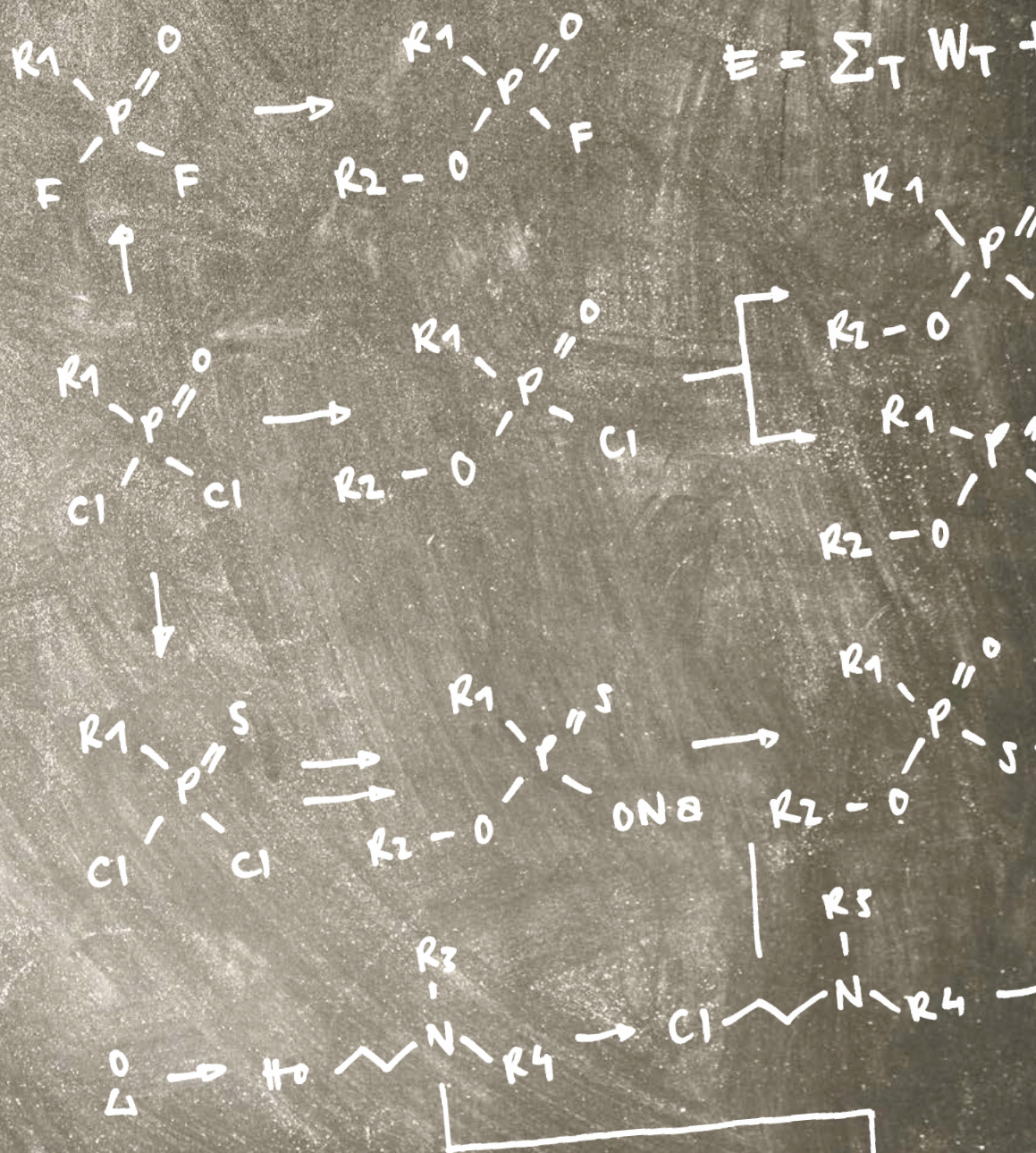


**Federal Office for Civil Protection FOC**  
SPIEZ LABORATORY

Annual report 2017

# SPIEZ LABORATORY





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**Images**

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Residents wear masks as they search for bodies after a shelling at al-Ansari area in Aleppo

## Dear readers,

In spring 2017, Switzerland strongly condemned the use of chemical weapons in Syria and stated that this constituted a war crime. A few weeks later, the United Nations appointed Stefan Mogl, head of our Chemistry division, as member of the leadership panel of the OPCW-UN Joint Investigative Mechanism that was tasked by the UN Security Council to identify those responsible for the use of chemical weapons in Syria. After six months of intensive investigations, the mechanism published a report, which, among other findings, attributed the attack with the nerve agent sarin in Khan Sheikhun to the Syrian Government. Unfortunately, a request to extend the mechanism's mandate failed because of Russia's veto in the UN Security Council. Therefore, at this time, there is no mechanism to identify those responsible for the use of chemical weapons or to hold the parties already identified to account (p. 41).

We hope the political standstill will soon be resolved. The use and proliferation of weapons of mass destruction continue to endanger the security of many regions in the world: Even after UN investigations, chemical weapons con-

tinue to be used in Syria. This also affects the risk assessment for Switzerland: According to the Swiss Federal Intelligence Service, terrorist groups remain interested in weapons of mass destruction and despite international disarmament efforts chemical weapons and the necessary chemicals remain available.

In North Korea, the regime has continued its weapons of mass destruction programs and successfully tested nuclear explosive devices, which the highly sensitive global measurement-system of the Comprehensive Nuclear-Test-Ban Treaty Organisation (CTBTO) was able to prove beyond doubt. Spiez Laboratory has been involved in the technical working groups of this organisation for years. The monitoring system can also be employed for important non-military purposes, for example as a warning system for tsunamis (p. 16).

We continue to develop our nuclear forensic capabilities to support the investigating authorities in the fight against nuclear terrorism. Nuclear forensics can help to determine the origin, the method of production or type of use of a radioactive source. Lead is generally used to



Dr. Marc Cadisch  
Director Spiez Laboratory

shield against radiation. The analysis of lead impurities and isotope ratios supports nuclear forensics. In addition, since the analytical techniques are the same as for conventional lead ammunition, these methods could support classical forensic investigations in connection with firearms (p. 23).

With a series of workshops in Spiez, we continue our efforts for a functioning international network of biological laboratories (p. 38), analogous to the Organization for the Prohibition of Chemical Weapons (OPCW), which celebrated its 20th anniversary in 2017 (p. 49).

Our Biology division is developing new strategies for the development of antiviral therapies against highly pathogenic viruses. Our biocontainment laboratory is the only research institute in Switzerland with the necessary infrastructure to evaluate substances against pathogens of the highest risk group (p. 27).

The NBC protection division develops and tests concepts on how cost-effective CBRE protection can be implemented in aboveground buildings (p. 52). We test the performance of

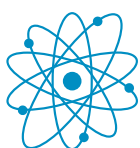
new materials for protective clothing (p. 56), and we conduct chemical and radiological decontamination studies – in some cases with astonishing results: our tests have shown that for the decontamination of radiologically contaminated buildings, water is generally equal to or even superior to commercially available products. It is therefore unnecessary to buy and store expensive decontamination products or to procure new ones after the expiry date (p. 20).

The role of Spiez Laboratory is undisputed, both in NBC prevention and protection as well as in dealing with possible NBC events. Switzerland's policy makers and the public support our work. We are doing everything we can to ensure that we continue to earn this trust.





The opened reactor in Mühleberg during an inspection



# Decommissioning of nuclear facilities: Measurement methods for decommissioning analysis

*Dr. José Corcho*

**In 2019, the nuclear power plant Mühleberg will enter the final phase of its life cycle. This decommissioning phase is a long and complex process (up to 15 years), involving activities such as decontamination, dismantling and demolition of equipment and structures, as well as the disposal of the resulting wastes. Central tasks during decommissioning are the radiological measurements to ensure the safety of people and the environment. For the dismantling of nuclear facilities, Spiez Laboratory has at its disposal a wide range of measurement methods for the determination of a large number of radionuclides. The last remaining gaps in this measurement methodology are now being filled under the current research programme.**

Throughout the entire decommissioning of the Nuclear Power Plant (NPP) Mühleberg, the same legal requirements will apply as during power operations. The objective of the decommissioning is to release the area for other uses without any restrictions.

Central tasks during the decommissioning phase will be the radiological measurements of materials and building structures, to verify and retain evidence demonstrating the radiological safety for humans and the environment as well as of the disposal of radiological and conventional waste materials (Swiss Federal Nuclear Safety Inspectorate, ENSI, 2017). The dismantling will require extensive analytical and measurements methods for the determination of a wide range of radionuclides in different components.

## **Need for new developments**

Spiez Laboratory (LS) has at its disposal a comprehensive set of technical measurement meth-

ods for the determination of a large number of relevant radionuclides (table 1). For a full and comprehensive monitoring of the decommissioning of nuclear power plants (NPP), however, certain additional methods will be required. At the moment, LS does not have accredited measurement methods for some of the radionuclides. Furthermore, the complex chemical composition of the materials present in a NPP (such as concrete, steel, aluminium, graphite) calls for sophisticated radio-analytical separation techniques.

### Supplementation of the measurement methodology

In order to close the gaps, the Nuclear Chemistry Division of Spiez Laboratory has set up a research programme for the development and improvement of radio-analytical methods for the decommissioning work. Within the framework of this programme, several radiochemical methods have been developed that now form part of the accredited analytical methods of LS. This includes, for example, the development of a radiochemical method for the sequential determination of several radionuclides (such as Pu, Np, U, Th, Am and Sr) in the same sample aliquot (Sahli et al., 2017). This method allows the simultaneous determination of several important radionuclides in the same sample, which is required for the estimation of the total radioactivity inventory during decommissioning activities. A further example is the method for the fast determination of Strontium radioisotopes in environmental samples (Corcho, 2015). Spiez has developed this method in collaboration with the ALMERA Network Laboratories of the International Atomic Energy Agency (IAEA). This new method results in significant gains in time and reductions in costs.

Furthermore, certain radiochemical methods have been further improved, such as the method for the determination of  $^{99}\text{Tc}$  in environmental samples (Sahli et al., 2017) as well as the determination of Tritium and gross-Alpha-/Beta emitters in water samples (Corcho, 2015).

### Relevant Radionuclides

At the beginning of decommissioning, the hazard potential of a nuclear power plant is mostly associated with the activity inventory of the fuel assembly present (more than 99 per cent of the total activity inventory; ENSI, 2017). Radioactive components and materials, such as fuel elements, will be removed first (figures 1 and 2). The remaining activity inventory (without the fuel elements) amounts to appr.  $10^{15}\text{Bq}$  and is distributed as follows:

- i) Appr. 99 percent is firmly bound in materials of the core installations, the reactor pressure tank and the biological shield, and thus cannot be released directly;

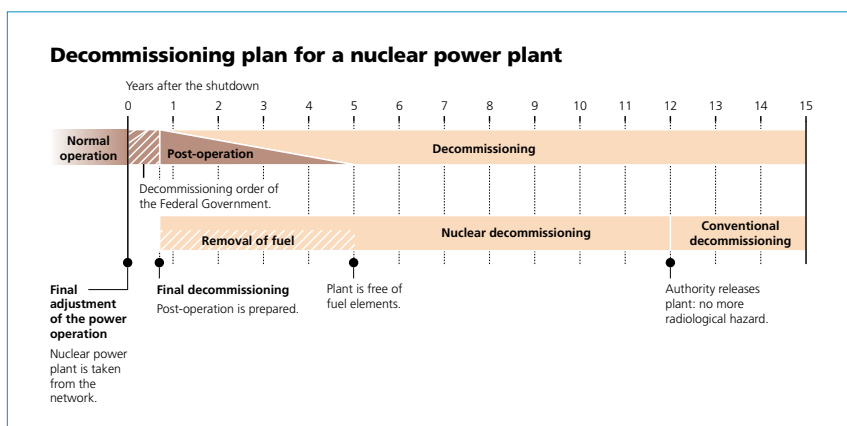


Figure 2: Selected decommissioning option for the NPP (BKW, 2015)

Decay	Radionuclide	Accredited measurement method in LS	Method
Gamma Emitters	$^{22}\text{Na}$ , $^{54}\text{Mn}$ , $^{65}\text{Zn}$ , $^{58}\text{Co}$ , $^{59}\text{Fe}$ , $^{60}\text{Co}$ , $^{94}\text{Nb}$ , $^{106}\text{Ru}$ , $^{108}\text{mAg}$ , $^{110}\text{mAg}$ , $^{125}\text{Sb}$ , $^{133}\text{Ba}$ , $^{137}\text{Cs}$ , $^{134}\text{Cs}$ , $^{144}\text{Ce}$ , $^{152}\text{Eu}$ , $^{154}\text{Eu}$ , $^{155}\text{Eu}$ , $^{241}\text{Am}$	Yes	No chemical separation Gamma spectrometry
Alpha Emitters	$^{234}\text{U}$ , $^{235}\text{U}$ , $^{236}\text{U}$ , $^{238}\text{U}$ , $^{238}\text{Pu}$ , $^{239}\text{Pu}$ , $^{240}\text{Pu}$ , $^{241}\text{Am}$ , $^{237}\text{Np}$	Yes	Chemical separation required Alpha spectrometry
	$^{243}\text{Cm}$ , $^{244}\text{Cm}$	No	Mass spectrometry Liquid scintillation counting
Beta Emitters	$^3\text{H}$ , $^{90}\text{Sr}$ , $^{99}\text{Tc}$ , $^{241}\text{Pu}$	Yes	Chemical separation required
	$^{14}\text{C}$ , $^{36}\text{Cl}$ , $^{41}\text{Ca}$ , $^{55}\text{Fe}$ , $^{63}\text{Ni}$ , $^{59}\text{Ni}$ , $^{93}\text{Zr}$ , $^{93}\text{Mo}$ , $^{129}\text{I}$ , $^{135}\text{Cs}$ , $^{166}\text{mHo}$	No	Mass spectrometry Liquid scintillation counting Gas proportional counter

Table 1: Typical measurement methods (Corcho, 2016)

- ii) Appr. 1 per cent is present as contamination, mostly located on the inner surfaces of systems, and thus cannot be released instantly (ENSI, 2017).

For the clearance measurement, only dose-relevant radionuclides will be included. To this end, first, radionuclide vectors (radionuclide inventory) have to be determined by conducting complete radiochemical analyses (Eikenberg et al., 2008). The radiological characterisation of facilities includes the measurement of special radionuclides such as alpha emitters, tritium,  $^{90}\text{Sr}$ ,  $^{63}\text{Ni}$ ,  $^{55}\text{Fe}$ , etc., depending on their relevance (table 2).

### Utilisation of analytical methods

Analytical methods that have proven successful in environmental monitoring can also be used for the analysis of samples in the context of clearance measurements. A large number of dose-relevant radionuclides can be determined with relatively little effort by means of direct measurements using high-resolution gamma spectrometry (in the laboratory or in situ) (Eikenberg et al., 2008; Corcho, 2016). For some radionuclides, activity measurements can only be accomplished after radiochemical separation methods (destructive analysis) (Corcho, 2016). Gamma spectrometry and LSC methods can be applied for more than 75 per

Radionuclide	Half life (years)	Emitter type	Origin
<sup>3</sup> H	12.3	β <sup>-</sup>	Activation
<sup>14</sup> C	5730	β <sup>-</sup>	Activation
<sup>22</sup> Na	2.6	B <sup>+</sup> , γ	Activation
<sup>36</sup> Cl	302 000	β <sup>-</sup>	Activation
<sup>41</sup> Ca	100 200	EC	Activation
<sup>55</sup> Fe	2.7	ε	Activation
<sup>59</sup> Ni	76 000	ε	Activation
<sup>60</sup> Co	5.3	β <sup>-</sup> , γ	Activation
<sup>63</sup> Ni	98.7	β <sup>-</sup>	Activation
<sup>90</sup> Sr	28.8	β <sup>-</sup>	Nuclear fission
<sup>93</sup> Zr	1.6 10 <sup>6</sup>	β <sup>-</sup>	Activation
<sup>93</sup> Mo	4.0 10 <sup>3</sup>	EC, X	Activation
<sup>94</sup> Nb	2.0 10 <sup>4</sup>	β <sup>-</sup> , γ	Nuclear fission
<sup>99</sup> Tc	2.1 10 <sup>5</sup>	β <sup>-</sup>	Nuclear fission
<sup>106</sup> Ru	1.0	β <sup>-</sup> , γ	Nuclear fission
<sup>108m</sup> Ag	438	EC, γ	Activation
<sup>125</sup> Sb	2.8	β <sup>-</sup> , γ	Nuclear fission
<sup>129</sup> I	1.6 10 <sup>7</sup>	β <sup>-</sup> , γ	Nuclear fission
<sup>133</sup> Ba	10.5	ε, γ	Activation
<sup>134</sup> Cs	2.1	β <sup>-</sup> , γ	Activation
<sup>135</sup> Cs	2.3 10 <sup>6</sup>	β <sup>-</sup>	Nuclear fission
<sup>137</sup> Cs	30.1	β <sup>-</sup> , γ	Nuclear fission
<sup>152</sup> Eu	13.5	ε, β <sup>-</sup> , γ	Activation
<sup>154</sup> Eu	8.6	β <sup>-</sup> , γ	Activation
<sup>155</sup> Eu	4.8	β <sup>-</sup> , γ	Activation
<sup>166m</sup> Ho	1200	β <sup>-</sup>	Activation
<sup>234</sup> U	2.5 10 <sup>5</sup>	α, γ	Raw material
<sup>235</sup> U	7 10 <sup>8</sup>	α, γ	Raw material
<sup>236</sup> U	2.3 10 <sup>7</sup>	α	Activation
<sup>237</sup> Np	2.1 10 <sup>6</sup>	α	Decay
<sup>238</sup> U	4.5 10 <sup>9</sup>	α	Raw material
<sup>238</sup> Pu	88	α	Activation
<sup>239</sup> Pu	2.4 10 <sup>4</sup>	α	Activation
<sup>240</sup> Pu	6.6 10 <sup>3</sup>	α	Activation
<sup>241</sup> Pu	14.3	β <sup>-</sup>	Activation
<sup>241</sup> Am	432.6	α, γ	Activation
<sup>243</sup> Cm	28.9	α	Activation
<sup>244</sup> Cm	18	α	Activation

Table 2: Relevant Radionuclides (half-life > 1 year) for the decommissioning of nuclear facilities (boiling water reactor) (IAEA, 1998; Eikenberg et al., 2008).

cent of the radionuclides that are present in the decommissioning of nuclear facilities (Eikenberg et al., 2008). If one adds to this alpha spectrometry and gas proportional counter methods, the radioactivity measurement methods cover 99 per cent of the analyses. Mass spectrometry is rarely used in decommissioning and amounts to less than one per cent (Eikenberg et al., 2008).

