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Proposal to the ISOLDE and Neutron Time-of-Flight Committee

Liquid β-NMR studies of the interaction of Na and K cations with DNA G-quadruplex structures

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B. Karg[1], A. Antušek[2],N. Azaryan[3], M. Baranowski[4], M. L. Bissell[5], L. Cerato[1], J. Croese[6,1], K. M. Dziubinska-Kühn[6,7], A.M. Gerami[8], Y. Gimbal-Zofka[1], R. D. Harding[9], A. Kanellakopoulos[10], M. Kowalska[6], M. Kozak[4], K. Kulesz[1,6], M. Madurga Flores[10], J. Matysik[7], G. Neyens[6,10], S. Pallada[12], J. Plavec[13], Z. Salman[14], A. Skrzypczak[15], P. Wagenknecht[10], S. Warren[1], T. A. Wesołowski[1], J. Wolak[4], Z. Xu[10], D. Zákoucký[16]

[1] *University of Geneva, Geneva, Switzerland*
[2] *Slovak University of Technology, Bratislava, Slovakia*
[3] *JINR, Dubna, Russia*
[4] *Adam Mickiewicz University, Poznan, Poland*
[5] *University of Manchester, Manchester, United Kingdom*
[6] *CERN, Geneva, Switzerland*
[7] *Leipzig University, Leipzig, Germany*
[8] *K.N. Toosi University of Technology, Tehran*
[9] *Nottingham University Hospitals Trust, Nottingham, United Kingdom*
[10] *University of Tennessee, Knoxville, USA*
[11] *KU Leuven, Leuven, Belgium*
[12] *HESSO, Geneva, Switzerland*
[13] *National Institute of Chemistry, Ljubljana, Slovenia*
[14] *PSI, Villingen, Switzerland*
[15] *Poznan University of Technology, Poznan, Poland*
[16] *Czech Academy of Sciences, Rez, Czech Republic*

Spokesperson: B. Karg [beatrice.karg@cern.ch], M. Kowalska [kowalska@cern.ch]
Contact person: M. Kowalska [kowalska@cern.ch]

Abstract: We request beams of ^{26}Na and ^{37,49}K to investigate the interaction of G-Quadruplex structures with alkali cations, including binding, folding and exchange reactions, using the β-NMR technique. These non-canonical DNA structures are formed in living systems, where they are associated with cell aging and oncogenesis but also with gene activation and oxidative damage repair. Alkali metals serve as their crucial folding partner, so understanding the alkali-DNA interaction will greatly facilitate G-quadruplex applications in therapeutical and technological areas. The method of choice to investigate such dynamic systems, NMR, suffers from signal broadening and lack of sensitivity for alkali metals. Therefore, we propose using high-field ultra-sensitive liquid β-NMR. Specifically, we plan to record NMR chemical shifts and relaxation times of ^{26}Na and ^{37}K or ^{49}K interacting with different oligonucleotides in various solvents, including close-to-native conditions.

Requested shifts: 29 shifts, (split into 3 runs over 2 years)

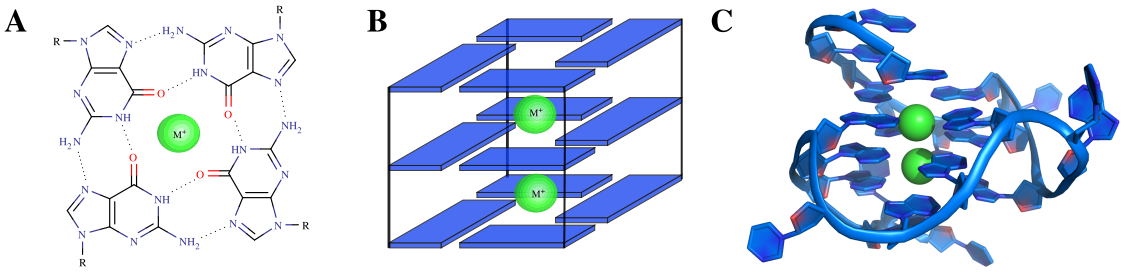

Figure 1: The G-Quadruplex structure: **A** Guanine bases coordinated in a tetrad around a central monovalent cation. **B** Schematic structure of G4 with metal ions located in-between tetrads coordinating the partial charges of 8 oxygen-groups. **C** 3-dimensional structure of a G4 (pdb: 1XAV)[8]

1 Motivation: alkali metals and G-quadruplexes

In spite of recent technical progress in nuclear magnetic resonance (NMR), the study of some nuclei like alkali metals in biologically relevant liquids remains difficult. One of the reasons is the strong broadening induced by quadrupolar interactions, since all stable alkali nuclei have spin $I > 1/2$. The resulting reduced signal intensity has created a significant obstacle in alkali-based studies. Nonetheless, their importance in biological processes is undisputed and investigation of such nuclei can grant a unique and often neglected view into these processes.

