EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

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

Interaction of Na ions with DNA G-quadruplex structures studied directly with Na β-NMR spectroscopy

31.05.2017

M. Kowalska¹, V. Araujo Escalona², M. Baranowski³, L. Cerato⁴, M. Bissell², W. Gins², R. Grzywacz⁶, F. Gustafsson⁵, R. Harding¹, L. Hemmingsen⁸, F. Hofmann⁵, H. Heylen², A. Kanellakopoulos², V. Kocman¹⁰, M. Kozak³, M. Madurga Flores⁶, G. Neyens², S. Pallada¹, J. Plavec¹⁰, N. Severijns², D. Szunyogh⁸, K. Szutkowski³, P. Thulstrup⁸, M. Walczak³¹, F. Wienholtz¹, J. Wolak³, X.F. Yang², D. Zakoucky²

1. EP-Dept, CERN, Geneva, Switzerland
2. IKS, KU Leuven, Leuven, Belgium
3. Faculty of Physics, Adam Mickiewicz University, Poznan, Poland
4. Faculty of Sciences, University of Geneva, Geneva, Switzerland
5. School of Physics and Astronomy, Manchester University, Manchester, United Kingdom
6. Dept. of Physics and Astronomy, University of Tennessee, Knoxville, Tennessee, USA
7. Department of Physics, University of York, York, United Kingdom
8. Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
9. MPIK, Heidelberg, Germany
10. Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia
11. Poznan University of Technology, Poznan, Poland
12. NPI, Czech Academy of Sciences, Rez, Czech Republic

Spokesperson(s): Magdalena Kowalska (kowalska@cern.ch)
Local contact: Magdalena Kowalska (kowalska@cern.ch)

Abstract

We request beams of $^{26-28}$Na to investigate the interaction of Na$^+$ cations with DNA G-quadruplex structures using the β-NMR technique. These DNA structures are formed in nature in nucleic-acid sequences rich in guanine, such as near the ends of the chromosomes, and alkali metal ions are known to play important roles in their formation, stability, and structural polymorphism. NMR can be a powerful tool to investigate such biological systems, however the direct detection of $^{23}$Na$^+$ NMR signals at physiological concentrations in G-quadruplex liquid samples is difficult. To overcome this challenge, we propose to use the β-NMR technique, which is up to which a billion-times more sensitive than conventional NMR. The experiments will take place at the laser-polarization and β-NMR line, which was commissioned in 2016. $^{26-28}$Na NMR chemical shifts and relaxation times will be recorded in different conditions, which should shed more light on the structure and dynamic of Na$^+$ interaction with these important biological systems.

Requested shifts: 15 shifts, (split into 1-2 runs over 1 year)
**Introduction and motivation**

Alkali metal ions play important roles in living organisms and Nuclear Magnetic Resonance (NMR) is a very powerful tool to investigate their interaction with proteins or nucleic acids. However, for some biological systems the direct detection of $^{23}$Na$^+$ NMR signals at physiological concentrations in liquid samples is difficult. Here we are proposing to address one class of such biological systems using the $\beta$-detected NMR technique, which is up to a billion-times more sensitive than conventional NMR. This extremely large increase in sensitivity comes from two sides. First, the hyperpolarization of nuclear spins up to 90% (compared to less than per-mille for conventional NMR), achieved in our case using optical pumping with lasers. Next, the detection of resonances is performed by observing the asymmetry in the emission of $\beta$ particles by radioactive probe nuclei interacting with a host.

One of the studies which can profit significantly from increased Na-NMR sensitivity concerns the interaction of alkali metal ions, such as Na$^+$, with DNA and RNA guanine-quadruplexes (known as G-quadruplexes). Such dynamic secondary structures are formed in nature in nucleic-acid sequences rich in guanine and are alternative to the usual double helix structures [Lar16]. They can be formed reversibly in regions of the genome with the specific DNA sequence, such as near the ends of the chromosomes and promoting regions of many genes. Alkali metal ions are known to play important roles in their formation, stability, and structural polymorphism, see e.g. [Won05, Lar16].