### Research programme supports decommissioning activities

In the future, the research will focus on the development of radiochemical methods for the determination of difficult-to-measure beta emitters, such as <sup>55</sup>Fe, <sup>63</sup>Ni and <sup>69</sup>Ni (by liquid scintillation counting) as well as <sup>135</sup>Cs (by mass spectrometry). To this end, a further collaboration is planned with IAEA ALMERA Laboratories. In the final phase of the research programme, the work will concentrate on the isotopes <sup>14</sup>C, <sup>36</sup>Cl and <sup>129</sup>I.

With this programme, LS will expand its analytical capabilities significantly. In this way, it can take on an important role in the verification and control of radiological characterisation activities in the context of the upcoming decommissioning of the NPP Mühleberg.

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# Nuclear Plant

## REACTOR DECOMMISSIONING RADIONUCLIDES

Activation of steel  $^{55}\text{Fe}$ ,  $^{59}\text{Ni}$ ,  $^{63}\text{Ni}$ ,  $^{60}\text{Co}$ ,  $^{92}\text{Zr}$ ,  $^{94}\text{Nb}$ .

Activation of graphite  $^{14}\text{C}$  and  $^{36}\text{Cl}$  (from trace Cl impurities)

Fission products in fuel from  $^{235}\text{U}$  fission e.g.  
 $^{79}\text{Se}$ ,  $^{90}\text{Sr}$ ,  $^{92}\text{Zr}$ ,  $^{95}\text{Zr}$ ,  $^{99}\text{Tc}$ ,  $^{107}\text{Pd}$ ,  $^{135}\text{Sn}$ ,  $^{129}\text{I}$ ,  $^{135}\text{Cs}$ ,  $^{137}\text{Cs}$ ,  $^{147}\text{Pm}$ ,  $^{151}\text{Sm}$ .

Actinides in fuel from neutron capture by U e.g.  
 $^{236}\text{U}$ ,  $^{237}\text{Np}$ ,  $^{238}\text{Pu}$ ,  $^{239}\text{Pu}$ ,  $^{240}\text{Pu}$ ,  $^{241}\text{Pu}$ ,  $^{241}\text{Am}$ ,  $^{242}\text{Cm}$ ,  $^{252}\text{Cf}$ .

Activation of concrete bioshield  $^3\text{H}$ ,  $^{152}\text{Eu}$ ,  $^{41}\text{Ca}$ .

## Main Elements

### REACTOR BUILDING

- 1 Control rods
- 2 Fuel assembly
- 3 Steam separator assembly
- 4 Steam dryer
- 5 Reactor vessel
- 6 Containment
- 7 Concrete shielding
- 8 Reactor circulation pump
- 9 Internal pressure reduction chamber
- 10 External pressure reduction chamber
- 11 Feedwater line
- 12 Steam line
- 13 Spent fuel pool

### POWER HOUSE

- 14 High pressure turbine
- 15 Low pressure turbines
- 16 Generator
- 17 Re-heater / Water separator
- 18 Capacitor
- 19 Feedwater pumps
- 20 Feedwater preheater
- 21 Cooling water supply (from the Aare)
- 22 Cooling water return (to the Aare)

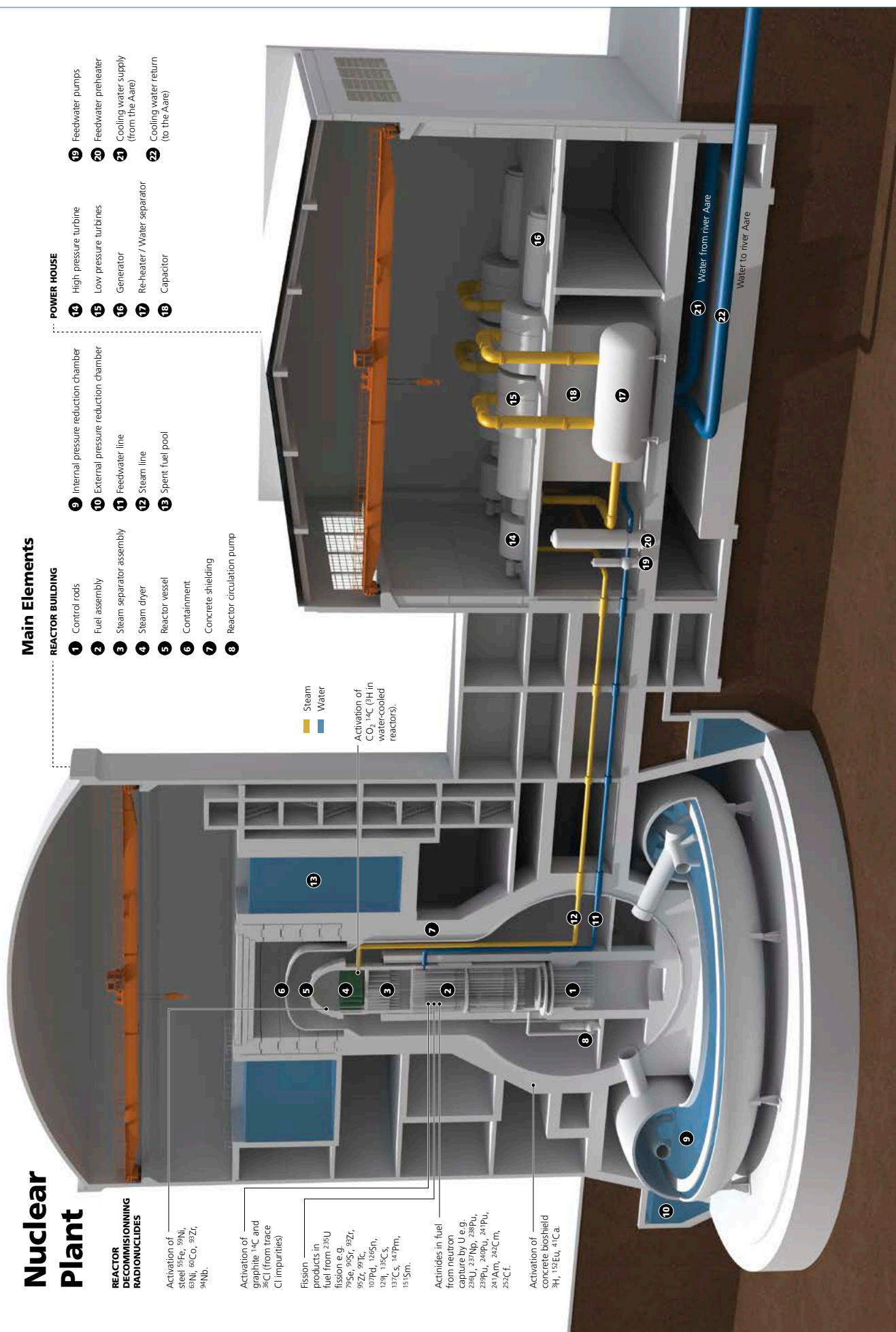


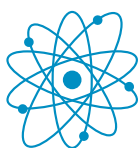
Figure 3: Nuclear reactor



# LABOR SPIEZ



On June 19, David Osborn, IAEA Director for Environmental Laboratories, presented the plaque for the IAEA-designation of Spiez Laboratory



## Spiez Laboratory is a Collaborating Centre of the IAEA

*Dr. Mario Burger*

**Spiez Laboratory has received the designation as Collaborating Centre of the International Atomic Energy Agency (IAEA). Collaborating Centres are scientific institutions that collaborate with IAEA Divisions, for example in the fields of environmental protection, food safety, resources management, public health, quality assurance and training. IAEA presently maintains such cooperation contracts with some 20 institutions worldwide. The collaboration with Spiez focuses on the enhancement of the quality and range of services provided by the laboratory network of the IAEA.**

A Collaborating Centre (CC) supports the IAEA in the implementation of specific tasks within the multiannual planning and the programmes of the Divisions of the Agency. Selection criteria for the designation as a Collaborating Centre include amongst others the scientific-technical reputation of the institute, the number of publications in the field of the collaboration, the degree of networking in the nuclear-scientific field and the stability of the respective Member State with regard to personnel, scientific activities and financial capacity.

The collaboration of Spiez Laboratory with the IAEA concerns, on the one hand, the ALMERA network.<sup>1</sup> This is a network of some 100 national laboratories with varying specialisations, which in cases of the release of radioactivity have the capability to analyse environmental samples. On the other hand, the collaboration

<sup>1</sup> ALMERA = Analytical Laboratories for the Measurement of Environmental Radioactivity



focuses on radio-analytical test methods of the highest standard.

### ALMERA

As a Collaborating Centre, Spiez Laboratory takes on a more active role within ALMERA that has been the case until 2016. The main function is to enhance the quality of the measurement results in the network. To this end, Spiez collaborates with the IAEA laboratories in Monaco and Seibersdorf as well as with the CC Hungary (reference materials) and the CC South Korea (methods development). Thereby, the development of robust analytical methods for environmental samples is being pursued, new measurement techniques are being introduced in the field, and the quality concept in general is to be promoted.

The CC Spiez also takes part in interlaboratory comparison tests. Every year, three to five analytically challenging materials are despatched to the laboratories that participate in ALMERA for testing. The laboratories are required to deliver a first analysis report within 48 hours and a comprehensive report within 3 months. Up to now Spiez Laboratory has passed all these proficiency tests with top marks.

The field measurement techniques include different methods, but specifically the *in situ* gamma spectrometry, which is to be introduced in ALMERA. Because this method is well established in Spiez, a basic course was organised already in 2015. In 2017, Spiez was a main contributor to a workshop in Hungary, and in 2018 there will be a course organised in Kazakhstan. The *in situ* gamma spectrometry is being utilised in the IAEA RANET network<sup>2</sup>, into which Spiez Laboratory is integrated as well.

### IAEA Fellowships

Because of its active participation in the IAEA networks and thanks to other projects in the field of nuclear security, and linked to its designation as Collaborating Centre, Spiez Laboratory has the possibility to integrate scientific fellowships of the IAEA into specific projects. During 2017, two fellowships have been implemented:

#### Fellowship 1

- Literature survey on Gamma Object Measurement (GOM)  
Utilisation of ISOCS/LABSOCS and simulation of radioactive materials
- Use of field measurement devices for the identification of radioactive materials of nat-

ural, medical, industrial and technical provenance

- Development of instructions and strategies for the measurement of radioactive material in the field, especially with regard to orphan radioactive material (containers, lorries, etc.) for use by the task force VBS (A-EEVBS).

#### Fellowship 2

- Development of radiochemical methods for the determination of Radium isotopes (228-Ra, 226-Ra, 224-Ra and 223-Ra) in water
- Literature survey and study of internationally adopted methods.  
Development of methods that meet the highest quality standards in accordance with ISO/EN 17025.
- Methods comparison with regard to detection limits, time needed etc. Preparation of a comprehensive laboratory note and preparations for a publication for ALMERA.

### Technical Cooperation Projects

For many years, Spiez Laboratory has supported technical cooperation projects of the IAEA, in particular capacity building in the areas of measurement techniques, sample collection, evaluation and quality assurance. During the reporting year, LS experts supported the following missions:

1. 08.02.–10.02.2017: IAEA data evaluation workshop on determination of low activity radio-caesium in freshwater, held in Vienna (Austria) (IAEA, 2016a).
2. 26.06.–30.06.2017: IAEA regional workshop on sampling procedures for water and sediment sample, held in Kozloduy (Bulgaria) (IAEA, 2016a).
3. 27.11.–01.12.2017: National training course in basic aspects of radiation protection and environmental radioactivity, held in Majuro (RMI) (IAEA, 2016b).
4. 04.12.–08.12.2017: Training on sampling and pre-treatment techniques and to collect samples as part of a baseline study, held in Majuro (RMI) (IAEA, 2016b).
5. 16.12.–23.12.2017: Training on analysis of noble gases and tritium, and support the groundwater sampling campaign, held in Thailand (IAEA TC Project THA7005).
6. Mission Marshall Islands (see Box)

In addition, Spiez Laboratory has supported several IAEA programmes for the determination of radioisotopes in challenging matrices, for example the determination of plutonium in sediments of oceans, coastal lagoons, salt-marshes etc. as well as in corals. The measurements adopted in Spiez allow a better un-

### Mission Marshall Islands

The Republic of the Marshall Islands (RMI) is situated between Hawaii and Australia and stretches across 29 coral islands. During the 1940s and 1950s, the US conducted 67 nuclear weapons tests on the Bikini and Enewetak atolls of the RMI, given that these islands are far away from all regular shipping lines and air traffic routes. The tests resulted in severe radioactive contamination of many of the islands and atolls. Because of major concerns of the local population, the government of the Marshall Islands with the support of the IAEA has launched a project for monitoring radioactivity in the atolls.

In December 2017, in the framework of this project, an expert of Spiez Laboratory acting as representative of the IAEA trained scientists of the local environmental protection agency and of the Authority for Marine Resources. The two-weeks training course focused on basic aspects of radiation protection and of environmental radioactivity (for example exposure pathways, critical radionuclides), as well as sample collection and sample processing techniques for the radioactivity analysis.

Using case studies, the participants could gain experience in the establishment and implementation of a monitoring programme for the environment, food and drinking water. The expert mission conveyed the theoretical basis of environmental sample collection, and demonstrated the necessary practical steps to secure representative samples and to prepare them for radioactivity analysis. In order to exercise the new skills, a small random sample was collected on Majuro atoll. Additional missions are being planned for the further instruction of the local scientists. The environmental samples are being analysed by several laboratories worldwide, including Spiez. It is also planned to train selected scientists from the Marshall Islands in Spiez.



Colour enhanced picture of the "Baker Test" of 1946 on the Marshall Islands. The main goal of the nuclear tests was to research the effect of nuclear weapons on naval vessels. The condensation cloud formed by the pressure wave has dissolved, which opens the view on the water column, the explosion mushroom and the flotilla of target ships. The trunks of the palm trees on the beach had been painted to allow the measurement of the tidal wave. (Image National Nuclear Security Administration/Nevada Field Office).

derstanding of the influence of varying sea levels or acid content on sensitive natural systems (Carnero-Bravo et al., 2016; Carnero-Bravo et al., 2018). Spiez is also involved in the work of thematic working groups of the IAEA. Examples include the reviews concerning Chernobyl and Fukushima, issues related to decontamination after radiological mega events as well as expert groups dealing with post-conflict issues (such as in Iraq) – often in the context of environmental assessments of the UN Environment Programme (UNEP).

### Support for Switzerland's Foreign and Security Policy

In order to contribute also in the future as a relevant actor in the themes of international nuclear energy policy as well a nuclear safety and security, Switzerland's engagement with the IAEA must rely on solid technical know-how. The involvement of Spiez Laboratory as one of the leading federal laboratories in the radiological field underscores the foreign and security policy objectives of Switzerland in the area of nuclear weapons, the peaceful application of nuclear energy, and the continuous improvement of global nuclear security.

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Measuring Radiation levels outside Sarajevo

# Investigations of the Migration of Depleted Uranium in Soil



**Spiez Laboratory increasingly receives requests from the United Nations to study the impact of Depleted Uranium (DU) on soil and ground water. This material is used in armor penetrating munitions. For these studies, Spiez has developed new detection methods. The research data so far show that increased values of the local dose rate as well as the uranium concentration in soil are limited to very short distances from the impact points of the munitions. Any long-term monitoring in contaminated regions is therefore only sensible with regard to water sources.**

Uranium (U) is a natural element with the atomic number 92. Natural uranium consists of three different isotopes: 99,2745 per cent are  $^{238}\text{U}$ , 0,7200 per cent  $^{235}\text{U}$ , and 0,0055 per cent  $^{234}\text{U}$  (table 1). For most applications in nuclear technology, the relatively small fraction of fissionable  $^{235}\text{U}$  in natural Uranium (0,7200 per cent) is insufficient, and needs to be increased using an enrichment process. The uranium material that remains after this isotope separation process is called Depleted Uranium (DU). The specific activity of DU, and thus its radiotoxicity, are approximately 40 per cent smaller than those of natural uranium.