Among the most intriguing systems relying on alkali metal binding are so-called G-Quadruplexes (G4). As the passive keeper of information, DNA is viewed as a constant in cell biology, but the G4 structures prove the versatility of this seemingly simple biomolecule: they are found in cells as contributors to aging[1], gene activation and silencing[2] and even in oxidative damage compensation[3] or beyond the living system as catalysts for chemical reactions[4] and therapeutics[5]. The alkali metal ion therein has a strong influence on the structure (Fig.: 1) and dynamics of the G4. This can result in completely different folds of the same nucleotide sequence like the human telomeric region[6], or slowed down folding kinetics up to days[7].

Methods like mass spectrometry or X-ray crystallography yield reliable results on the number of cations involved in the G4-formation[9]. However, they are restricted to snapshots of an interrupted process when entering the observable state (which can additionally be distorted by stacking effects during the crystallization process or water-deficiency in vacuum). In contrast, NMR can be performed in the liquid phase and remains the method of choice for the study of dynamic processes by observing chemical shifts over time, relaxation and exchange times. Still, the aforementioned quadrupolar broadening and lowered signal intensity makes the measurement of chemical shifts challenging. This has redirected the field to indirect methods, like the measurement of relaxation times of G4 groove-bound ions[10] to detect drug binding events. Nonetheless, there has been progress in direct ^{23}Na and ^{39}K detection[11]–[13], though these are up until now limited to single-fold systems and already finished folding events due to the low Signal-to-Noise-ratios, long accumulation and measurement setup times.

Therefore, we herein propose to study the interaction of alkali metals with G4 structures

by using the liquid $\beta-$detected NMR technique at the VITO beamline[14], [15]. The technique is up to a billion-times more sensitive than conventional NMR and it appends the canonical nuclei catalog with new probe nuclei with complementary properties, e.g. smaller or no quadrupole interactions. The large increase in sensitivity comes from two sides. First, the nuclei are hyperpolarized by optical pumping with circularly polarized light. Second, the detection of NMR signals is performed by observing the asymmetry in the emission of $\beta-$particles by radioactive probe nuclei.
Specifically, this approach provides the unique opportunity to measure the G4 folding with superior sensitivity. The high signal intensity allows the observation of low-populated species (intermediate states and minor side-products) especially during the folding event to finally be visible. Consequently, gaps in the folding landscape of G4s only accessible by molecular dynamics simulations[16], could be mapped experimentally. Filling these gaps is an essential step in understanding and consequently making efficient use of G4s in therapeutics and in applications.

2 Technique: Liquid β-NMR

We will employ liquid-state β-NMR, because it combines the billion-fold enhanced sensitivity of β-NMR with narrow resonances known from conventional liquid NMR, required to resolve Na and K chemical shifts in dozen ppm range. For liquid β-NMR to be applicable for determining chemical shifts in the ppm range, a dedicated experimental setup[14], [15], [17], including a stable ^{1}H reference NMR probe, has been constructed at ISOLDE. A proof-of-principle measurement using a laser-polarized ^{26}Na beam has demonstrated that ppm precision can be reached for determining the NMR resonance frequencies of ^{26}Na in different liquids [17].
For the measurements proposed here we envisage implementing several upgrades to the above liquid β-NMR setup [17]. These upgrades have been already presented for aproval to the INTC and ISCC committees [18], [19], and to the ISOLDE technical teams. Among them, the new Bruker 4.7 T superconducting magnet has opened up advantageous regions to the experiment with improved field homogeneity (< 1 ppm) at substantially higher fields, which lead to higher resolution. This has allowed improvements to the detector systems that take full advantage of the high degree of β focusing that occurs due to the strong solenoid field. The focusing has been modeled using CST and GEANT4 simulation tool-kits guiding the new detector geometry and positioning. The other upgrades take full advantage of new beam optical devices installed upstream of the charge exchange cell to maximise the beam envelope overlap with the sample. In addition, a new differential pumping system has been extensively modeled using COMSOL and MOLFLOW++ to optimise the gas-density gradient from beam to sample, reducing beam scattering and loss of polarisation from the residual gas.