![Fig 1 Schematic of a G-quadruplex. Left: top view of 4 guanine bases forming a G-quadruplex. Middle: side view of alkali-metal cations bound inside G-quadruplex channels. Right: top view of cations interacting with guanine bases with DNA backbone around them.](image)

Until recently, detection of alkali metal cations in G-quadruplexes has relied on either solid-state techniques such as X-ray crystallography and solid-state NMR, or indirect solution NMR methods using spin-1/2 probes such as $^{15}$NH$_4^+$ and $^{205}$Tl$^+$, see [Won05] and references therein. Based on these studies, two types of alkali metal binding sites were identified in the G-quadruplex structure: one being loosely coordinated to phosphate groups and the other bound tightly inside the G-quadruplex channel. Compared to crystallography and solid-state NMR it is much more difficult to study alkali metal ion binding to G-quadruplexes in solution. Until recently the ‘channel’ ions were even considered to be ‘invisible’ in solution-NMR, due to low signal intensity and unfavourable quadrupole spin relaxation properties. Only in 2005 the first direct alkali-NMR studies in solution were performed [Won05] and several similar studies were reported since then. As a result, despite a number of crystal and NMR structures for G-quadruplex DNA available in the literature, still little is known about the alkali metal cation dynamics in this important class of DNA structures [Lar16].

$\beta$-NMR could provide a new approach to direct NMR studies of G-quadruplex interaction with alkali ions in solution and could contribute to a better understanding of the dynamics of these complexes. Such studies would provide unique information to draw to a more consistent picture after combining results of different available experimental techniques.
**β-NMR technique and experimental setup**

β-detected NMR relies on the fact that the emission of β particles from spin-polarised nuclei is anisotropic in space, with their angular distribution given by \(W(\theta) = 1 + a_\beta \frac{v}{c} P_t \cos(\theta)\), where \(a_\beta\) is the degree of angular asymmetry and \(\theta\) is the angle between the direction of particle emission and the magnetic field, and \(v\) is the velocity of the emitted β particle. What is observed directly in the experiment is the β-decay asymmetry, which is defined as the normalised difference in the number of beta particles detected parallel and antiparallel to the magnetic field direction.

Beta-NMR probe nuclei are polarized outside of the host material, in our case via the laser optical pumping. Spin polarization via optical pumping relies on multiple resonant excitations of the ion or atom by circularly polarized laser light, in order to polarize the atomic spins. Due to the hyperfine interaction between the electron spin and the spin of the nucleus in free atoms (or ions), polarization of the atomic spins results also in the polarization of the nuclear spins, with the polarization axis along the laser beam axis. The observed nuclear polarization is reached after the adiabatic decoupling of the spins in a gradually increasing static magnetic field.

The β-NMR technique makes mostly use of continuous-wave (CW) RF, i.e. each incoming ion/atom bunch is exposed to a continuous RF signal of a certain frequency (several frequencies or frequency modulation are also used). Unlike conventional NMR, where an RF pulse is generated to induce a change in the spin-polarization, whose return to equilibrium is then observed as a function of time, here we induce a continuous change in the polarization. This change is detected by observing the time-integrated β-particle asymmetry during about three half-lives of the probe, so that most nuclei have time to emit a β particle and thus can contribute to the signal. When the next particle bunch is implanted into the sample, the RF frequency is changed, and this is how a resonance is recorded. With this procedure, already 10 RF steps with about \(10^5\) β-particles per step are sufficient to obtain a signal-to-noise ratio above 5. The exact value depends on the starting polarization \(P_t\) and the asymmetry factor \(a_\beta\). The β-detectors cover about 10% of the solid angle, resulting in about \(10^7\) radioactive probe nuclei required for one NMR spectrum. That is about \(10^9\) times less than in conventional NMR, where typically one needs \(10^{16}\) probe nuclei.