*Dr. José Corcho*

## **Military Applications**

Because of its high density, the military uses DU in the field predominantly as armor or in armor penetrating munitions. The use of DU is creating concerns about the possible health and environmental risks of this material (SCHER, 2010). In order to evaluate the potential health and environmental risks related to the use of DU, its composition must be determined as accurately as possible. To this end,

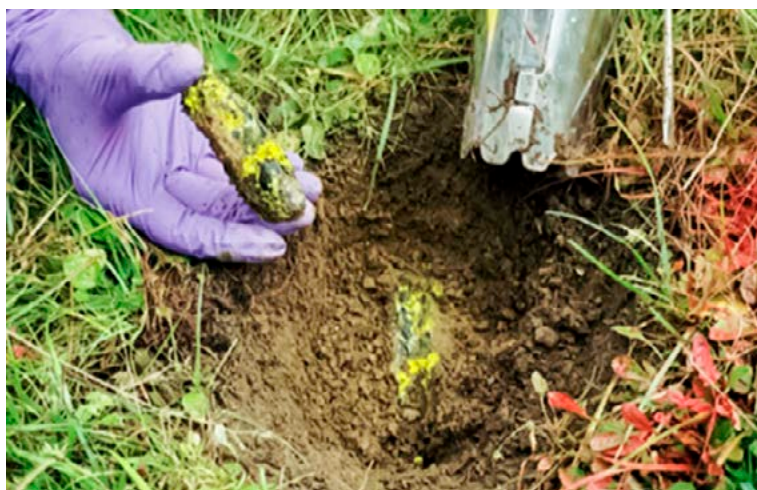


Figure 1: Corroded DU penetrators

Table 1: Typical values for the concentration of uranium isotopes depending on the degree of enrichment or depletion of the material

Isotope	Half life (years)	DU mass ratio (%)	Natural U mass ratio (%)	LEU mass ratio (%)	Weapon Grade mass ratio (%)
$^{234}\text{U}$	$2,455 \cdot 10^5$	0,00066	0,0055	0,0478	0,934
$^{235}\text{U}$	$7,037 \cdot 10^8$	0,199	0,7200	5,0	93
$^{238}\text{U}$	$4,468 \cdot 10^9$	99,8	99,2745	94,9518	6,07

Spiez Laboratory has developed and validated several methods for the detection and characterisation of DU materials (in particular DU penetrators). This research included on the one hand radiometric measurements, and on the other hand trace analyses using mass spectrometric methods.

In DU munitions left behind from the Balkan war, for example, Spiez Laboratory has determined the uranium concentration as well as the isotope composition of the uranium. Indeed, traces of plutonium, neptunium and technetium could be detected in the DU materials investigated (Sahli et al., 2017). Spiez Laboratory has determined that the plutonium contained in the DU munitions did not pose an additional health risk. The isotope composition correlates well with that of weapons grade plutonium. The contribution of this contamination to the total radiation dose, however, is negligible.

#### Weathering of DU munitions

For many years, there has been a question about the longer-term fate of DU munitions and remnants thereof in the environment, and in

particular in soils. DU munitions in soil are subject to weathering and corrosion (figure 1), however there is a lack of adequate knowledge about the degree and speed of these processes. These factors are essential, however, for the evaluation of long-term risks.

The migration of uranium in soils is being addressed more and more in the context of uranium mines. Population protection in urban areas close to uranium mining areas is being taken more and more seriously. Spiez Laboratory and other specialised institutes have received an increasing number of requests by the United Nations to conduct investigations of these issues (UNEP 2001, 2002, 2003; LS 2010).

The migration of uranium in surface and ground waters is an issue of particular attention in Europe and in Switzerland as well, because the material is present in certain agricultural fertilisers.

#### International Cooperation

In order to deal with these problems, Spiez Laboratory has involved other specialised insti-



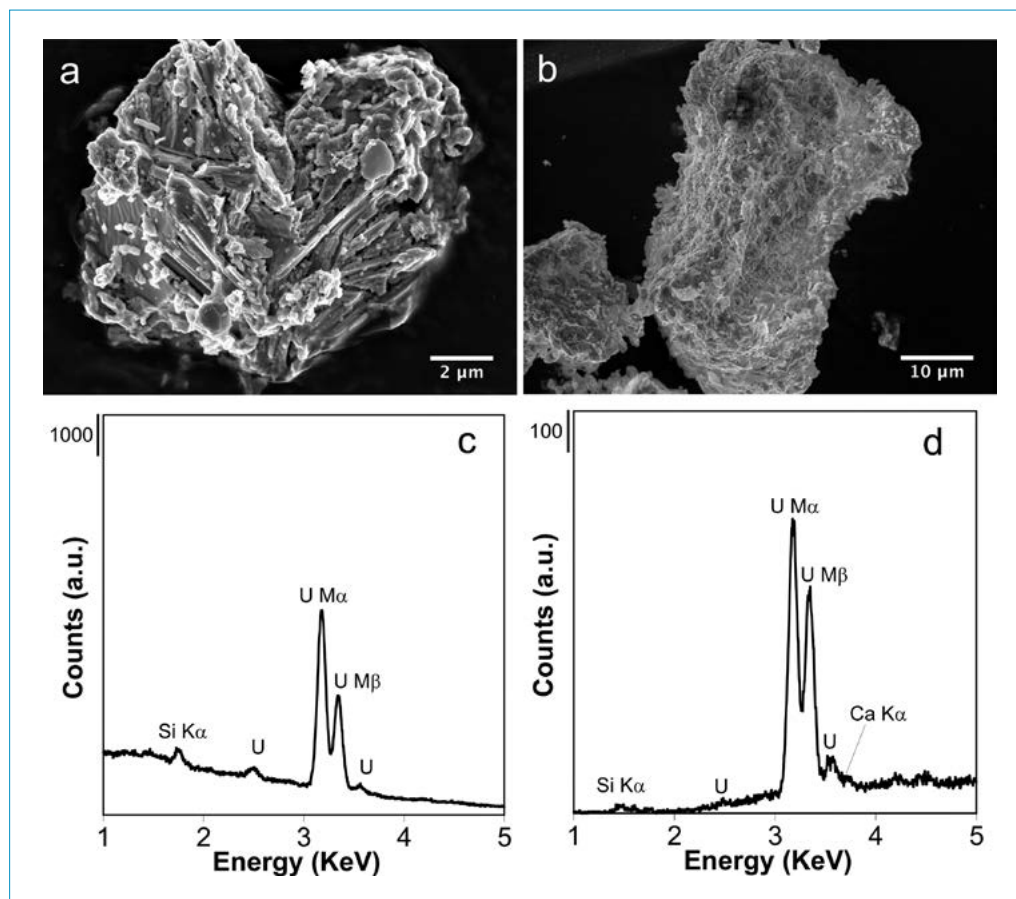


Figure 2: Electron microscopic observation data of uranium bearing particles in the corrosion product of a DU penetrator (Wang et al., 2016)

tutes and conducted studies in collaboration with the Environmental Microbiology Laboratory of the Ecole Polytechnique Fédérale in Lausanne – EPFL (Professor Dr Rizlan Bernier-Latmani) as well as the Department of Environmental Geosciences of Vienna University (Professor Dr Stephan Krämer). In the framework of this collaboration, the corrosion products of intact DU penetrators under field conditions over longer periods have been characterised (von Gunten, 2014; Wang et al., 2016). Two DU penetrators were studied in the context of this project; these were made available by Spiez Laboratory. The two penetrators, both heavily corroded, had been laying in the ground in Bosnia and Herzegovina for more than 7 years (figure 1).

A combination of X-ray diffraction (XRD) and electron microscopy (SEM) conducted at EPFL (figure 2) and X-ray absorption spectroscopy (XAS) conducted at the British Diamond Light Source Synchrotron (figure 3) was used. The analysis of the corrosion products showed a uranium content of 77 per cent. The results of mineralogy studies showed that Metaschoepite

was a main constituent of the two DU corrosion products (Wang et al. 2016). In addition, Studtite and Becquerelite were identified in the corrosion products (Wang et al., 2016). Their formation through a conversion of Metaschoepite was the result of the geochemical conditions under which the DU penetrators had corroded. The transformation of Metaschoepite into Becquerelite or Studtite in the DU corrosion products appears to reduce the potential mobilisation of uranium from the corroded DU penetrators, if they are exposed to similar environments in post conflict regions (Wang et al., 2016). The discovery of Studtite in the corrosion product was a surprise, as it had not been previously reported in corroded DU penetrators (von Gunten, 2014).

In order to investigate the behavior of projectile material in the environment and in soils, corrosion products of DU penetrators were placed into columns filled with different soils to study the leaching behavior. The columns were filled with typical soils, set up in an air-conditioned laboratory and watered weekly. The concentration of uranium isotopes in the column eluate

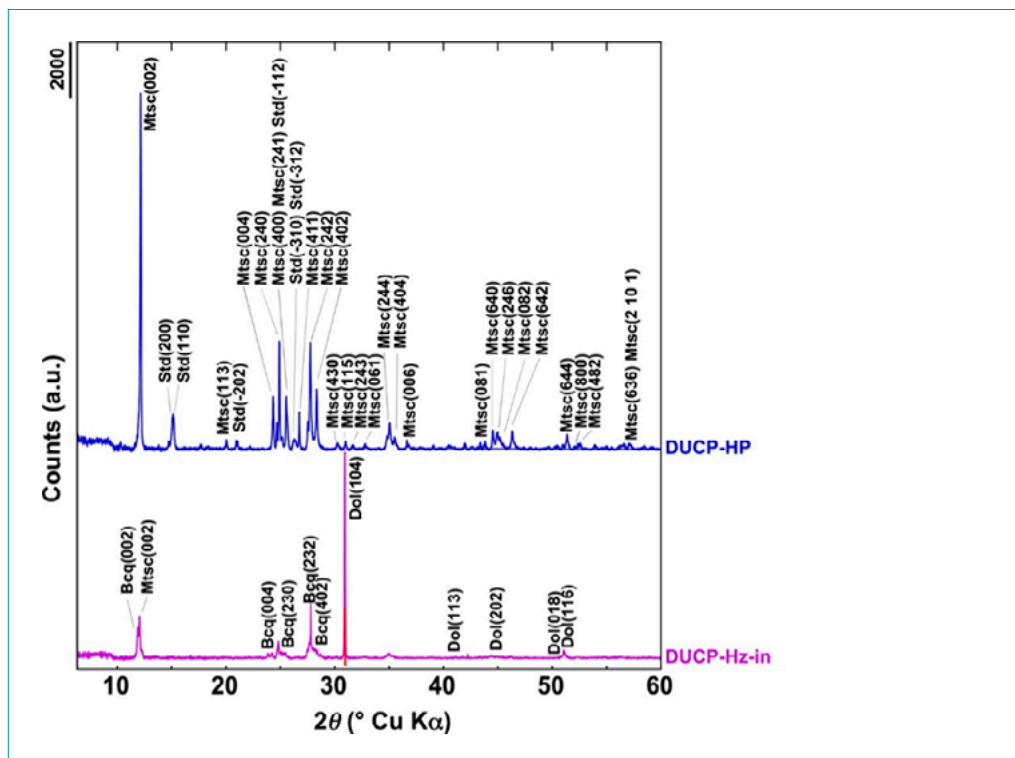


Figure 3: XRD patterns of the DU corrosion products (DUCP-HP) and a soil sample (DUCP-Hz-In).  
Note: Mtsc-metaschoepite, Std-studtite, Bcq-bequerelite, Dol-dolomite (Wang et al., 2016).

was monitored before and after the insertion of the DU munitions. The experiments simulated the release of uranium from the corrosion products into the soil, depending on the geochemical conditions of the soil.

The column studies showed the following:

1. The rain, the geochemical parameters of the soils (such as calcium, iron, total organic carbon and inorganic carbon, pH) and the amounts of sand, sludge and clay all influence the mobility of uranium in soils;
2. Sandy para-brown earth (luvisol, often used in agriculture) shows a high uranium mobility;
3. Good uranium retention was found in rich organic histosols (acidic and neutral) as well as carbonate rich soils (rendzina, fluvisol).

For each soil, aliquots were collected after several months of exposure to DU corrosion products. Micro X-ray absorption spectroscopy ( $\mu$ -XAS), micro X-ray fluorescence ( $\mu$ -XRF), micro X-ray diffraction ( $\mu$ -XRD) and micro X-ray absorption near-edge-spectroscopy ( $\mu$ -XANES) were conducted on soil samples using the MicroXAS Beamline at Swiss Light Source (Paul Scherrer Institute). XANES data showed that a significant amount of the

uranium immobilised in the soils is present as hexavalent form (figure 4a; von Gunten, 2014).  $\mu$ -XRF,  $\mu$ -XRD and  $\mu$ -XANES demonstrated that uranium hotspots in soil samples are present mainly in the form of Uranitite and/or non-crystalline U(IV) (figure 4b), in particular in places where organic material (plant material) was available (von Gunten, 2014).

### Health risk negligible

The investigations of soil and water samples have shown that even in locations with confirmed DU shelling, there is no large-area contamination with DU (UNEP 2001, 2002, 2003). Increased values of the local gamma dose rate as well as the uranium concentration in the soil are limited to very short distances from the impact points of the DU munitions. In summary, it can be concluded that the health risk is negligibly low. Essentially, only the ingestion route has a certain importance for the risk of possible incorporation. The inhalation route merely plays a minor role. If at all, any long term monitoring in contaminated regions is only sensible with regard to water sources.



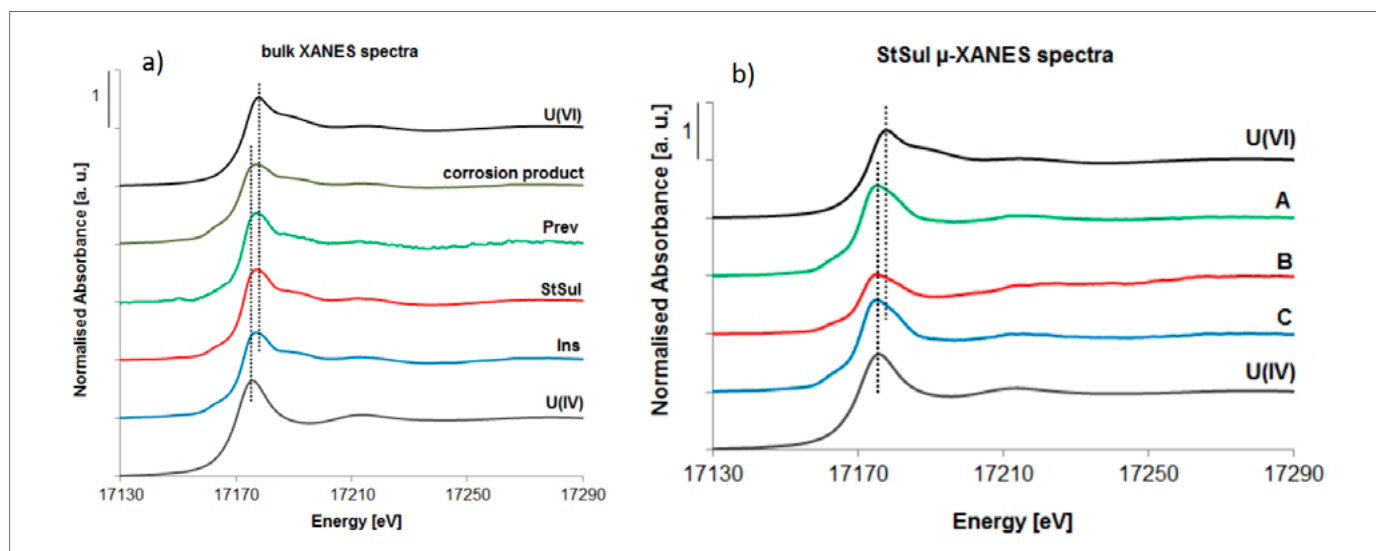


Figure 4:

- a) Normalised U LIII-Edge XANES data for the DU corrosion product and various soil samples (Prev, StSul, Ins)  
 b)  $\mu$ -XANES spectra of a uranium hotspot in a soil sample. The U(IV) spectrum of the corrosion product and the U(IV) spectrum of a non-crystalline sample were used as standards.

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## North Korea's Nuclear Weapons Tests – Why Do We Know What We Know?