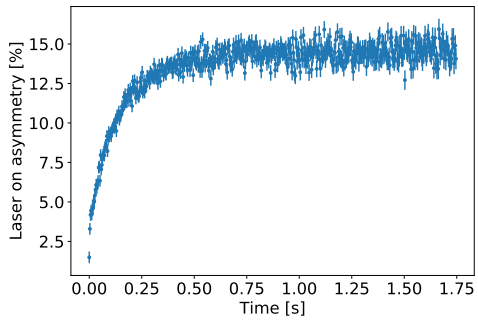

Figure 2: T_1 measurement of ^{26}Na in BMIM-HCOO

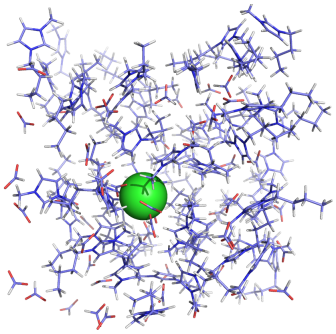

Figure 3: Molecular dynamics simulation of BMIM-HCOO and Na^+

3 Previous studies

3.1 Liquid β-NMR

Previously, our 1.2-Tesla β-NMR setup has been used to measure ^{26}Na signals in ionic liquids and deep eutectic solvents with the aim of observing the binding of G4s to sodium ions in those low-vapour pressure liquid hosts[20], [21]. Unfortunately, the observed signal broadening in the solvents, imidazole-based ionic liquids and glycholine-based deep eutectic solvents, used in November 2018 did not allow for direct confirmation of binding events by chemical shift referencing. However, relaxation time measurements (T_1) are revealing valuable information about the alkali environment (Fig.: 2).
As the relaxation time in low magnetic fields directly correlates to the electric field gradient (EFG), calculations of this parameter can yield precise information about the environment of the nucleus. Currently, high-level *ab initio* molecular quantum mechanical computations are underway within our collaboration using density-functional theory (DFT) and frozen density embedding theory (FDET) with snapshots of nuclei in different environments created by molecular dynamics simulations (Fig.: 3).

3.2 Linking β-NMR to conventional NMR

In order to take the full advantage of liquid β-NMR, one has to be able to express the results in the same way as it is done in conventional NMR, namely via chemical shifts. Our first step was to determine frequency ratios of short-lived Na isotopes relative to stable ^{23}Na in the same hosts with ppm precision[17]. Such precision is required because the magnitude of the expected chemical shifts is in the order of tens to hundreds of ppm. In addition, *ab initio* calculations of the shielding constants using the non-relativistic coupled cluster method allowed us to improve on the accuracy of $\mu(^{23}\text{Na})$. We could thus determine the magnetic moment of ^{26}Na with ppm accuracy, which is two orders of magnitude better than before. The resulting resonance frequencies for non-shielded nuclei give us the opportunity to calculate absolute shielding both in ^{26}Na β-NMR and ^{23}Na NMR. Furthermore, we improved by one order of magnitude the accuracy of the magnetic moments of $^{27-31}$Na as compared to previous measurements with solid-state β-NMR [17].

3.3 K laser polarisation

Collinear Laser Spectroscopy measurements of the hyperfine spectra of various potassium isotopes have been preformed several times at the COLLAPS setup [22]–[24]. In addition, potassium was spin-polarised at COLLAPS by Georg, Keim, Neugart and Neuroth in 1994 [unpublished data]. This study has shown efficient polarisation of ground-state ^{36,37}K in the D1 line at 770.11 nm. Among the recorded β-asymmetry hyperfine spectra of ^{37}K, the highest asymmetry (8%) was achieved in the SmCo crystal. This is comparable to 11 % asymmetry recorded more recently also in the D1 line at the BECOLA beamline at NSCL/MSU [25]. Moreover the asymmetry factor of the main decay branch (97.99%) of the ground state of ^{37}K (I = 3/2) to the ground state of ^{37}Ar (I = 3/2) has recently been determined precisely to be $-0.5706(7)$ [26]. Another K isotope interesting for β-NMR, because of its nuclear spin of 1/2, is ^{49}K (see details later). It has never been polarised, and has a very fragmented decay scheme with missing spin assignments, making it impossible to theoretically predict the expected isotopic beta decay asymmetry. Therefore, this asymmetry should be determined experimentally.

4 Proposed studies

We envisage to record relaxation-time and resonance spectra in liquid hosts and different G4-forming oligonucleotides from polarised Na and K isotopes to determine the local electronic properties of these alkali metals and thus their interaction with G4s.