The experimental setup is shown in Fig. 2. Its main components have been commissioned online in autumn 2016 when spin polarization of \(^{26,28}\)Na was achieved at levels around 50%. This is achieved using a single laser frequency, but the polarization can be further enhanced up to 80% using a second laser frequency using an electro-optic modulator (EOM) so that both ground-state hyperfine components can be excited at the same time. The details on the setup and on the commissioning beamtime are given in [Kow17]. Once the ions from ISOLDE and the laser light are overlapped, the ion beam passes via a set of acceleration-deceleration electrodes which can change the energy of the incoming ions by up to several keV, in order to Doppler-tune them into resonance with the laser light. Next comes a charge-exchange cell, in which the ion beam is neutralized by collisions with a Na or K vapour as it passes through. The cell is used only for species which are polarized more efficiently as neutral atoms (e.g. Li, Na, or Ar). It is followed by a chamber housing a photomultiplier tube positioned perpendicular to the beam axis, which can be used to detect fluorescence light emitted by the excited ions or atoms (to allow for a quick resonance search for cases where this is not known). Directly behind, a 2-m optical-pumping section is used to induce the spin polarization. Finally, the radioactive beam reaches the implantation chamber placed between the poles of the NMR magnet.

To maintain the spin polarization along the beam axis and to avoid polarization losses due to stray magnetic fields and magnetic material in neighbouring setups, a longitudinal magnetic field of \(20\) Gauss is present along the beamline. It is followed by small solenoids giving a gradually increasing field to allow an adiabatic spin rotation to the transversal field of the NMR magnet. At the present stage of the setup we have concentrated on polarising neutral atoms, since this is also interesting for the \(^{35}\)Ar experiment, which has seen the first online tests in May 2017 [Vel14].
Aqueous solutions require pressures of several mbar to keep them liquid (e.g. pure water at 5°C has a vapour pressure of 8.6 mbar), while the $^{26-28}$Na beams travel in $10^6$ mbar vacuum to avoid transmission losses by collisions with rest gas. Two challenges thus arise: efficient transport of the atoms into the liquid sample and minimal loss of their polarization on the way. At present, we are bridging the pressure difference via a differential pumping system which consists of a set of small pinholes, segmented beam diagnostics, and powerful vacuum pumps. This system is part of the PhD thesis of R. Harding who has finalised the design of the system and who is in the process of ordering parts. When the ordered vacuum pumps arrive this summer, the system will be tested first without and then with stable beam. This will allow determining the pressure in the subsequent stages of the differential pumping system and optimising the beam transmission. (Short access to radioactive beam will allow determining the degree of polarization). With the implemented solutions, the system should allow at least a few percent of beam transmission and a few percent of the original beam polarization in mbar pressures required for aqueous samples. With the above efficiencies, it should be still possible to record beta-NMR spectra within a reasonable time, since $^{26-28}$Na are well produced and show very good decay asymmetries.

The $^{26-28}$Na beam will be finally implanted into a small drop of the G-quadruplex solution. To avoid additional beam losses on nm-thin windows, the drop will be suspended directly from a liquid capillary, in which precise dosing and temperature control will be possible. A small coil providing the RF signals will surround the probe. The chamber and all ancillaries are presently being prepared by the Poznan group.

The magnet to be used is an electromagnet, which has $< 5$ ppm homogeneity in the central 1 cm$^3$ region and can provide fields up to 1.7 T, which will help resolving neighbouring NMR signals. A superconducting magnet providing 4.7 T and a sub-ppm field homogeneity is also available within our collaboration and we are investigating ways how to integrate it into the experimental setup. We will also use a new beta-detection system, which is presently being manufactured in the laboratories of the Tennessee group, consisting of two sets of two organic scintillators: 2-mm thin one followed by a 8-mm one, both read out by Si photomultipliers connected directly to readout electronics. This configuration will allow low noise and possibility of recording coincidences between the thin and thick detector.
Envisaged biological studies

Here we propose to investigate the interaction of Na ions with DNA G-quadruplex structures using the beta-NMR technique. We aim to perform Na NMR studies using $^{26}$Na, $^{27}$Na, and $^{28}$Na probe nuclei, whose relevant properties are shown in Table 1.