*Dr. Christoph Wirz*

**The globally distributed network of measurement stations of the Organisation of the Comprehensive Nuclear Test Ban Treaty (CTBTO) can pinpoint precisely the point of origin of underground explosions. The North Korean nuclear weapons tests at the end of 2017 demonstrated clearly that this international measurement system functions outstandingly well in practice. Switzerland participates actively in the work of the CTBTO and contributes to the budget of the Organisation. In addition, Switzerland maintains a seismic measurement station in Davos, as part of the monitoring system.**

The parties of the “Comprehensive Nuclear Test Ban Treaty” (CTBT) have undertaken to renounce nuclear explosions, to prevent them in their sphere of influence, and neither to participate in nuclear explosions nor to assist other parties in this regard. The CTBT aims at hindering the further development of nuclear weapons in those States that possess them. Compliance with a treaty must be verifiable. The Preparatory Commission of the CTBTO with seat in Vienna today works on a global monitoring system (figure 1) which is designed to reliably detect and localise a nuclear explosion in the atmosphere, underground or under water in any place on earth and at any point in time. Once completed, the monitoring system will comprise the following:

- 170 seismological stations
- 11 stations for hydro-acoustic monitoring (recording sound waves in the oceans)
- 60 infrasound stations for the measurement of smallest variations in air pressure



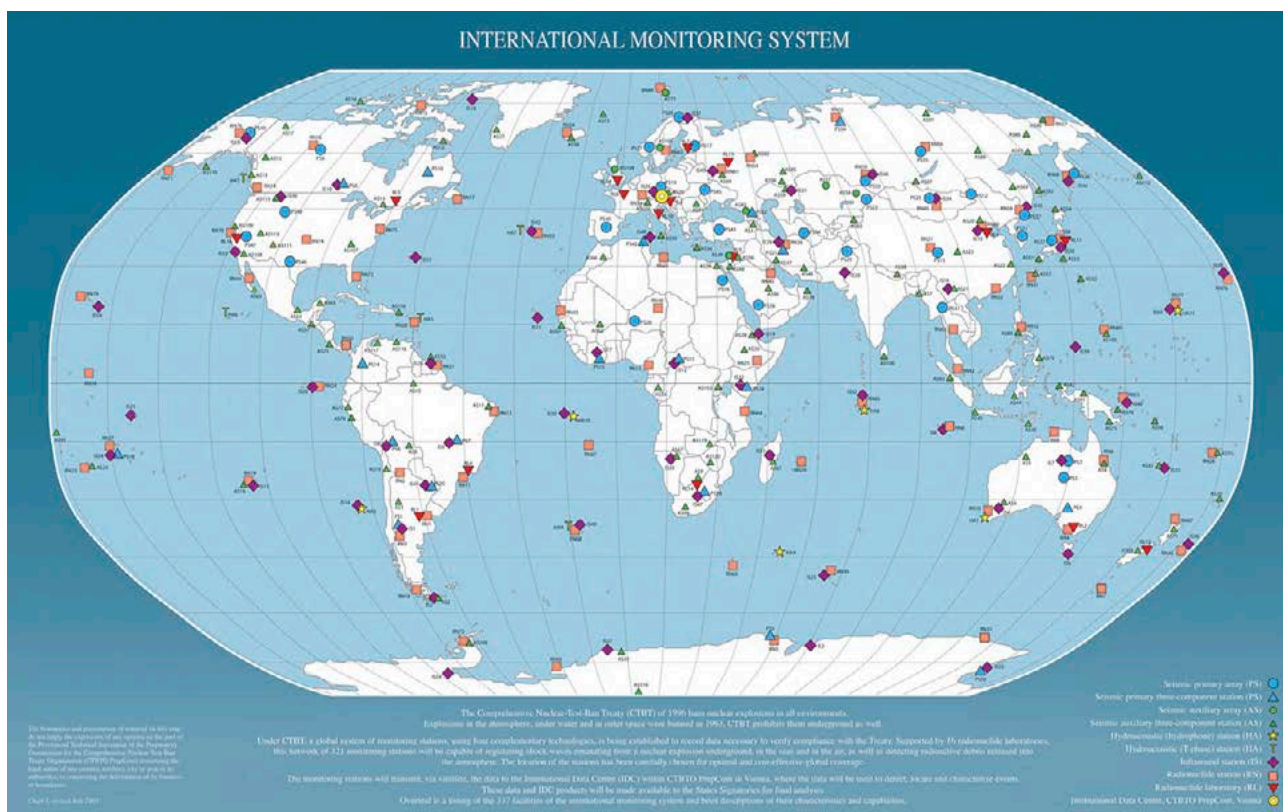


Figure 1: International monitoring system of the CTBTO

- 80 stations with radionuclide detectors for the measurement of special radioactive particles, as well as for the measurement of the concentration of radioactive noble gases; in addition there will be 16 radionuclide laboratories
- A data centre in Vienna where all data will be collected and evaluated.

At this point in time (April 2018), 294 of these 337 measurement stations have been installed and certified. The data centre in Vienna is operational and is constantly improving the software and methods used for data evaluation. The raw data as well as evaluation results are made available to the Member States. It is the Member States who verify compliance with the treaty, using the data of CTBTO, and that can request an on-site inspection on case of a suspicion of an underground test.

### The North Korean Nuclear Weapons Test

At the beginning of September 2017, North Korean state television reported that the test of a hydrogen bomb on 3 September 2017 had exhibited an “unprecedented power” and has been a “total success”. North Korea could also deploy this weapon using a long-range missile. The successful detonation had been a “very important step” to “complete the State nuclear power”, further explained the speaker of the State television.

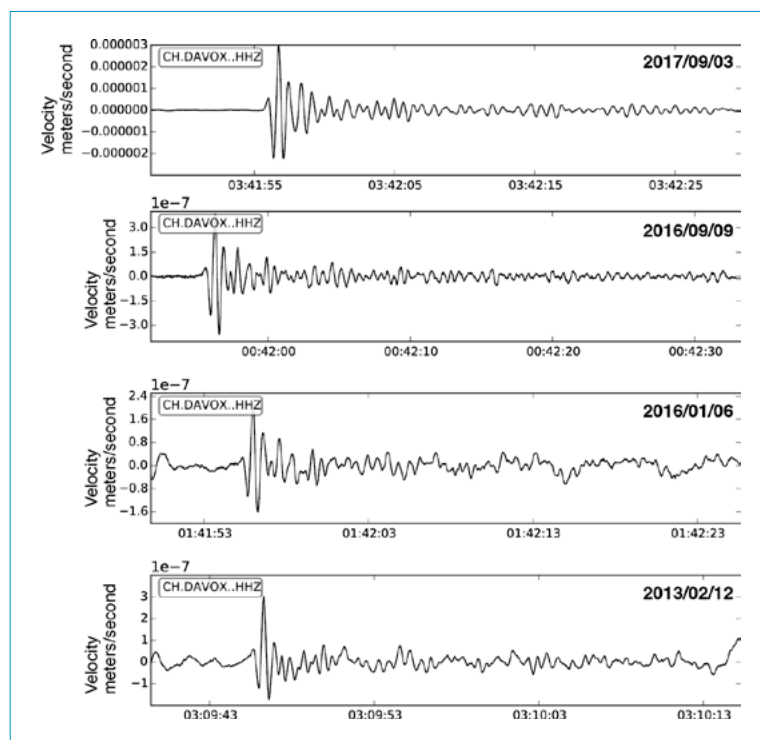


Figure 2: Seismological signals, recorded by the CTBTO station in Davos.  
<http://www.seismo.ethz.ch/de/home/>

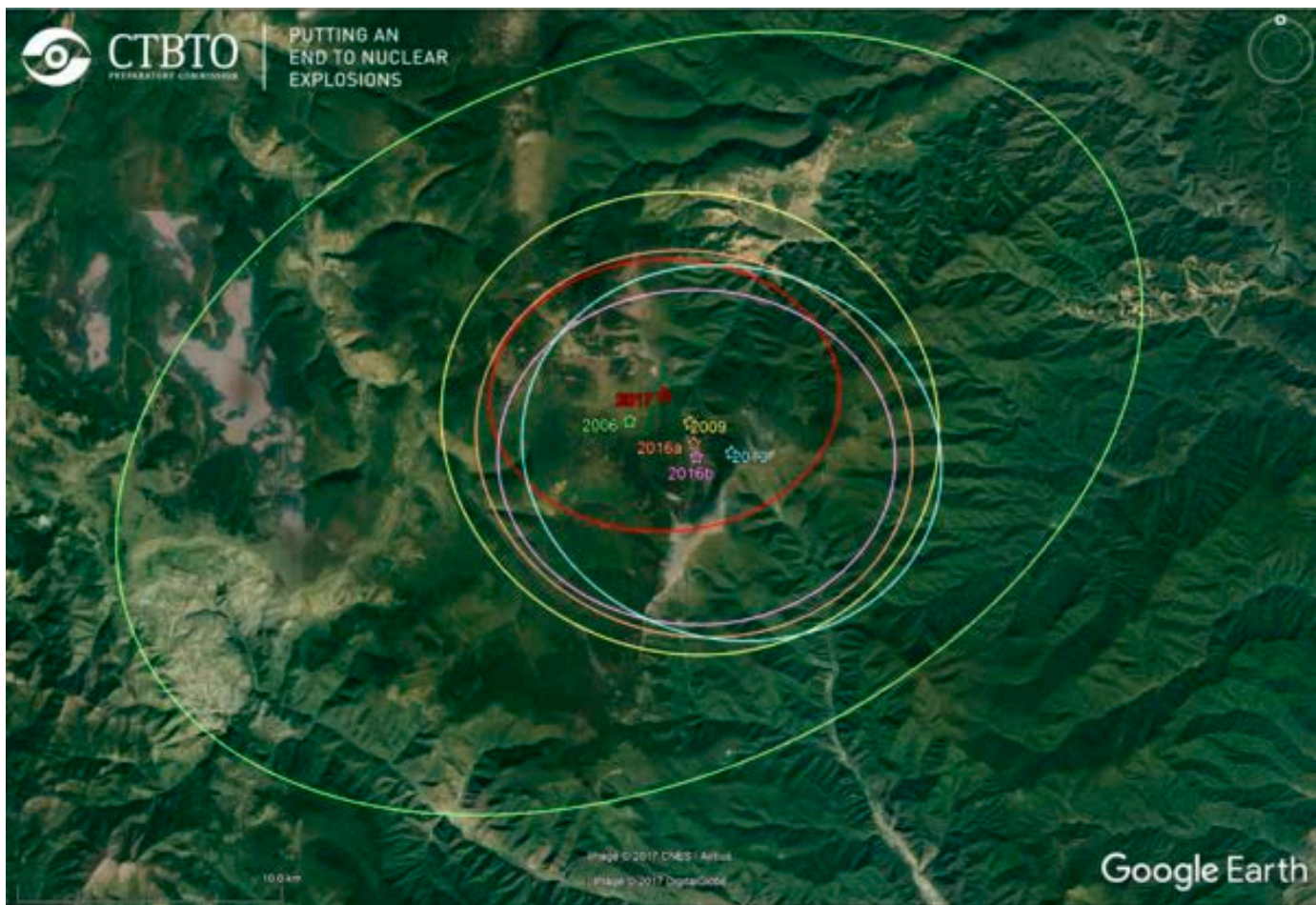


Figure 3: The calculated position of the North Korean tests with error ellipses. The position of the 2017 tests was the most accurate given that the establishment of the International Monitoring System IMS had progressed farthest.

<http://pws.ctbto.org/the-treaty/developments-after-1996/2017-sept-dprk/technical-findings/>

Seismological stations of the CTBTO, including the Swiss station DAVOX (figure 2), recorded the signals of the explosion. By analysing the shape of the seismic signals using the software of the CTBTO, it was possible to ascertain that the tremors were indeed caused by an explosion rather than an earthquake.

Using the measurements of 130 stations, the CTBTO was able to calculate the location and magnitude of the explosion. Infrasound as well as hydro-acoustic data, too, were included in the calculations (figure 3).

#### **“Comprehensive Nuclear Test Ban Treaty (CTBT)”**

Of a total of 196 States, 183 have signed this treaty, and 166 have ratified it (as of April 2018). However, despite these numbers the treaty has yet to enter into force. It will only enter into force once it has been ratified by all of the 44 States specifically listed in the treaty (the so-called Annex 2 States). Egypt, China, Iran, Israel and the USA are still missing – they have signed but not yet ratified the treaty; also missing are India, North Korea and Pakistan who have yet to sign the treaty. Switzerland ratified the treaty in 1999.

Apparently, North Korea had conducted this sixth, as well as the previous five, nuclear weapons tests at the testing ground Punggye-ri in the North East of the country. It was by far the strongest North Korean test explosion, releasing the energy of approximately a quarter of a megaton. This corresponds to the explosive equivalent of 250 000 000 kilograms of the conventional explosive TNT (table 1).

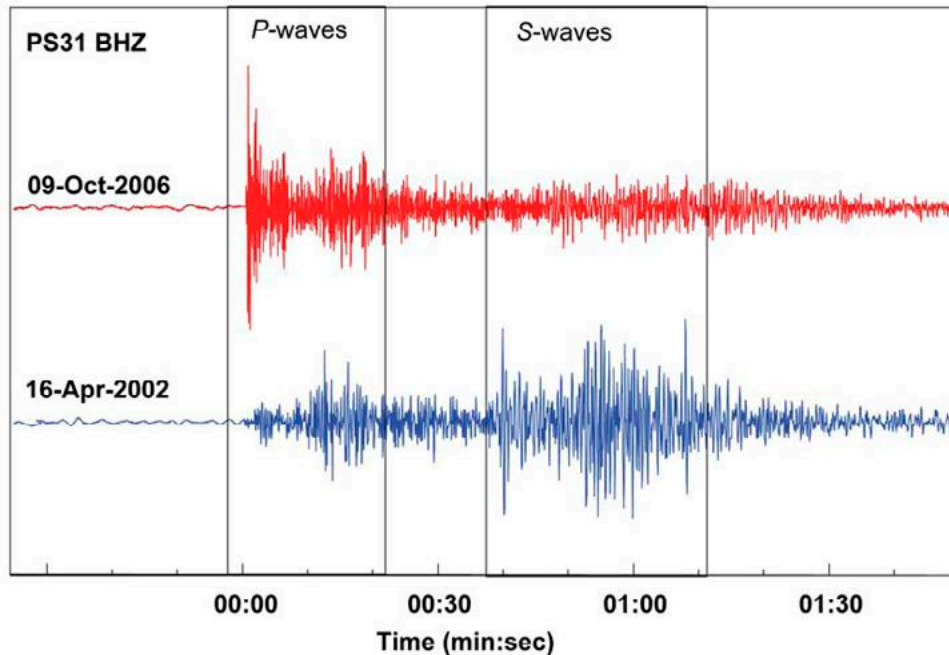
The high-precision comparison of the seismic signals of the six explosions yields, in addition, the relative positions of the tests in relation to each other – with a resolution of a few 100 meters. A combination of these data with satellite images, furthermore shows that the latest test had taken place underneath the 2205-meter high Mt. Mantap. Whether this was a hydrogen bomb test as claimed by North Korea cannot be assessed conclusively. Such calibres, indeed, are typical for two stage nuclear weapons (fission and fusion), which would support the North Korean claim to have detonated a hydrogen bomb. This does not however



### Earthquake or explosion?

A seismogram contains information about the event and the paths of the different waves from the place of the quake or explosion to the monitoring station. In the case of an earthquake, the seismogram initially shows smaller P (primary) waves and subsequently larger S (secondary) waves. In case of an explosion (red) on the other hand, the monitoring instruments respond initially strongly when the shock wave arrives. P and S waves travel through the globe. They differ in their direction of vibration (longitudinal versus transversal). P as well as S waves precede the slower, more energy-rich surface waves (not shown) which normally cause the greatest damage.

<http://pws.ctbto.org/verification-regime/the-international-data-centre/waveform-data-processing-and-analysis/>



prove the matter – during the 1950ies there were nuclear weapons tests (at the time still in the atmosphere) with even stronger weapons that used fission alone.

The isotope ratios of the radioactive compounds that leak from the bedrock allow drawing conclusions about the nature of the nuclear explosion. The noble gas Xenon, in particular, would be a good indicator. Xenon is one of the substances that escape particularly quickly after a possible release – for example from nuclear power stations or isotope manufacturing facilities for medical applications. Xenon is not washed out of the atmosphere by rain and thus can be transported easily across larger distances. The North Korean test, however, did take place very deep in the underground. This is why attempts failed to detect traces of radioactive fission products in the atmosphere, and thus no precise conclusions are possible about the nature of the explosion.

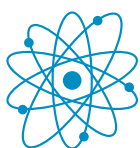
Time of the nuclear weapons test	Body Wave Magnitude mb according to CTBTO	Calibre [kt]
October 2006	4.08	0.5
May 2009	4.51	1–3
February 2013	4.92	10
January 2016	4.82	5
September 2016	5.09	10–15
September 2017	6.07	200–300

Table 1: Body wave magnitude mb of the North Korean tests according to the CTBTO, and estimated calibre based on these data

Although the monitoring system of the CTBTO has been developed for the detection of nuclear weapons tests, its “civilian” applications today are equally important. This includes the Tsunami warning system, climate studies, the tracking of icebergs, the localisation of meteor impacts, or in the case of a release of radioactive material the determination of the release site and the estimation of the amounts released into the atmosphere.



