction, of relevant sources of uncertainty, including those relating to dosimetry, was made during the course of CURE. This has generated a substantial amount of information (Giussani et al 2016), however, the treatment of such information within any future analysis (including the best way to assign uncertainty distributions to groups and individuals) is still to be finalized and its definition would constitute an entire part of a future research project.

A protocol has been prepared that would allow the testing of the integration of biological indicators into epidemiological studies of uranium exposed workers, by conducting molecular epidemiology studies. The next step before moving to a large scale molecular epidemiology study would be to conduct one or more pilot studies for field testing. Whilst launching such pilot studies was beyond the time frame of the CURE project, these would be of value, particularly to assess the potential participation rate among workers, the appropriate completion of questionnaires, the applicability of SOPs in field conditions in a full scale study (e.g. for several workers per day) and the quality of resulting samples for the proposed biomarker analyses. The feasibility of implementing molecular epidemiology studies in some settings depends on practical constraints, for example, the biological sampling scheme needs to be compatible with the usual organization of medical check-ups, and with local infrastructures. All these conditions were carefully checked as part of the preliminary feasibility studies, and the logistic strategies were defined accordingly.

Obtaining agreements from stakeholders for molecular epidemiology studies (employers, doctors, workers, workers’ representatives) can be challenging, although previous experiences demonstrated that it is feasible (Zolzer et al 2012b, Abilmazhinova et al 2014, Li et al 2014, Takhauov et al 2015). Biological sampling is only possible if there is interest in and support of, the project by the workers who would provide biological samples, and also the medical centres where samples are intended to be collected. Agreements from employers are needed if sample collections are organized during occupational medical check-ups. Local acceptability may also depend upon the social and economic context. The personal views of occupational physicians about the study goals can also determine their willingness to authorize certain kinds of biological sampling (e.g. sputum collection). Communication with all stakeholders (workers, workers’ representatives, doctors, employers) is therefore a key issue.

The levels of uranium exposure for workers currently active in Western Europe are now much lower than those encountered in the same occupational settings a few decades ago. It might be informative to also conduct pilot studies in areas outside Western Europe, where workers have had higher levels of uranium exposure in recent years, but where more stringent standards (important to the conduct of ethical research) are being, or are about to be, implemented. As part of CURE, contacts have been established with research teams involved in research projects on uranium effects outside Europe: USA (Anderson et al 2015, Leng et al 2016), Kazakhstan (Kazymbet et al 2012) and Russia (Takhauov et al 2015). Conducting pilot molecular epidemiological studies in these countries would provide a wider, scientifically pertinent, perspective.

As for any molecular epidemiology study, our proposed pilot study carries potential risks. The number of workers that could be included in prospective cohorts for molecular epidemiology will be dependent on many factors. If insufficient, this might result in a lack of statistical power to investigate certain biomarkers. Also, because the knowledge about the biological effects of uranium is still evolving, it might be concluded in the future that the biomarkers currently proposed for the pilot study (which we believe are the best ones available to date) are not the best possible ones or do not cover all the potential biological effects of uranium. However, as mentioned in section 3.2) the state-of-the art joint biobanking strategy proposed
by CURE would also allow in the future for a multitude of analyses of other, emerging, biomarkers out of the biospecimen to be collected.

The successful founding of a new state-of-the-art joint biobank and/or the validation of biomarkers of exposure or effects, would establish important precedents and open the way to the creation of large-scale international biobanks. In the future, such biobanks could become major infrastructures for integrated radiobiological and molecular epidemiology research on the effects of chronic exposure to internal emitters and, more generally, for radiation protection research. The proposed project would allow the basis of a road map toward the future integration of radiobiological and epidemiological research to be defined and, as a result, the potential impact of the project would extend far beyond the issue of uranium exposure.

In conclusion, future projects, building on work conducted within the concerted action CURE, would be able to further improve the estimates of health risk related to exposure, not only to uranium, but also more generally to alpha particles and to low doses of ionizing radiation, through the integration of dosimetry, biology, epidemiology and biostatistics.

Conflict of interest

None of the authors declared conflict of interest related to this work.

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340
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341
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