Table 1 Properties of $^{26-28}$Na relevant for the present proposal [Kei00], [Kow17] and ref. therein.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Radioactive half-life</th>
<th>Nuclear spin</th>
<th>Magnetic moment ($\mu_N$)</th>
<th>Quadrupole moment (mb)</th>
<th>Observed $\beta$–asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{26}$Na</td>
<td>1.1 s</td>
<td>3</td>
<td>2.86</td>
<td>-5</td>
<td>30 %</td>
</tr>
<tr>
<td>$^{27}$Na</td>
<td>0.3 s</td>
<td>$5/2$</td>
<td>3.89</td>
<td>-7</td>
<td>30 %</td>
</tr>
<tr>
<td>$^{28}$Na</td>
<td>30 ms</td>
<td>1</td>
<td>2.43</td>
<td>40</td>
<td>40 %</td>
</tr>
</tbody>
</table>

All three nuclei offer several advantages for the envisaged studies:

1. Due to favourable atomic transitions their nuclear spins have been polarized at the level of 30-60%, both by us ($^{26,28}$Na) [Kow17] and by the COLLAPS group ($^{26,27,28}$Na) [Kei00].

2. Their $\beta$-asymmetry factors $\alpha_\beta$ are close to 1. This means that the maximum achievable $\beta$-asymmetry is high, see Table 1, which will be beneficial for the actual measurement in the liquid.

3. The two above points mean that one can easily observe $\beta$-NMR resonances, even if only a small fraction of the original polarization could be maintained after implantation in the liquid sample, e.g. due to polarization losses when going through the differential pumping section discussed later.

4. Their production rates at Isolde are very good, allowing to record one NMR resonance within a few minutes. They can be also produced using several target materials while only needing surface ionization, giving few scheduling constraints.

5. Their quadrupole moments are considerably smaller than that of stable $^{23}$Na ($Q(\text{^{23}Na}) = +100$ mb), leading to weaker quadrupole interaction with the environment. This in turn will lead to longer relaxation time and thus to smaller resonance broadening.

6. Their quite different half-lives allow probing different timescales. For example, $t_{1/2}$ of $^{28}$Na is already comparable with the relaxation time observed in one of the few G-quadruplex liquid $^{23}$Na NMR studies [Won05].

7. They are compatible with the present stage of our experimental setup which allows to optically pump with visible and infrared laser light and to polarize neutral atoms.

G-quadruplex interaction with Na$^+$ seems to be very suitable to be studied with the beta-NMR technique: G-quadruplexes bind very strongly to alkali metals, the reaction is extremely fast – expected to take place within the radioactive half-life of $^{26}$Na and even $^{28}$Na, the relaxation times of tens of ms were reported (again, well compatible with $^{26,28}$Na $t_{1/2}$), the chemical shifts reported before in conventional Na-NMR are in the 20 ppm range [Won05], which will be resolved with our magnetic field homogeneity.

The goal of the studies is to determine how and at what time scale Na$^+$ binds to several G-quadruplex structures which can be easily synthesized and have been studied in detail with NH$_4^+$ by the Ljubljana group co-authoring this proposal [Ske05, Ske012, Tra09]. We would like to determine the dissociation constant of the reaction, and thus to observe the Na$^+$ aqua ion at low concentrations and then the Na$^+$- quadruplex complex at higher concentration. We also envisage measuring the relaxation times of the observed signals, distinguishing between different positions occupied by the Na ions. $^{26-28}$Na NMR chemical shifts and relaxation times will be recorded in the following hosts:
1. **Ionic liquids**, since they exhibit a very low vapor pressure, and are therefore straight forwardly used in a high vacuum beam line, thus avoiding any losses in beam transmission or beam polarization connected to bad vacuum. We will start with only one ionic liquid (EMMIM-Ac) and will then pass to mixture of two ionic liquids chosen in such a way that the observed chemical shift is in the range expected for G-quadruplexes (10 to 20 ppm), e.g. EMMIM-PF₆ and EMMIM-Ac (signals about 25 ppm apart) [Sch12]. Experiments are presently underway in Copenhagen and Poznan to identify which ionic liquids will mix well and which show relaxation times above ms, so that they can be measured with ²⁶⁻²⁸Na probe nuclei. The data will be taken at several values of vacuum, between 1e-5 mbar all the way to 5 mbar needed later for aqueous solutions, to determine at which level of vacuum the beam transmission and observed β-decay asymmetry make the resonances very difficult to see. In this way, the experiments will serve as a proof of principle for the Na-β-NMR experiments, showing that the recorded chemical shifts agree with conventional NMR.