Workers decontaminate the roof of the Okuma town office near Fukushima Daiichi nuclear power plant in December 2011



## Decontamination in Urban Environments

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**An attack with a radiological bomb in a big city would have severe consequences for the economy if entire quarters had to be sealed off for extended periods and the population had to be evacuated. For this reason, it is useful to know how to efficiently decontaminate an urban area. Studies have shown that radioactive material penetrates only slowly into building materials. It also is unnecessary to apply expensive decontamination agents. Water achieves the same results.**

After the catastrophes of Chernobyl and Fukushima, studies were conducted of the decontamination of building materials, yet it is not possible to draw conclusions from the literature that would apply to the situation in urban areas in Switzerland because in Switzerland, other building materials are sometimes used such as sandstone. For this reason, Spiez Laboratory, in collaboration with the Institute for Facility Management of Zurich University for Applied Sciences (ZHAW), has been undertaking a research project on the effective decontamination in urban environments.

In this project, building materials typical for the historic centres of Swiss cities were contaminated with Caesium, artificially aged in the climate chamber of Spiez Laboratory and exposed to the weather. At different times, the spread of the Caesium in the material and its leaching due to weather conditions was examined. Simultaneously, the material samples were decontaminated with pure water, two different latex membranes as well as several decontamination foams also used by the Swiss army.



These experiments used building materials such as concrete, sand stone and unglazed roof tiles. The samples consisted of disks of material in the case of concrete and sand stone, as well as of whole tiles. In addition, cubes were cut out from these materials using a diamond saw. A similar behaviour could be observed for all materials: as a rule, the contamination penetrates merely into the upper third of the investigated layer thickness (1cm). With few exceptions, only small amounts of Caesium chloride can be determined in the bottom-third layer (approximately 10 per cent of the surface concentration). Irrigation promotes the migration of the Caesium and, for example, leads to a three-times higher final value in the lower segment of concrete. Similarly, more Caesium can also be determined in the lower layers of unglazed tiles.

#### **Contamination of the material samples**

In order to achieve an as realistic as possible simulation of the impact of a radiological bomb, some of the material samples were contaminated in the detonation bunker of armasuisse in Thun, using a bomb explosion (2kg plastit) to which 200 grams of Caesium chloride (CsCl) had been added. As expected, the different materials were contaminated unevenly.

The remainder of the material samples were contaminated in the laboratory, using a solution of 13g CsCl in one litre water. 10 microliters of this solution were deposited on each material sample using an Eppendorf pipette. The cubes were each contaminated with one drop, larger samples with one drop every 3cm.

#### **Aging of the samples in the climate chamber**

After an incident leading to radiological contamination, large areas usually cannot be decontaminated right away. Therefore, all material samples were artificially aged in the climate chamber. Except for extraction, all samples were stored at 25 degrees Celsius and 85% relative humidity. Some of the samples were irrigated once per day with 3mm/m<sup>2</sup> of drinking water. To this end, the test specimens – placed on SBB (Swiss federal rail) palettes/plywood – were irrigated in alignment with their contamination orientation (horizontal/vertical) using an irrigation apparatus with automatic dosage. The climate was monitored and documented. The samples were investigated 1, 2, 7, 14, 28, 35, and 140 days after contamination.

#### **Sample preparation and analysis**

Samples designated for analysis were processed depending on which series they belonged to: massive discs/plates were bored directly by dry drilling with a diamond drill (inner surface 1cm<sup>2</sup>) without water-cooling. Plates that had been contaminated by pipette were bored directly over the centre of the contamination spot. Plates contaminated by explosion were bored randomly across the sample surface. The pre-cut cubes were processed as a whole.

In order to determine the migration into the material, the drill cores and the cubes were sliced into three segments of 3mm thickness each using a fine diamond disc; in case of material that was too weak, e.g. in some cases sandstone, only top and back layer were separated.

Subsequently, the samples were broken up and pulverised in a crusher and transferred into polypropylene vials. After each sample processing, the crusher was cleaned using sea sand, in order to prevent contamination carry-over.

The environmental analysis team of Spiez Laboratory subsequently analysed the samples using the EPA method 3015A: the milled construction material samples were homogenised. The Caesium contained in approximately 500mg of the sample was extracted with concentrated nitric acid using a microwave-supported method at 175 degrees Celsius for 5 minutes. The resulting solution was filtrated and after dilution analysed using mass spectrometry.

#### **Results**

First, the migration of Caesium into the material was investigated. The results show that the weather effects will only begin to contribute significantly to a reduction of the contamination after 140 days. For one month after contamination, hardly any reduction is detectable in comparison to the starting value.

In parallel to investigating the Caesium migration, the construction materials were treated with different decontamination agents: Two Latex materials (Belfor and Latex), three decontamination foams of the Swiss army (BX24, SX34 and BX40), and tap water.

The two Latex products were applied onto the material samples and dried for one day. Subsequently, the Latex layers were peeled off. The commercially available decontamination foams of the Swiss army were used in accordance with the instruction manual. The tap water was sprayed onto the material sample. After decontamination, the samples were tested for residual amounts of Caesium.

The results of the decontamination tests after 28 days<sup>1</sup> show unambiguously that the two Latex products have very limited suitability for the decontamination of sandstone.<sup>2</sup>

It was equally clear that the commercial decontamination foams do not have an effect superior to normal tap water. For the operational capability, this brings a relief: water is available in sufficient amounts even in crisis situations.

The results of decontamination of concrete are even more clear-cut. In the case of concrete, the results with Latex are as good as those using the decontamination foams BX24 and SX34. At that point, it was unfortunately no longer possible to test BX40, as the stock was depleted. In any event, the decontamination effectiveness using water was superior by a factor of almost two.

### Conclusions

- Decontamination can reduce significantly the Caesium load. Depending on the time of decontamination and the building material, the effects achieved varied from 50 to 95%.
- A complete decontamination was not possible in any of the cases. Normal water, as a rule, was as effective or even superior to commercial decontamination agents. It is not necessary, therefore, to procure decontamination agents, to stock them and purchase resupplies after their expiry date.

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<sup>1</sup> Decontamination does not necessarily have to take place immediately after the event. The experiments did not indicate any disadvantages of delaying decontamination, on the contrary: over longer periods, the effects of weather contribute to the washing out of part of the contamination. This process, however, is uncontrolled and the contamination as a rule will be transferred to other areas with the wastewater. This, then, speaks in favor of conducting the decontamination as early as possible.

<sup>2</sup> Even though the two Latex products are not suitable for decontamination, they can nevertheless be useful: the application of a Latex layer can prevent the carry-over of the contamination, which may be desirable.





Lead container for shielding radioactive material

# Lead Analysis in Nuclear Forensics



**Nuclear forensics provides authorities that fight against nuclear terrorism with important evidence for the tracing of radioactive sources. Switzerland supports these efforts as a partner of the *Global Initiative to Combat Nuclear Terrorism*. Lead is a preferred material to shield radioactive sources. In this context, the inorganic analysis of impurities in lead as well as the identification of isotope ratios supports nuclear forensics. Because the analytical techniques used for lead as shielding material are the same as for conventional lead core cartridges, these techniques could also support classical forensic investigations of incidents involving firearms.**

In nuclear forensics, samples are analysed in order to gain information about their chemical composition and isotope ratios. From these parameters, it is possible to draw conclusions about the degree of enrichment, the chemical composition, and any impurities as well as by-products. These data can help determine the origin, the manufacturing process or mode of use of a radioactive source or nuclear material, which can provide authorities, such as the Federal Intelligence Service, the Federal Office of Police (fedpol) or the Federal Prosecutor's Office with evidence they can use to trace a radioactive source. Switzerland is making these efforts, amongst others, in the framework of the *Global Initiative to Combat Nuclear Terrorism* (see box).

Marc Stauffer,  
Cédric von Gunten

## **Analysis of the radioactive source**

The radioactive source itself is a typical investigation item:

Which radionuclides are present? What are their relative isotope ratios? Has the object been exposed to neutron flux in a reactor? Are the decay products in radiological equilibrium? Is it



Figure 1: Machining of the Lead specimen



Figure 2: Addition of high-purity nitric acid to the Lead samples in quartz vessels

The Global Initiative to Combat Nuclear Terrorism (GICNT) is a voluntary partnership of 88 Nations and five International Organisations, which advocate the strengthening of global capacities for the prevention of and response to nuclear terrorism.

The partner countries, amongst them also Switzerland, have pledged voluntarily to implement the GICNT principles – a range of comprehensive security objectives which include a range of deterrence, prevention, detection and response goals. These principles aim at developing the capacity for collaborations and partnerships in the struggle against nuclear terrorism, in concert with national authorities and in accordance with international agreements on the combat of nuclear terrorism, the Convention on the Physical Protection of Nuclear Materials, as well as Resolutions 1373 and 1540 of the United Nations Security Council.

The USA and Russia act as co-chairs of the GICNT whilst Finland sub-leads the Implementation and Assessment Group (IAG). The GICNT is open for all nations that actively stand up for the determined and systematic combat against nuclear terrorism.

possible on that basis to establish the manufacturing date of the source?

#### Lead as shielding material

Additional information can be gathered from the shielding or the container of the source. The shielding is often composed of lead because this metal absorbs well Gamma and X-ray radiation given its high density and atomic mass.

For forensic investigation, the lead can be analytically characterised based on the following parameters:

- Alloy constituents (e.g., hardening of the Lead by addition of Antimony)
- Impurities caused by accompanying elements (e.g., Arsenic, Bismuth, Copper, Indium, Tin, Tellurium, Thallium)
- Isotope ratios between  $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$  and  $^{208}\text{Pb}$

Based on the impurities as well as the relative isotope ratios the mineral deposit from which the Lead has been extracted can be determined or excluded, if the corresponding databases exist. Alternatively, Lead samples can



Figure 3: Lead isotope ratios measured with ICP quadrupole MS

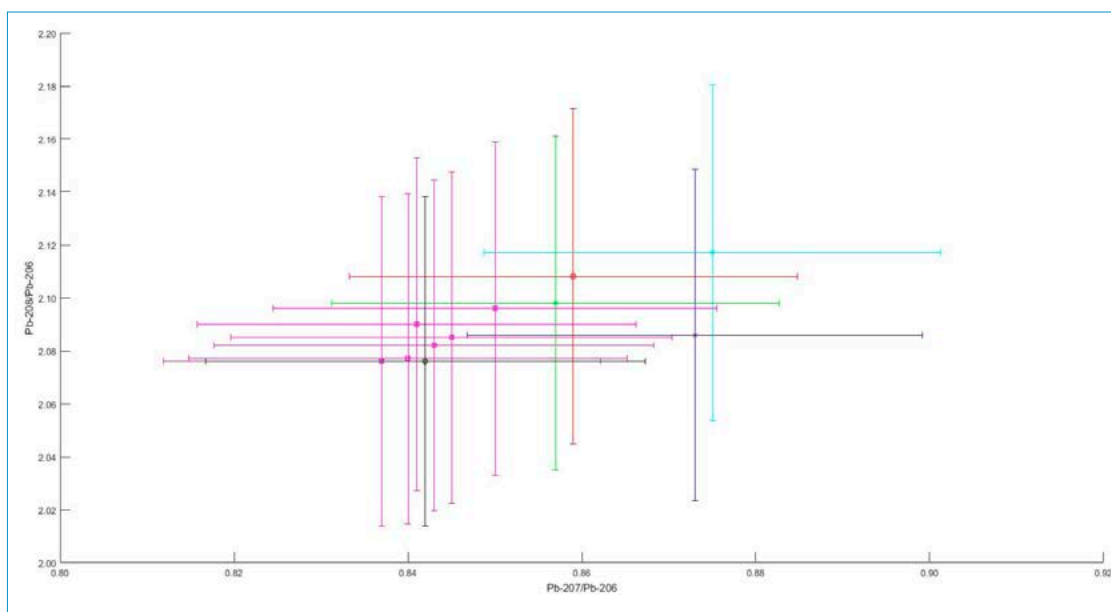
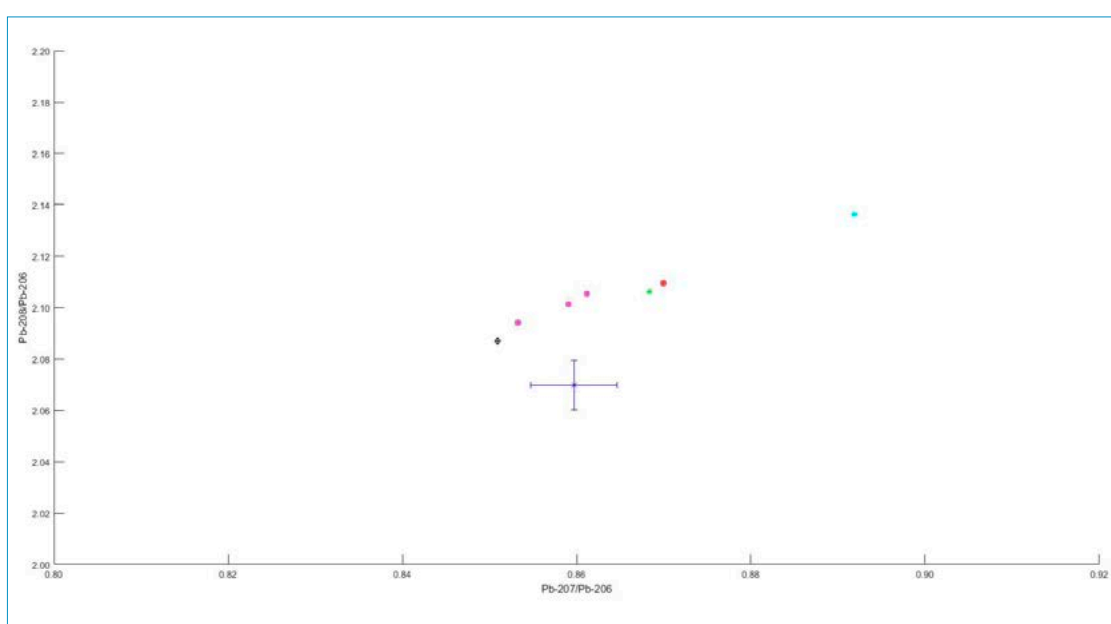


Figure 4: Lead isotope ratios measured with ICP multi-collector MS



be compared to known (reference) samples. For example, unknown Lead samples can be matched against known production batches or ideally against samples that have been secured from the environment of a suspect.

### Analytical steps

The analytical approach is executed in accordance with the following scheme:

- Sample preparation (machining and comminution)
- Digestion (dissolution with nitric acid and complexing with citric acid)
- Inductively coupled plasma – optical emission spectrometry (ICP-OES)
- Inductively coupled plasma – multi-collector mass spectrometry (ICP-MC-MS)

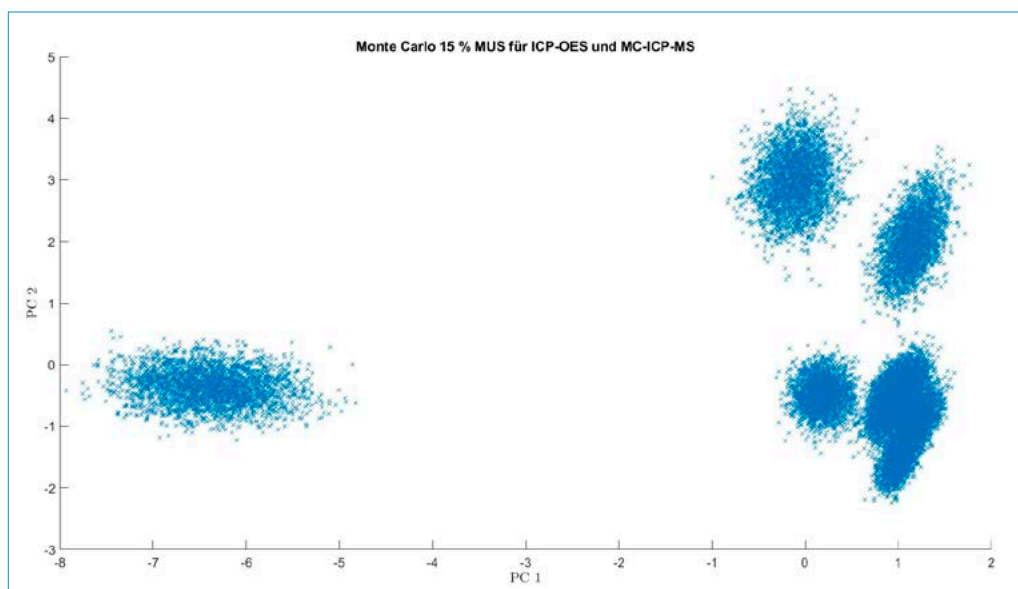
During sample preparation by machining (see figure 1), care was taken to achieve a reproducible multiple sampling of the Lead specimen. In addition, the element composition of

the drill piece must be taken into account, as it will influence the subsequent high-performance analysis.

The chopped up Lead cuttings are weighed and dissolved in open digestion by the addition of nitric acid and citric acid (figure 2). The nitric acid dissolves the matrix (the Lead) as well as most of the impurities. Certain elements such as Antimony are dissolved by nitric acid yet the resulting antimonate would not be captured as it forms an insoluble deposit and would thus not be accessible for subsequent analysis. The addition of citric acid prevents the precipitation of Antimony and other metals in the digestion solution. It is important to perform the digestion across all experiments in a standardised manner to ensure reproducibility.