4.1 Studies employing ^{26}Na

Considering the many technological applications for G4-structures[27], [28] and the need for non-aqueous environments for many chemical reactions, studies of G4s in ionic liquids are still needed. With the higher magnetic field and the differential pumping setup, resonance broadening in ionic liquids will be significantly reduced, thus we will be able to observe G4 folding in ionic liquids which are known to create extremely stable structures[29]. The expected difference in chemical shift $\Delta\delta$=17 ppm[12] between free and G4-bound sodium will prove the binding site and therefore the folding.
While ionic liquids are highly interesting solvents for technological aspects of nucleic acids, biological studies demand native environments, e.g. water. Therefore, in the proposed ^{26}Na studies we intend to also use mixtures of glycerol/water and polyethylene glycol/water. Necessarily, these have an adjustable low vapour pressure up to the sub-mbar region allowing for the differential pumping system to operate in a broader range (our previous system allowed to see resonances in up to 0.5 mbar pressures). Furthermore, they will affect the G4 final structure in a favorable way as they function as molecular crowding agents, simulating a close-to cell-like environment[30]. The folding event observed will thus be strongly related to a native in-cell G4 formation.
Direct implantation of polarised Na in solutions of disordered single strand oligonucleotides will allow us to instantaneously observe G4 folding dynamics (Fig.: 4**A**). In combination with precise chemical shifts the reaction rate can be used to reconstruct the binding event. Ideal targets for these dynamics studies would be a NHE III promoter sequence element

(specifically the *c-myc* sequence)[2] and the human telomeric sequence (*ht*)[31]. Apart from their biological relevance these sequences show extreme fast folding kinetics[32] upon cation-binding, guaranteeing a binding event within the ^{26}Na decay time.
Additionally, ^{26}Na implantations into solutions of G4 already folded with stable Na will give the direct measurement of the ion exchange reaction, which in the case of G4s depends on the tetrad opening reaction[33].
Specifically, parameters measured in these studies will be related to the structure (by chemical shift), the dynamics (by time until observation) and affinities (by ^{26}Na implantations thus concentrations) as a function of water content and also depending on the sequence chosen.

4.2 Studies employing K-isotopes

Other interesting alkali elements such as potassium, with stable ^{39}K and ^{41}K isotopes, show in addition to their quadrupolar broadening a decreased sensitivity for NMR studies, because their magnetic moments are small (leading to very low polarisation). But as almost all G4-forming sequences accept potassium as a central cation, employing K-isotopes will open up our studies to the vast G4 folding landscape. From a kinetic point of view, folding dynamics with potassium tend to be greatly enhanced, so even very slow processes - like the folding of bimolecular G4s - can be observed within the decay time of the β-NMR isotope.
Specifically, we intend to exploit potassium's strong binding affinity to observe less stable sequences like *TBA* (Thrombin binding aptamer, an aptameric sequence used in phase II clinical trials)[34] which are not stable enough to be observed with sodium as a binding partner. As opposed to sodium, potassium is a lot more sensitive to its environment, thus we expect chemical shift differences bound and free for ^{39}K nuclei of $\Delta\delta = +50 - -200\,\mathrm{ppm}$[11]. With such a broad range it might even be possible to observe intermediate states during the complexation of potassium in the G4-core.
Additionally, studying sequences that fold both with Na^+ and K^+ in water-deprived conditions will shed light on the actual binding event and the contribution of dehydration energies by comparison to previous results from ^{26}Na (Fig.: 4).
We plan to work with one or two radioactive K isotopes, ^{37}K and ^{49}K (see Table 1 for their relevant properties). ^{37}K has a suitable half-life (1.2 s), a production rate up to 10^6 ions/s, spin 3/2 and a quadrupole moment in the same range as stable ^{39}K[35]. It has shown β-asymmetries around 10 % for laser polarisation at ISOLDE and MSU [25]. ^{49}K is an interesting isotope, because it has a suitable half-life (1.3 s) and most importantly, a nuclear spin $I = 1/2$, so no additional quadrupolar interaction can broaden the NMR resonances. Its ISOLDE yields are however at the level of 2×10^4/s and it has not yet been laser polarised. Therefore, we would like to first measure the reachable β-decay asymmetry and relaxation times in liquids for this isotope, to assess if it is suitable for our studies.
Additionally, as described for $\mu(^{26}\mathrm{Na})$, Larmor frequencies of ^{37}K and ^{49}K in the liquid host will be detected with β-NMR and directly compared with conventional NMR results in the same host, in order to determine their magnetic moments to ppm precision. Increasing the precision of $\mu(^{37}\mathrm{K})$ and $\mu(^{49}\mathrm{K})$ will increase the accuracy of their NMR standards.