2. **High purified de-ionised water** and 0.1 M aqueous solution of NaCl, which is conventionally used as a definition of 0 ppm shift in Na NMR.

3. Optionally, **crown ethers**, since they can be used as simple analogues for biological channels or diffusive carriers for alkali metals. We can use 15-crown-5 since it can be dissolved both in ionic liquids and water and it binds with high selectivity to Na⁺.

4. Finally, **simple DNA G-quadruplex solutions** at different concentrations and temperatures. One of the possible sequences d(TAG3CG3AG3AG3A2) (present in the N-myc gene) was already synthesized and studied by our Ljubljana co-authors [Traj12]. We expect to observe a signal around -18 ppm (compared to a signal from free Na⁺ in solution at 0 ppm), reported at this position for several types of G-quadruplex structures [Won05, Ida08]. This signal has been shown to be due to Na⁺ ions residing inside the G-quadruplex structures (between G-quadruplex sheets). Measurement of relaxation times will provide additional information on the position of Na⁺ ions, since they were shown to be very different for ions inside the DNA channel and outside it.

   If needed, for easier interpretation of the obtained spectra, we envisage performing quantum chemical simulations similar to those reported in [Ida08] and expertise in such calculations already exists within our collaboration.

**Beamtime request**

The shift request takes into account the ²⁶⁻²⁸Na yields (Table 2) and is based on the times required previously to record good quality NMR spectra using the COLLAPS setup, which was 5-10 min for β asymmetries above 5% and some 20-30 min for asymmetries of below 5%, rather independent of the ISOLDE production yields.

Thus, we assume that we will need: 1 shift to establish the optimal settings of the charge exchange cell and post-acceleration electrode for the 1st polarization signal and subsequent scanning of a β-NMR resonance on the best-produced ²⁸Na (based on commissioning beamtime in 2016). We also know that in good vacuum 1 h should be enough to record several NMR resonances, whereas for worse vacuum required for aqueous samples 1 h might be needed for one scan (due to very low expected asymmetry). In addition, between measurements in different hosts, we assume that 1 h will be needed to remove the previous liquid, titrate the new one, as well as achieve the right pressure and temperature. We also need some time for contingency.

Thus, we would like to request 1-2 runs of in **total 15 shifts**, split in the following way:

- 3 shifts (24 h) to perform preparatory NMR measurements with ionic liquids, with one and two liquids, and at several pressures: 1 shift to see 1st NMR spectrum, 2 shifts to record some 20 spectra.
- 3 shifts (24 h) to record NMR resonances in water and crown ethers, with min 1 spectrum per hour.
- 9 shifts to investigate the G-quadruplex interaction with ²⁶⁻²⁸Na, again with min 1 spectrum/hour.
Table 2 Expected yields from several ISOLDE targets in ions/uC of protons [yield].