For the subsequent quantification of the impurities using optical emission spectrometry (ICP-OES), the digestions are filled up quantitatively

Figure 5: PCA analysis using Monte Carlo simulation using an extended measurement uncertainty of 15 %



to an analytical volume, filtrated and diluted. For the determination of the isotope ratios, on the other hand, the digestions simply have to be diluted to a level of approximately 50–100 µg/L Lead, because all analytical steps including filling up and dilution have no further impact on the equilibrated isotope ratio.

The analysis by ICP-OES was done by measurements using external multi-element calibration. The analysis was validated by using spiking and dilution experiments as well as by analysing certified Lead reference standards alongside. Because of its higher matrix tolerance, the ICP-OES method is better suited for the analysis of impurities in dissolved metal samples than ICP quadrupole mass spectrometry (ICP-Q-MS). This advantage of ICP-OES compared to ICP-Q-MS is the result of ICP-OES analysing light emissions, which means that no actual material transfer is required through a cone system and the ion optics.

For the analysis of the isotope ratio, however, mass spectrometry is indispensable. Figures 3 and 4 show the Lead isotope ration analyses using quadrupole and multi-collector MS instruments. Figure 3 demonstrates that the precision of the isotope ratio measurement in the quadrupole MS instrument does not suffice to provide a statistically sound answer to the forensic task. On the contrary, the error bars of the multi-collector MS measurements in figure 4 are hardly discernible. ICP multi-collector mass spectrometry is best suited for the analysis of isotope ratios given its measurements simultaneity.

The data so collected – composed of mass fractions of impurities of the Lead as well as the isotope ratios of Lead nuclides – now form a multidimensional picture. One possible

way of representing and visualising the match of a measured sample with a comparative sample is the Principle Component Analysis (PCA). PCA is a method of multivariate statistics that allows the visualisation of multiple-dimensional data sets in the two or three dimensions familiar to us.

Figure 5 shows a PCA analysis of five different Lead samples which after analyses of the impurities and isotope ratios can be clearly differentiated, using a conservative, extended measurement uncertainty of 15 %, at least optically.

If an unknown Lead sample was picked up, it could be indicatively associated with one of the five groups shown, or excluded from them.

In addition to the actual nuclear forensic data, the method described provides additional information from the inactive, inorganic analysis. These can be used as complementary data to further support investigation authorities. It is conceivable that, after further and in-depth statistical analyses, the same analytical approach can also be used by traditional forensic services for the investigations of Lead cartridges.





BSL 2

1

Development of cell based test systems suitable for high throughput analysis

2

Screening of chemical compound libraries for the identification of candidate molecules

3

Evaluation of candidate molecules for cell toxicity



BSL 3

4

Establishing test systems with wild-type hantaviruses

5

Validation of candidate molecules with wild-type hantaviruses

6

Investigating mechanism of action

New strategies for the development of anti-viral therapies

# Development of antiviral therapies against highly pathogenic viruses



**The spread of highly pathogenic viruses signals a genuine threat given that today, there are no vaccinations or effective therapies to treat against the majority of them. Research involving these life-threatening viruses, however, can only be conducted under the strictest safety precautions. Spiez Laboratory with its biocontainment laboratory is the only research institution in Switzerland that meets the technical requirements to evaluate antiviral substances against the particularly dangerous human pathogenic viruses of the highest risk groups, and to develop countermeasures against them.**

The outbreaks of SARS, MERS, West Nile Virus, influenza and Ebola during the past 15 years have demonstrated that the spread of highly pathogenic viruses constitutes a genuine threat situation. In addition, the intensive travel volume leads to the regular introduction by returning travellers of exotic diseases into Switzerland – diseases which only rarely can be treated causatively. For almost all highly pathogenic viruses, neither protective vaccinations nor effective therapies are available. Thanks to intensive research, effective therapeutic strategies have been established in recent years for a growing number of viral diseases, such as HIV/AIDS or Hepatitis C. The development of therapy approaches against highly pathogenic viruses, on the other hand, for the most still remains in its infancy. One reason for this is that research involving highly pathogenic viruses can only be con-

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Dr. Sylvia Rothenberger,  
Dr. Stefan Kunz (Lausanne University Hospital)*

ducted with high safety precautions and that few institutes have at their disposal the required infrastructure. The manufacturing of medicines for the treatment of diseases that are relatively rare on a day-to-day basis is not profitable for the pharmaceutical industry, without support from public entities. This is why there is a need for alternative strategies and the engagement of public institutions.

Spiez Laboratory with its biocontainment laboratory has at its disposal prerequisites like no other laboratory in Switzerland to evaluate antiviral substances against the particularly dangerous human pathogenic viruses of the highest risk groups. In the framework of the public mandate of the Federal Government to develop measures against highly pathogenic viruses, Spiez Laboratory is investigating a broad spectrum of substances with antiviral properties, with the following priorities:

- Validation of synthetic compounds and decontamination processes for use in the inactivation of viruses in the environment or in the laboratory setting.
- Assessment of the virus-neutralising properties of vaccination serums, for example in the frame of international vaccination studies against Ebola.
- Development of antiviral strategies.

These research activities are being conducted in close collaboration with partners in universities or companies that have a specific interest in therapies of infections with highly pathogenic viruses. In recent years, substances and strategies against highly pathogenic Corona viruses (SARS and MERS), influenza (H5N1), Ebola and hantavirus have been evaluated in the framework of these research activities.

To investigate antiviral substances, Spiez Laboratory uses a well-equipped infrastructure and has developed the required methods. Cell culture systems are available for a large number of highly pathogenic viruses, which permit efficient virus propagation. The availability of relevant cell culture systems is crucial for the investigation of the replication strategy of viruses in cells. In addition to cell lines that are used for the primary analyses, so-called organotypic cultures are being used for advanced investigations. These cultures are composed of different cell types that naturally constitute the corresponding organs, and reproduce these tissue types in terms of morphology as well as functionality. In this way, substances can be evaluated using the organotypic cell systems as a model for the corresponding tissues. The effect of the active compound on the replication of

the viruses is assessed in a first stage, through quantification of the replicated viral genomes by molecular biological means, and in a second stage, the number of infectious particles is estimated by limiting dilutions in cell cultures. In addition, immunological staining allows visualising and evaluating the virus growth within the cell. The physiological status of the cells is monitored throughout the experiment in order to register toxic effects of the test substances on the cells and related secondary effects on the virus replication. This is accomplished by measuring so-called physiological biomarkers – molecules that can be used as reference points for the cell status. The potential build-up of resistance caused by changes in the genome sequence of the virus can be elucidated in real time by modern whole-genome sequencing technology. Advanced investigations of the mode of action are being conducted in the context of basic research in close collaboration with university partners. For these investigations of the interaction between viral proteins and cell molecules, inactivated cell extracts can be removed from the high containment laboratory through the chemical containment barrier for subsequent investigations in partner laboratories.

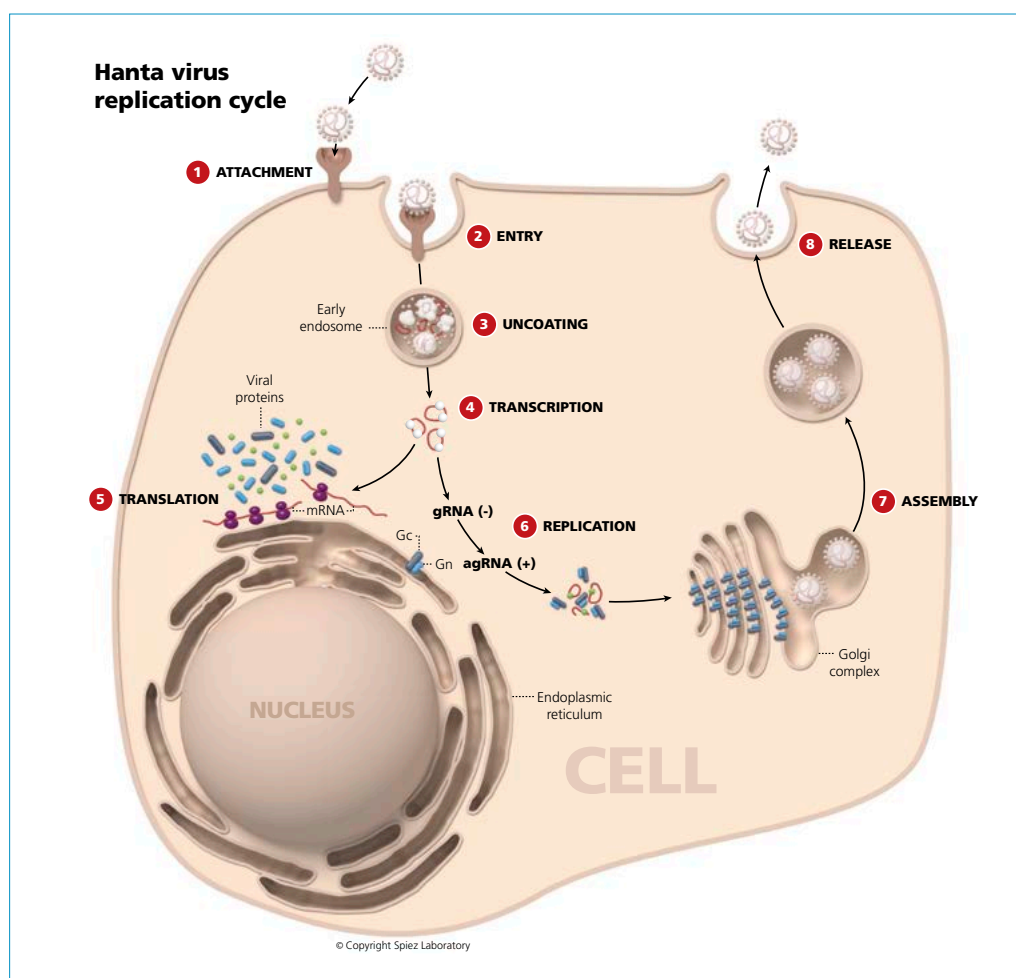
A primary challenge in the developments of new antiviral substances is to identify a leverage point for the intervention. Different from bacteria, viruses do not possess their own metabolism but replicate exclusively inside the infected cells, utilising the biosynthesis apparatus of these cells. Because of the participation of cellular proteins in the multiplication of the viruses, substances that suppress the replication of viruses frequently also affect normal cell functions, which manifests itself in side effects. An additional problem is that viruses display an enormous variability, so the viral drug target structures are constantly changing.

The identification of suitable target structures depends on a sound understanding of the entry and replication mechanisms of the viruses. These are being worked out in meticulous fine-tuning in the framework of basic research. Based on this knowledge, suitable target structures and intervention strategies can be developed.

#### **Development of antiviral substances against hantavirus infections, a promising collaboration with the Microbiological Institute of the CHUV**

In close research collaboration with the Microbiological Institute of the University Hospital Lausanne (CHUV), Spiez Laboratory pursues





The basis of modern antiviral drug design is the identification of viral proteins and their cellular binding partners in order to specifically block their interaction. An early starting point in the infection and replication cycle of viruses is the infiltration of the cell. In order to enter the cell, the virus has to undergo a series of (virus-specific) processes. (1. Attachment) of the viruses onto the host cell follows a lock-key principle involving cellular receptors, and can be blocked by suitable molecules. The further infiltration (2. Entry) of the viruses into the cell and the removal of the viral envelop (3. Uncoating) are active processes, which can be targeted as starting points for an intervention. The antiviral substances Amantadine and Rimantadine, for example, inhibit the release of the virus genome of influenza viruses in the cell, thereby significantly reducing the extent and course of an infection. Inside the cell, predominantly viral proteins (polymerases) are involved in the synthesis of viral RNA or DNA (4. Transcription) and proteins (5. Translation) and the multiplication of the viral genomes (6. Replication). Since the 1980ies, an increasing number of molecules have been developed – predominantly nucleotide and nucleoside analogues – which are composed similarly to the building blocks of RNA and DNA but which due to chemical modifications inhibit the viral polymerases, thereby terminating the viral RNA or DNA synthesis. This principle is used in Aciclovir – one of the first substances developed to treat herpesviruses – as well as by Ribavirin, which is used, for example, in the treatment of Hepatitis C, arenaviruses and adenoviruses. Further strategies target later steps in the virus cycle, such as the virus (7. Assembly) and (8. Release) of infectious virus particles from the cell. For example, the medicines for influenza virus treatment Zanamivir (Relenza) and Oseltamivir (Tamiflu) block the viral protein Neuramidase which is located on the surface of influenza viruses and is responsible for the release of virus particles.

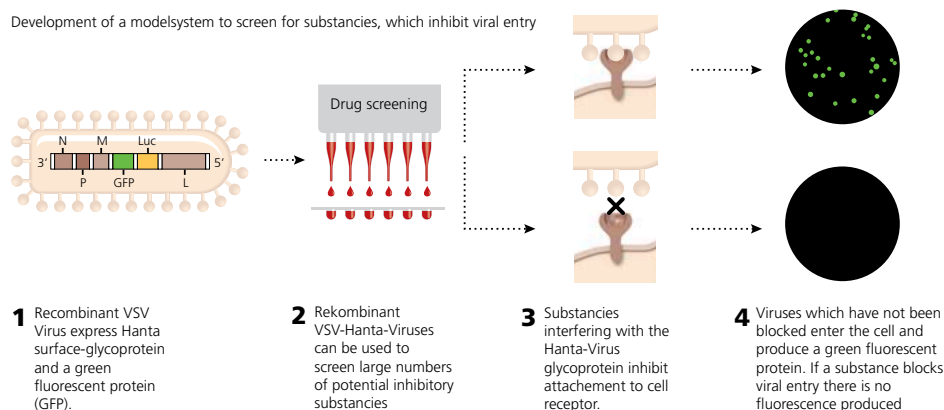
the goal of developing antiviral strategies for the treatment of hantavirus infections. Hantaviruses are prevalent in Europe, Asia and America. The highly pathogenic hantaviruses cause severe diseases, and hitherto neither prophylaxes nor treatments are available. The collaboration with the group of Professor Kunz and Dr. Rothenberger has made it possible to profit from profound knowledge on molecular virology and molecular biology technics. On this basis, investigations could be conducted of the interactions of viral proteins with cellular structures occurring during virus entry and replication inside the cell. The acquired knowledge is laying the ground for the establishment

of infection and replication models of hantaviruses, which will allow the screening of large compound libraries for effective candidates as well as to evaluate selected, already-established antiviral substances. In a subsequent phase, the selected candidate drugs are being investigated in the biocontainment Laboratory in Spiez for their effectiveness against infectious hantavirus isolates.

To study the process of cell entry of hantaviruses, so-called Hanta pseudoviruses have been generated. These pseudoviruses utilise recombinant particles of the harmless vesicular Stomatitis virus (VSV) as carrier for the

## Drug screening

Development of a modelsystem to screen for substances, which inhibit viral entry



surface proteins of highly pathogenic hanta-viruses, which mediate cell entry. In addition, the viruses were modified by inserting the genetic information for a fluorescent protein (eGFP) and a luciferase protein (Dr Gert Zimmer, IVI) so that infected cells could be made visible by fluorescence and the virus infection could be quantified. The pseudovirus system therefore is very well suited for the fast screening of libraries of chemical compounds for molecules that inhibit the invasion of cells by Hanta viruses.

With the use of recombinant VSV-Hanavirus-pseudotype system it was already possible to identify cellular receptors and other proteins that hantaviruses require for cell entry. The identified cellular receptors recognise a conserved component of the viral envelope and might thus be involved in the entry process of several other enveloped viruses. The targeted inhibition of these receptors using inhibitory antibodies or their over-expression in lung cells demonstrated for the first time that the entry of hantaviruses into human lung epithelia cells could happen via these receptors. Based on these understandings, a selection of well-characterised inhibitors was used to identify cellular factors that participate in the virus entry. Using these tests, it was possible to show that different types of Hanta virus share a common entry mechanism: the so-called macropinocytosis, which normally serves the uptake of dissolved substances into the cell. The cellular factors that are required for cell entry are being confirmed in detail at the BSL-3 laboratory using infectious hantaviruses. In addition, there are plans to conduct a screening of a large chemical library based on the hantavirus pseudotype system, in order to identify inhibitory compounds.

In a complementary strategy, the replication mechanism of hantaviruses is being investigat-

ed to identify viral proteins that may be suitable as targets for a therapeutic approach. The viral proteins responsible for transcription and replication, in particular the viral polymerase, are highly conserved and constitute the most promising targets for the development of anti-viral medicines. Studies at the University Hospital Lausanne and in other laboratories have shown that hantavirus polymerases play an important role in the processing of viral RNA – this is of central importance for viral replication. Its inhibition by low-molecular-weight compounds is a promising antiviral strategy. We have succeeded in establishing a robust cell-based biochemical test for endonucleases of hantaviruses, which allows to quickly test inhibitors for possible effect. The test protocol allows the swift screening of comprehensive “chemical libraries” for new inhibitors of Hanta virus endonuclease. Subsequently, candidate substances will be further evaluated at the BSL3/4 high containment laboratory in Spiez using authentic hantaviruses. A major goal of the project is to combine anti-viral drugs that target different steps of the viral life cycle. Attacking the virus from different angles reduces the chance for drug resistance.