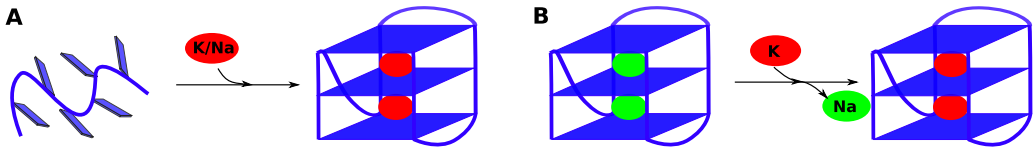

Figure 4: A: Adding alkali metals to unfolded single stranded DNA. B: Exchange of alkalis due to higher affinity

Table 1: Properties of stable and radioactive isotopes relevant for this proposal[17], [21], [22], [24], [36]–[40]

Nucleus	Radioactive half-life	Nuclear spin I	Magnetic moment (μ_N)	Quadrupole moment (mb)	Observed β-asymmetry
^{23}Na	-	3/2	2.217499(7)	104	-
^{26}Na	1.077 s	3	2.849378(20)	-5	25%
^{37}K	1.237 s	3/2	0.20320(6)	100	8-11%
^{39}K	-	3/2	0.39147(3)	60	-
^{49}K	1.260 s	1/2	1.33868(8)	-	-

4.3 Planned measurements and requested shifts

The number of required shifts is based on our experience with liquid β-NMR with ^{26}Na, and it does not depend very much on the yield.
First, we would profit from running in parallel or interchanged with other users, for the following reasons:
(i) We use only every 3rd or 4th proton pulse. This is because we have to minimise β background due to the decay of nuclei from the previous proton pulse, whose spin polarisation has relaxed significantly (T_1 of ^{26}Na in ionic liquids is around 0.5 s). Half-lives of ^{26}Na, ^{37,49}K are around 1 second. This means that about 50 % of proton pulses can be used by other users in parallel to us. E.g. when we use HRS, solid-state users can perform implantations at GLM/GHM.
(ii) We need 4 to 6 hours to change between one liquid sample to another (slowing down turbo-pumps, opening the chamber, replacing the sample, closing up and pumping). During this time other users could use all protons allocated to ISOLDE.
(iii) We might need time to prepare the most suitable biological solution, based on the result of the previous measurement.
The number of shifts required for different tasks is given below:
1 shift (for each used isotope): determining the highest pressure with the differential pumping system when signals are still visible.
1 shift at the start of every beamtime: establishing laser polarisation by HFS scans, optimising laser-atom overlap.
0.5 shift for every change of the liquid sample): see details above.
0.3-0.5 shift (for each solvent and G4 configuration): measuring T_1 in one liquid sample, with different parameters optimised.
0.5-1 shift for each solvent and G4 configuration: performing several NMR scans in one

liquid sample (depending on the number of peaks and observed β-asymmetry).

1. Sodium (^{26}Na) interaction with G4:
   ^{26}Na is produced at the level of 10^7 ions/s from Ta, Ti, or UC targets. We will start by determining relaxation times T_1, chemical shifts and widths of NMR resonances for pure solvents. This is because we will use some solvents for the first time (like glycerol mixtures) and the resolution at higher field will be greatly increased. These studies will allow us to distinguish later between signals from the solvent and signatures of Na binding to G4.
   Next, we will look at T_1 times and NMR spectra for solutions of G4 (*ht, c-myc*) in the above solvents. As described before, we will use both unfolded and pre-folded G4 (by the presence of stable ^{23}Na) and will perform the measurements for different experimental conditions (e.g. G4 concentration). (Fig.: 4**A**)
   We request 1 run with 11 shifts, which should allow to address about 10 different solvent-G4 combinations (about 1 shift per system). The 11 shifts should be spread over a period of 5-6 days.