<table>
<thead>
<tr>
<th>beam</th>
<th>Yield from UCx</th>
<th>Yield from Ta</th>
<th>Yield from Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>26Na</td>
<td>3.0e7 [Kei00]</td>
<td>4e6</td>
<td>1.5e6</td>
</tr>
<tr>
<td>27Na</td>
<td>8.5e6</td>
<td>1.2e4</td>
<td>1.7e5</td>
</tr>
<tr>
<td>28Na</td>
<td>9.6e5</td>
<td>7e3 (interpolated)</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

References:
[Lar16] E. Largy et al., in The Alkali Metal Ions: Their Role for Life, Springer 2016, p 203
Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

The experimental setup comprises:

<table>
<thead>
<tr>
<th>Part of the</th>
<th>Choose an Item</th>
<th>Availability</th>
<th>Design and manufacturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>[permanent laser-polarization setup]</td>
<td>☑ Existing</td>
<td>☑ To be used without any modification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ New</td>
<td>☑ Standard equipment supplied by a manufacturer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>☑ CERN/collaboration responsible for the design and/or manufacturing</td>
<td></td>
</tr>
<tr>
<td>[beta-NMR setup]</td>
<td>☑ Existing</td>
<td>☑ To be used without any modification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ New</td>
<td>☑ Standard equipment supplied by a manufacturer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>☑ CERN/collaboration responsible for the design and/or manufacturing</td>
<td></td>
</tr>
<tr>
<td>[COLLAPS laser system]</td>
<td>☑ Existing</td>
<td>☑ To be used without any modification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ New</td>
<td>☑ Standard equipment supplied by a manufacturer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>☑ CERN/collaboration responsible for the design and/or manufacturing</td>
<td></td>
</tr>
</tbody>
</table>

HAZARDS GENERATED BY THE EXPERIMENT

Hazards named in the document relevant for the fixed laser-polarization setup

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Charge-exchange and post-acceleration chamber</th>
<th>Optical detection and optical pumping section</th>
<th>ISOLDE-standard focussing triplet</th>
</tr>
</thead>
</table>

Thermodynamic and fluidic

<table>
<thead>
<tr>
<th>Pressure</th>
<th>[pressure][Bar], [volume][l]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum</td>
<td>1e-6 mbar</td>
<td>1e-6 mbar</td>
</tr>
<tr>
<td>Temperature</td>
<td>150 C</td>
<td></td>
</tr>
<tr>
<td>Heat transfer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal properties of materials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryogenic fluid</td>
<td>[fluid], [pressure][Bar], [volume][l]</td>
<td></td>
</tr>
</tbody>
</table>

Electrical and electromagnetic

<table>
<thead>
<tr>
<th>Electricity</th>
<th>+/- 1kV, [current][A]</th>
<th>+/- 1kV</th>
<th>+/- 2 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static electricity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetic field</td>
<td>[magnetic field][T]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capacitors</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ionizing radiation

| Target material     | [material]                     |                                    |                                 |
|---------------------|--------------------------------|                                    |                                 |
| Beam particle type  | (e, p, ions, etc)              |                                    |                                 |
| Beam intensity      |                                 |                                    |                                 |
| Beam energy         |                                 |                                    |                                 |
| Cooling liquids     | Galden by Solvay               |                                    |                                 |
| Gases               | [gas]                          |                                    |                                 |
Calibration sources:
- Open source
- Sealed source [ISO standard]

Use of activated material:
- Description
- Dose rate on contact and in 10 cm distance [dose][mSV]
- Isotope
- Activity

Non-ionizing radiation
- Laser
- UV light
- Microwaves (300MHz-30 GHz)
- Radiofrequency (1-300MHz)

Chemical
- Toxic [chemical agent], [quantity]
- Harmful [chemical agent], [quantity]
- CMR (carcinogens, mutagens and substances toxic to reproduction) [chemical agent], [quantity]
- Corrosive [chemical agent], [quantity]
- Irritant [chemical agent], [quantity]
- Flammable [chemical agent], [quantity]
- Oxidizing [chemical agent], [quantity]
- Explosiveness [chemical agent], [quantity]
- Asphyxiants [chemical agent], [quantity]
- Dangerous for the environment [chemical agent], [quantity]

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]

0.1 Hazard identification

3.2 Average electrical power requirements (excluding fixed ISOLDE-installation mentioned above):