Ricinus Communis

# International Round Robin Test for Ricin



The poison of the plant *Ricinus communis* is one of two naturally occurring toxic chemicals that the Organisation for the Prohibition of Chemical Weapons (OPCW) has included in the Schedules of Chemicals of the Chemical Weapons Convention (CWC). In 2017, the OPCW organised the first international inter-laboratory comparison test for Ricin. The Toxinology Branch of Spiez Laboratory – with the support of the colleagues of the Organic Analysis Branch – has successfully participated in this test. The international character of such tests helps the Toxinology Group to maintain an extensive European network and to offset its own limited personnel capacity.

Toxins are poisonous substances of natural origin that are produced by animals, plants or microorganisms: Ricin is a glycoprotein that occurs in the seeds of the plant *Ricinus communis* as a natural feeding protection [1,2]. As a macromolecular glycoprotein, Ricin is easily available from the residue of seed pressing resulting from castor oil production. Worldwide castor oil production amounts to appr. 1,5 million tones per year, in particular in China, Brazil and India. In Switzerland, *Ricinus communis* is found as an ornamental garden plant.

Ricin belongs to the group of so-called type-2 Ribosome inactivating proteins (RiPs). The Ribosome inactivation leads to an irreversible blockage of protein synthesis in the body cells. This in turn leads to cell necrosis. In humans, less than 1 milligram of Ricin is lethal, if the poison is inhaled. The lethal dose for intravenous or intramuscular uptake is more or less the same. If the poison is ingested orally, several hundred milligrams are lethal.

Marc Avondet



Figure 1: The six samples of the first OPCW Ricin Exercise

Sample Code	Chem ID	Chemical Name	Conc. (µg/ml)	Schedule Number
A171/xx	A	Ricin	10	A.A.08
A172/xx	B	Ricinus communis agglutinin (RCA120)	100	N.S.
A173/xx	A & B	Ricin & Ricinus communis agglutinin (RCA120)	100	1.A.08
S174/xx	C	Crude ricin	100	1.A.08
S175/xx	D	Castor bean extract	–	1.A.08
S176/xx	E	Deactivated crude ricin	100	N.S.

Figure 2: List of samples and their composition (A171/A172/A173 = aqueous solutions, S174/S175/S176 = wet laboratory sand)

#### SUMMARY: REPORTED CHEMICALS

Original Sample Code*	Chemical ID assigned by the Laboratory**	Chemical name
A173/13	C-1	Ricin Estimated concentration = $85 \pm 4.8 \mu\text{g}/\text{mL}$
S174/13	D-1	Ricin Castor bean e Estimated concentration = $120 \pm 2.3 \mu\text{g}/\text{g}$
S175/13	E-1	Ricin Estimated concentration = $6.2 \pm 0.18 \mu\text{g}/\text{g}$

Figure 3: Summary of the LS measurement data (shown are Ricin positive samples with  $> 1 \mu\text{g}$  Ricin per gram/millilitre sample)

Spiez Laboratory has been focussing for several years on the establishment of identification methods for four toxins – in addition to Ricin these are Butolinum toxin A, staphylococcus enterotoxin B, and Saxitoxin.

The start of analytical work on Ricin at Spiez was rather complicated because at the beginning, no reference materials were available and the nature of the analytical methods to be used remained uncertain. The literature described gel electrophoresis and immunological methods as necessary measurement methods. But for these techniques, adequate antibodies were lacking. Thanks to the cooperation with Professor Uwe Pfüller of the Institute for Phytochemistry at the University Witten-Herdecke in Germany, Spiez Laboratory was able to acquire 500 milligrams of Ricin (purity  $>95\%$ ) in 2003, as well as several hybridoma cell lines

for the manufacturing of monoclonal anti-Ricin antibodies. Data of comparative measurements undertaken in the autumn of 2017 have shown that even 14 years after their manufacturing, the Ricin reference standard remained of good quality. In 2009, the group ZBS3 of the Robert-Koch-Institute Berlin organised a first Ricin round robin (inter-laboratory comparison) test [4].

#### OPCW Biotoxin Exercise Ricin 2017

Remarkable progress in the development of high performance analytical identification systems as well as intensive methods development allow today the integration of Ricin into the verification activities of the OPCW. To this end, the OPCW correspondingly launched a Ricin exercise in 2017. Prior to the conduct of Proficiency Tests with new performance requirements (such as the investigation of clinical





Figure 4: The co-workers of the Analytical Chemistry Group (OA) and Toxinology Group who participated at the evaluation meeting at the OPCW in The Hague (from left to right: Christian Müller, Dr Peter Siegenthaler (OA), Dr Martin Schär (OA), Marc Avondet

samples or bio-toxins), it is common practice that the OPCW conducts so-called “exercises”. In this way, Designated Laboratories or laboratories that aspire to obtain designation can practice and establish the necessary techniques. At the same time, the necessary criteria for verification can be worked out.

The Analytical Chemistry Branch (OA) of Spiez Laboratory has proved itself regularly and for many years as a very successful Designated Laboratory for the analysis of classical chemical warfare agents in environmental samples. At the beginning of 2017, the Toxinology Branch – with the support of the OA Branch – took part in this first Biotoxin Exercise of the OPCW. The objective was to test the Ricin analysis established in Spiez and to support the OPCW's stock taking with regard to methods for Ricin analysis.

On 16 January 2017, six samples – three liquids and three wet sand samples – arrived in Spiez (see figure 1). The sample set was complemented by three reference materials – Ricin, Ricin-agglutinin, and a Ricin peptide.

According to the request for analysis, primarily a qualitative assessment of the samples was expected (identification of Ricin) and, if possible, also a quantification. After appropriate sample preparation, a sensitive Ricin-ELISA

screening was conducted, which already provided quite specific evidence to answer the *Ricin yes or no*-question and to gauge its approximate concentration. By a skilful combination of different analytical methods, it was possible to ascertain the identification of Ricin and achieve an *unambiguous identification*.

According to information from the OPCW, the minimum spiking level in the Ricin positive samples was 10 µg/g. In addition, the participants had been advised that at least one sample would be free of Ricin (blank). During the analysis in Spiez, however, Ricin was detected in all samples, albeit the estimated Ricin concentration in the three samples A71/A172/S176 was significantly below the declared minimum spiking level of 10 µg/g and was consequently not mentioned in the report by the LS (see tables 2 and 3).

The second half of the available time was spent on the documentation of the extensive results in an analysis report – given the rigid reporting rules of the OPCW this is associated with a lot of work. Just before the deadline, the report [4] – counting 113 pages – was delivered to the OPCW after four weeks of intensive work.

In the middle of April, the OPCW issued a “Preliminary Evaluation Report” [5]. Finally, on 24



May 2017, a meeting was convened at the OPCW headquarters in The Hague to discuss the results among the representatives of the 26 participating laboratories. During the meeting, there were also discussions about identification criteria and options for the documentation of analytical results, as well as of how to proceed further. For the toxinologists, it was an exciting experience to visit OPCW headquarters and to participate together with the co-workers of the Organic Analysis Branch in the meeting. This first interdisciplinary work on Ricin samples involving the Branches Organic Analysis and Toxinology was an opportunity for both sides to gather valuable experiences, and it allowed them to identify further potential for the optimisation of the Ricin analysis.

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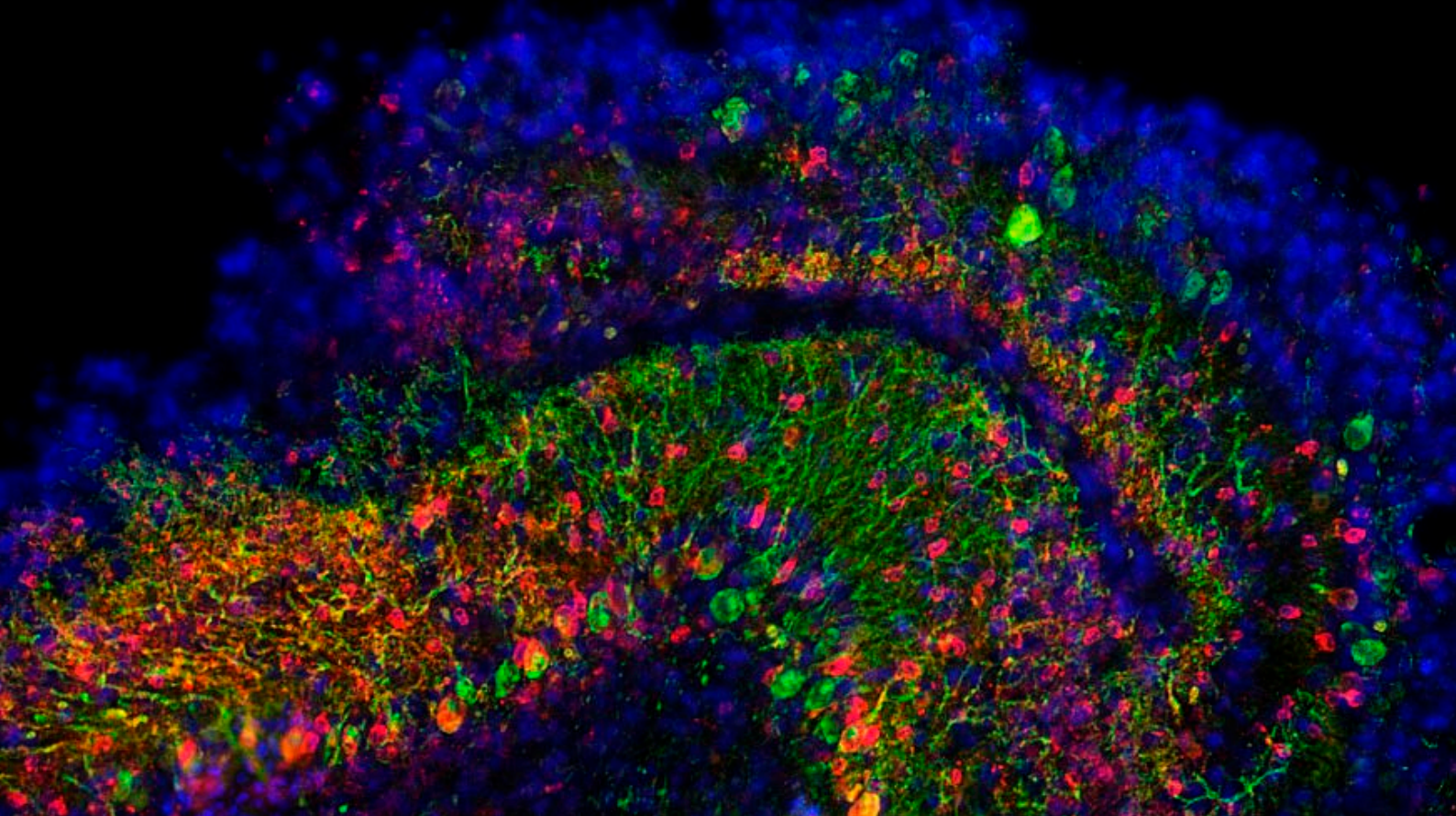
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- [3] LS Bericht 2010–03 1<sup>st</sup> International Proficiency Test on the Quantitation of Ricin in Aqueous Solution 2009 (SCM/AV)
- [4] Report of the First Biotoxin Sample Analysis Exercise 2017 (Laboratory Code 13)
- [5] Report of the First Official OPCW Biotoxin Analysis Exercise; Volume III: Preliminary Evaluation Report
- [6] <http://eurobiotox.eu>

#### International network for toxin analysis

The EU research project EuroBioTox, which has a duration of five years and a budget of 8 million Euro, was launched in 2017; Spiez Laboratory is one of the consortium partners. A welcome effect of EU research projects is that they result in the further expansion and nurturing of a comprehensive European network in the field of toxins. The international cooperation allows the Toxinology Group of Spiez Laboratory to compensate for the limitations of its own personnel capacity. Almost 60 per cent of the EuroBioTox budget is earmarked for the development of certified reference materials, the procurement of reagents, standardised analytical methods and realistic samples as well as for the organisation of interlaboratory comparison tests. One additional goal of the consortium is that in the framework of different repositories, reference materials are available for an efficient Europe-wide toxin analysis. These repositories will be complemented by training courses for instruction in toxin identification methods. Spiez Laboratory will take on a repository function for the certified reference material Ricin.



Figure 5: EuroBioTox and the participating consortium partners.



Immunofluorescent staining of TBE virus-infected organotypic cerebellum cultures.  
Blue = cell nuclei; green = Purkinje cells, important target cells of TBE viruses; red = TBE virus

# Genetic Plasticity underlying the high Adaptability of Emerging Viruses



**Most novel emerging infectious diseases are transmitted from animals to humans (zoonosis) and caused by RNA viruses. Since the RNA genomes of these viruses are replicating with high mutation rates, these viruses exhibit significant genetic diversity and can therefore adapt rapidly to changing environments. One of these emerging viruses is the tick-borne encephalitis virus (TBEV), which may cause central nervous system diseases. In order to better understand the adaptability (plasticity) of RNA viruses, TBEV isolates are being adapted to different environments and characterised by molecular biological and classical virological methods. This work is carried out in the framework of a Swiss National Science Foundation project.<sup>1</sup>**

Tick-borne encephalitis (TBE) is the most important viral disease of the central nervous system transmitted by ticks in Europe. In Switzerland, the tick species *Ixodes ricinus* is the vector for the causative agent of the disease, the tick-borne encephalitis virus (TBEV). In 70 % of cases, infections in humans are asymptomatic and thus remain undetected. In 30 % of the cases, flu-like symptoms develop 1–2 weeks after the tick bite; this disease stage lasts for 1–8 days and frequently ends with complete recovery. About one third of the symptomatic cases, however, proceed to a secondary disease stage involving an infection of the central nervous system. The symptoms of this infection of the meninges or brain include fever, headache, back and neck pain, un-

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Dr. Nicole Lenz*

<sup>1</sup> This research has been conducted in the context of a Sinergia project of the Swiss National Science Foundation under the title *Viral Plasticity Underlying Tropism and Pathogenesis/Innate Immune Evasion of Emerging Viruses* in collaboration with the Institute of Virology and Immunology, the Institute of Infectious Diseases at the University of Bern, as well as the Institute of Microbiology at the University of Lausanne.

Figure 1

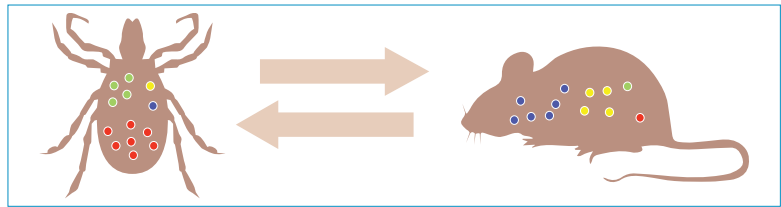
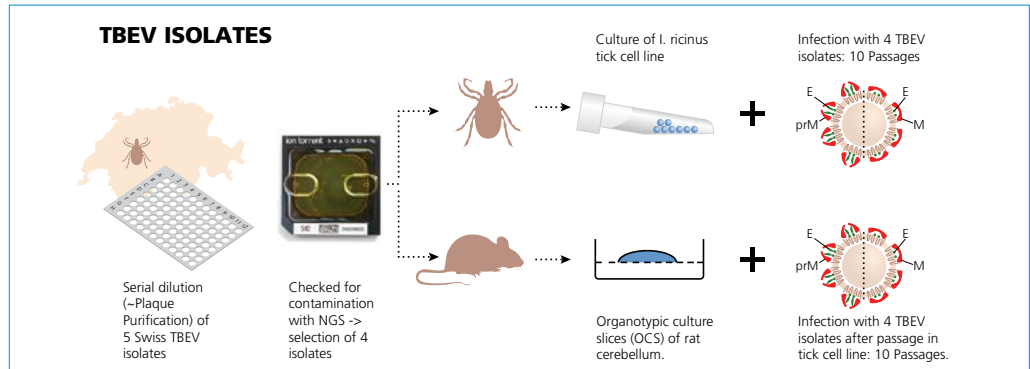


Figure 2



consciousness and paralyses. 3–11% of the patients suffer from neurological sequelae; approximately 1% of the cases are fatal. In Switzerland, a total of 100 to 250 cases of illness are reported every year [1, 2, 3].

TBEV belong to the group of RNA viruses; this group of viruses utilizes RNA (ribonucleic acid) to store their genetic information. In contrast to DNA (deoxyribonucleic acid), which is for example used for the storage of genetic information by plants and animals, the replication mechanism of RNA is susceptible to mutations (changes in the genetic sequence). Because of the high mutation rates, the replication process generates a large genetic diversity. This swarm of genetic variants is called the quasispecies population. The quasispecies population allows the virus to rapidly adapt to changing conditions, such as the transmission from the vector (*Ixodes ricinus*) to the host (human, rodent). The most fitting variant of the quasispecies population is selected and increasingly replicated, whilst less suitable variants are produced in smaller number only (figure 1)[4].

In the framework of a three-year research project in collaboration with the universities of Bern and Lausanne, the genetic adaptability of TBEV as a response to different host cells and under the influence of mediators of the immune system are being characterised using the newest molecular biological as well as classical virological methods. A better understanding of the plasticity of RNA viruses might contribute to estimating the risks of transmission to humans.

### Methods

Since 2009, Spiez Laboratory has organised five large-scale tick-screening studies.