2. ^{49}K polarisation tests and determination of the precise magnetic moments of ^{37,49}K:
   We will require the Ti target to produce and surface-ionise ^{37}K (yield of 7×10^6 ions/s) and UC_x for ^{49}K (2×10^4 ions/s). Similar to the measurement of the magnetic moment of ^{26}Na[17], we will record ^{37,49}K β-NMR resonances in a well understood solvent, e.g. one of the previously used ionic liquids. We will then compare the resonance frequency to conventional ^{39}K NMR signals in the same host. With new NMR shielding calculations we will also improve the accuracy of the stable references ^{39,41}K. By combining the experiment and theory, we will obtain accurate magnetic moments of ^{37,49}K for reference purposes.
   We request 7 shifts in 1 run with Ti and UC targets scheduled very close to each other. This will allow us to set up our beamline and the laser only once. We will need 3 shifts to determine the highest ^{49}K β-decay asymmetry. During the other half we will record ^{37,49}K NMR resonances in several solvents, to determine precisely their magnetic moments and to compare which isotope is more suitable for our studies (longer T_1, higher asymmetry, etc). The shifts should be spread over minimum 4-5 days.

3. Potassium (^{37}K or ^{49}K) interaction with three G4-forming sequences in selected solvents (Fig.: 4**A** and **B**)
   The studies with potassium will be performed in a similar way as for sodium (described above) using the aforementioned sequences *ht, c-myc* and *TBA*.
   We request 1 run with 11 shifts in total to investigate about 10 systems, scheduled separately from: (i) Na runs due to a different polarisation laser and neutralisation vapour, and (ii) previous K run, to allow preparing the most favourable solvents based on the resonances recorded in the first potassium run. The shifts should be spread over minimum 5-6 days.

Summary of requested shifts: 29 shifts split into 3 runs over 2 years.

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Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

The experimental setup comprises: (*name the fixed-ISOLDE installations, as well as flexible elements of the experiment*)

Part of the	Availability	Design and manufacturing
VITO	☒ Existing	☐ To be used without any modification ☒ To be modified
	☒ New	☒ Standard equipment supplied by a manufacturer ☒ CERN/collaboration responsible for the design and/or manufacturing

HAZARDS GENERATED BY THE EXPERIMENT Hazards named in the document relevant for the fixed VITO installation (EDMS 1961554) and the planned superconducting magnet (EDMS 2326693).

Additional hazards:

Hazards	VITO laser polarisation beamline	SC magnet	liquid β-NMR setup
Thermodynamic and fluidic			
Pressure			
Vacuum	1e-6 mbar		1e-6 mbar
Temperature		LN2, LHe	
Heat transfer			
Thermal properties of materials			
Cryogenic fluid		LN2, 1 bar, 70 l; LHe, 1 bar, 100 l	
Electrical and electromagnetic			
Electricity			1 kV
Static electricity			
Magnetic field		4.7 T	
Batteries			
Capacitors			
Ionizing radiation			
Target material [material]			
Beam particle type (e, p, ions, etc)	ions		ions
Beam intensity	1e4-1e7 ions/s		1e4-1e7 ions/s
Beam energy	60keV		60keV
Cooling liquids			

Gases	Ar		He
Calibration sources:	□		
• Open source	□		
• Sealed source	□ [ISO standard]		
• Isotope			
• Activity			
Use of activated material:			
• Description	□		
• Dose rate on contact and in 10 cm distance	[dose][mSV]		
• Isotope			
• Activity			
Non-ionizing radiation			
Laser	yes		yes
UV light			
Microwaves (300MHz-30 GHz)			
Radiofrequency (1-300 MHz)			yes
Chemical			
Toxic	[chemical agent], [quantity]		
Harmful	[chem. agent], [quant.]		
CMR (carcinogens, mutagens and substances toxic to reproduction)	[chem. agent], [quant.]		
Corrosive	[chem. agent], [quant.]		
Irritant	[chem. agent], [quant.]		
Flammable Na and K vapour	[chem. agent], [quant.]		
Oxidizing	[chem. agent], [quant.]		
Explosiveness	[chem. agent], [quant.]		
Asphyxiant	[chem. agent], [quant.]		
Dangerous for the environment	[chem. agent], [quant.]		
Mechanical			
Physical impact or mechanical energy (moving parts)	[location]		
Mechanical properties (Sharp, rough, slippery)	[location]		
Vibration	[location]		
Vehicles and Means of Transport	[location]		
Noise			

Frequency	[frequency],[Hz]		
Intensity			
Physical			
Confined spaces	[location]		
High workplaces	[location]		
Access to high workplaces	[location]		
Obstructions in passageways	[location]		
Manual handling	[location]		
Poor ergonomics	[location]		

Hazard identification:

Average electrical power requirements (excluding fixed ISOLDE-installation mentioned above): [make a rough estimate of the total power consumption of the additional equipment used in the experiment]