Ticks were collected and investigated for the presence of different pathogens, including TBEV, using real-time polymerase chain reaction assays (real-time [RT] PCR). From PCR-positive tick samples, different pathogens could be isolated, including 65 TBEV isolates [5, 6]. Five of these isolates were selected for the current project. For studies on viral quasispecies populations, a clearly defined, uniform virus population must be used as starting material to allow for the identification of mutations having resulted as a consequence of the experimental setup. Therefore, at the beginning of the experiment, the five TBEV isolates were serially diluted to yield a virus stock ideally originating from one single virus. Because viruses isolated from ticks may be contaminated with other pathogens, these virus stocks were analysed using a Next Generation Sequencing (NGS) protocol developed at Spiez Laboratory. The sequence analyses showed that one isolate was contaminated with other pathogens. Consequently, it was not used for further experiments. The remaining four isolates were serially passaged ten times in embryonic *Ixodes ricinus* tick cells [7] to adapt them to conditions they would naturally find in their vector (*Ixodes ricinus*). Thereafter, the virus cultures were transferred to 400 µm thick tissue cultures of rat cerebellum – so-called organotypical cerebellum cultures (OCS) – and again serially passaged ten times. In this second serial passaging, isolates were adapted to conditions they would encounter in the human host. TOCS, containing all their cells in their original distribution and micro architecture, were used as surrogates for the human cerebellum tissue, which is the brain region most prominently affected by the disease. In order to study the effects of the innate immune system and the corresponding evasion mechanisms of TBEV



isolates, serial passages in OCS were conducted in parallel with Interferon treatment. This cytokine (regulatory protein) is considered to be a significant mediator of the innate immune response against viruses. The quasispecies population of TBEV isolates from passages zero, five and ten of the vector cells as well as host tissue are then characterised in detail using NGS, plaque test and immunofluorescence staining (figure 2).

## Results

Ten serial passages of four TBEV isolates in tick cells have been completed successfully. It is known that after prolonged incubation periods, viruses primarily accumulate within the tick cells whereas mainly defective virus particles are being released to the culture medium. However, since the consequences of this effect on the quasispecies population dynamics was not clear, serial passaging was conducted in two different ways, namely:

a) passaging virus material from homogenate (a mixture of cell culture supernatant and tick cells) and b) passaging cell culture supernatant only. In all passages, the virus titre was measured in plaque forming units (PFU) – 1 PFU roughly corresponds to one virus (figure 3). In addition, from passages zero, five and ten, immunofluorescence staining was performed, and the corresponding samples were prepared for NGS analyses. Although for each passage,  $10^5$  PFU were used for infection, viral titres fluctuated between  $10^4$  and  $2 \times 10^7$  PFU/ml. Immunofluorescence staining showed that TBEV accumulates in specific regions of the cell around the cell nucleus. This remained constant over ten passages.

The tick-adapted tenth passage is now used to continue serial passaging of the isolates in OCS. By November 2017, 5 such passages had been performed (figure 4).

## Conclusions

In this study, so far four TBEV isolates were successfully adapted to vector cells by serial passaging. Similar studies have concluded that the fluctuation in the virus titres is a typical behaviour of viral quasispecies populations and an indicator of viral plasticity [8]. The vector-adapted virus isolates are now being passaged ten times on organotypical rat cerebellum sections and thereby adapted to host cells. The analysis of the sequence of the quasispecies population using NGS allows identifying mutations that are essential for the vector-to-host transmission of highly pathogenic RNA viruses.

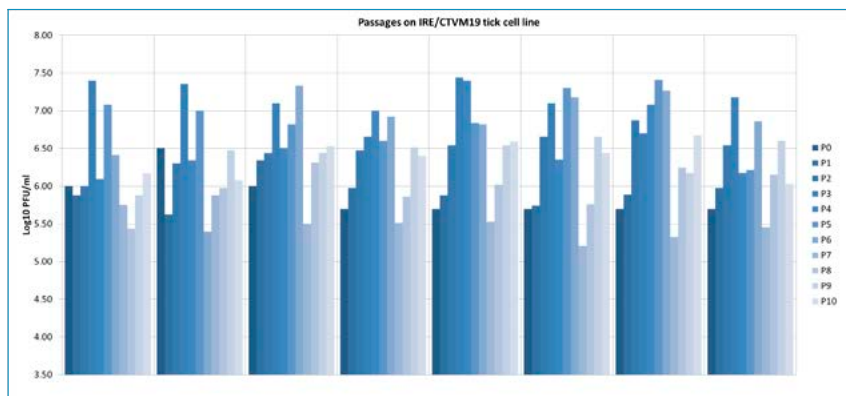


Figure 3

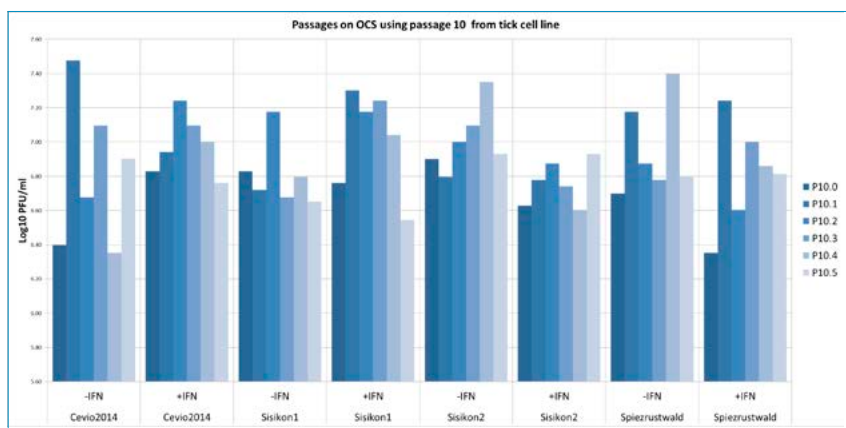


Figure 4

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UNSGM Designated Laboratories should include generalist and specialist laboratories to provide a rich mix of laboratory capabilities.



## Third UNSGM Designated Laboratories Workshop

*Dr. Cédric Invernizzi,  
Dr. Nadia Schürch,  
Dr. Beat Schmidt*

**The United Nations Secretary-General's Mechanism (UNSGM) is an important instrument for the international community to investigate allegations of use of chemical, biological or toxin weapons. Switzerland<sup>1</sup> started in 2015 an initiative aimed at strengthening the roster of designated laboratories that would support investigations of alleged use of biological and toxin weapons. The third workshop in this series took place from 20–22 June 2017 in Spiez.**

The objective of the UNSGM Designated Laboratories workshop series is the development of practical steps towards a functional network of trusted laboratories designated under the UNSGM to support investigations of alleged use of biological and toxin weapons. Building on previous workshop outcomes, participants discussed the value of a scoring system for laboratory methods and explored best practice approaches in a table-top exercise format. Three expert groups from the classical fields of virology, bacteriology, and toxinology started to develop a common understanding on adequate analysis and quality assurance criteria as well as defining a reasonable way forward in practical terms.

For any kind of UNSGM investigation that includes an analysis of samples by roster laboratories, the ultimate goal is the establishment of a clear sample provenance, a fully re-

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<sup>1</sup> Spiez Laboratory supported by the Division for Security Policy in the Federal Department of Foreign Affairs, and International Relations Defence in the Federal Department of Defence, Civil Protection and Sports

spected chain of custody, and a demonstrable technical competence for the analytical tasks required and performed, which would thereby reduce or eliminate the scope for political challenge. This is particularly relevant in the context of biological weapons investigations, given the absence of a dedicated and resourced international organisation. A collaborative network that provides confidence and trust in each laboratory's scientific competence and analytical skills as well as in its applied quality assurance systems is therefore of utmost importance.

In an investigation of alleged biological weapons use, isolation and cultivation of the causative agent may not always be achievable. To reach an acceptable level of confidence in laboratory results, analysis might therefore have to rely on the application of orthogonal complementary methods, which, in turn, would necessitate the assignment of values. Although such a scoring system on its own would likely not be suitable as independent proof of biological weapons use, it could, when applied in a flexible manner, still serve as a guidance tool for the unambiguous identification and deeper characterisation of a causative agent. In the end, such scored results contribute to the overall evidence a UNSGM mission would have at its disposal to conclude whether an incident was the result of deliberate use or a natural event.

To develop a functional network of trusted laboratories designated under the UNSGM, practical steps that take into account the demon-

stration of laboratory competence and the conduct of inter-laboratory calibrations, as set out in the UNSGM Guidelines and Procedures, are required. The three expert groups identified several key factors for acceptance of laboratory results, particularly in the political context of a UNSGM investigation. High standards in quality assurance were considered of prime importance and should include accreditations, the use of quality-assured reference standards and library data as well as appropriate controls. Participants recognised that an unambiguous identification of a causative agent in the setting of a UNSGM mission will depend on both context and mandate. Depending on the scenario, there would be merit in a UNSGM Designated Laboratory acting as hub laboratory and thus providing reach-back capability for the field team, especially when inherently complex technical questions related to sampling, laboratory analysis, and any processes in-between need to be answered. In order to tackle several of these aspects practical work is required, such as the conduct of confidence building exercises which was deemed conducive by the participants. Piggy-backing on existing external quality assurance exercise schemes should also be encouraged. This approach would allow for the development of appropriate reporting standards that, at the same time, need to remain adaptable to a given context and mandate.

Several factors that would need to be taken into account, in order to successfully move towards a trusted network of UNSGM Designated Laboratories, were highlighted. Inclu-



siveness, both in terms of scientific scope and geographical representation, was deemed key. The UNSGM roster of laboratories should include both generalist as well as specialised laboratories, in order to reflect a comprehensive mix of laboratory capabilities. Although a UNSGM investigation differs significantly from a public health/veterinary response to a disease outbreak, a close collaboration with other existing laboratory networks would still be desirable and add value. Not only would such a laboratory network act as a platform to share good practices, it would also function as a curator of generally accepted performance criteria based on validated and mutually accepted analytical methods, reference materials, and reference data. In the absence of adequate staffing and financial allocation in the regular budget of the UN Office of Disarmament Affairs, leadership and support for these technical aspects will eventually have to be provided by the laboratories themselves.

Workshop participants identified a number of initial practical steps of a “not yet” fully developed roadmap. These steps included preparation of a checklist containing the minimum requirements that laboratories should meet, continuation of the discussion on the value of a scoring system for laboratory methods and increased confidence through the conduct of external quality assurance exercises. Further steps would be the development of sample guidelines taking into account existing sample acceptance criteria of laboratories, the elaboration of a reach-back concept that would involve a UNSGM Designated Laboratory as hub laboratory, further discussions on the curation of reference databases and materials, development of training packages, as well as assessment of the nomination status to the UNSGM laboratory roster. Finally, through bolstering the efforts of the Organisation for the Prohibition of Chemical Weapons (OPCW) more support for the UNSGM could be achieved.

As a consequence, a number of activities at working level were offered as immediate follow-up steps. In October 2017 Germany organized a meeting in Berlin in order to further the discussions on the value of a scoring system for laboratory methods and on whether designated laboratories should meet minimum requirements. This was followed in November 2017 by a first confidence building exercise with clinical and environmental samples potentially containing inactivated bacteria. A second effort that will start in 2018 is geared to the conduct of yet another confidence building exercise in the form of dry lab tests that would use artificial virus sequencing data. In the field of toxins, the preparation of

an input paper on priority toxins is expected which could also serve as a basis for encouraging and empowering the OPCW to include other relevant toxins in future exercises. Finally, a web-based solution for sharing documents electronically amongst participants and serving as a repository of knowledge was set up.

Switzerland will host a fourth workshop in the second week of September 2018 that will take stock of the progress made in the intersessional period at working level. It will discuss next steps towards a robust quality assurance system for UNSGM Designated Laboratories in order to further develop them into a global trusted network.

*This text was adapted from the Executive Summary of the Third UNSGM Designated Laboratories Workshop report. The full report is available under*  
<https://labor-spiez.ch/en/rue/uno/index.htm>



Edmond Mulet (front left), head of the OPCW-UN Joint Investigative Mechanism (JIM) on the use of chemical weapons in the Syrian Arab Republic, briefs the Security Council. Judy Cheng-Hopkins (1<sup>st</sup> from left) and Stefan Mogl (2<sup>nd</sup> from left), Head of Chemistry division of Spiez Laboratory, were appointed by the UN as member of the JIM Leadership Panel 2017.

# Chemical Weapons Uses in Syria – The OPCW-UN Joint Investigative Mechanism



**On 16 November 2017, the mandate of the investigative body that had been set up for the UN Security Council to investigate the uses of chemical weapons in Syria and identify the perpetrators responsible for them expired. In two cases, the state of evidence was sufficient to attribute culpability. The Syrian regime as well as the Islamic State could be held responsible for particular chemical weapons attacks. Russia, however, used its veto in the UN Security Council to block a one-year extension of the mandate of the investigation team. As a consequence, there no longer remains a mechanism to determine those responsible or to bring to account those who have been identified as culpable.**

With its Resolution 2235, the Security Council created the investigative body named OPCW-UN Joint Investigative Mechanism, abbreviated JIM, and gave it the mandate over the period of one year to collect facts and evidence concerning cases of the use of chemical weapons in Syria that had already been confirmed by the OPCW. The leadership panel of the JIM, composed of three individuals, was asked to review the evidence in order to identify those responsible for the uses of chemical weapons and to name individuals involved in them, to the extent that the evidence so allowed.

*Stefan Mogl*

The JIM was the response of the Security Council to the reports by the OPCW, which beginning in 2014 had confirmed the use of chemical weapons in the Syrian armed conflict. The developments were of grave concern. After all, the international community had responded to the chemical weapons attack of

21 August 2013 in Ghouta and the horror images of people poisoned with Sarin: Syria had acceded to the Chemical Weapons Convention in October of the same year and had surrendered its chemical weapons programme to the control of the OPCW. The Syrian chemical weapons arsenal had been eliminated in a major international action; the most important chemicals had been moved out of the country and the production and storage facilities of the Syrian regime had been destroyed. For the time being, it seemed as if the danger of chemical weapons being used again after 2013 had been eliminated. This hope turned out to be wrong, and reports about attacks using chlorine gas – amongst others with the use of barrel bombs delivered from the air – grew in number, prompting the Security Council to take action and adopting resolution 2235.

#### **JIM 2015–16**

In the first report to the Security Council<sup>1</sup> (February 2016), the head of the JIM, Virginia Gamba of Argentina, confirmed that the JIM was operational since November 2015. Of the 116 reports about possible cases of chemical weapons use, the OPCW has investigated 29 cases by the beginning of 2016, and confirmed 23 of them as uses of chemical weapons. The JIM was not able to investigate all these cases in the time available, and made a selection from these many cases. In its third<sup>2</sup> and fourth<sup>3</sup> report, the JIM described nine cases of chemical weapons uses that it had investigated and that the OPCW had confirmed. In four of these cases, the evidence status was sufficient for the JIM to identify those responsible: for three attacks using chlorine gas, the Syrian air force was named as the responsible party, and for one attack using mustard agent the so-called Islamic State.

When the JIM mandate expired in the autumn of 2016, the Security Council initially was not able to agree on an extension. As a consequence, the JIM offices at the OPCW in The Hague were vacated and the contracts of employment ran out.

While the OPCW Fact-Finding Mission (FFM) continued to investigate and confirm uses of chemical weapons in Syria, the Security Council now no longer had at its disposal a mechanism to bring those responsible to account. This situation was unacceptable for many States. Behind the scenes, therefore, a new resolution was wrestled over and on 17 November, the Security Council with Resolution 2319 renewed the JIM mandate for one further year.

The UN Office for Disarmament Affairs (UNODA) and Virginia Gamba immediately made efforts to re-establish the JIM. In December 2016, UNODA contacted Switzerland and invited Stefan Mogl from Spiez Laboratory to take on the investigation sector in the three-headed leadership panel. The recruitment of new staff and the setting up of the new JIM organisation took several months thereafter. Only on 1 May was the JIM fully ready to start its work again, synchronised with a change in leadership. Edmond Mulet of Guatemala became the new head of the JIM in New York and Stefan Mogl took over the supervision of the investigative section of the JIM in The Hague. Judy Cheng Hopkins (Malaysia) completed the three-person leadership panel a few weeks after.

In the meantime, on 4 April 2017, a chemical weapons attack with Sarin – resulting in many victims – had taken place in the city of Khan Shaykhun north of Homs. The incident attracted huge media attention and the United States of America held the Syrian government responsible for the chemical weapons attack. On 7 April, the US bombed the air force base Al Shayrat southeast of Homs, from where according to the US the Sarin attack had been launched, using 59 cruise missiles. The magnitude of the chemical weapons incident in Khan Shaykhun and the military reaction of the US increased the political tensions in the Security Council as well as at the OPCW, creating a difficult political environment at the start of the work of the JIM.

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1 UN S/2016/142, 12 February 2016

2 UN S/2016/738, 24 August 2016

3 UN S/2016/888, 21 October 2016



### Methodology of work of the JIM 2017

A precondition for the JIM to start an investigation, in accordance with its mandate, was that the OPCW FFM had confirmed that a chemical weapons attack had taken place. Based on the report of the OPCW, the JIM developed a specific strategy to direct the investigation activities. In order to ensure that the search for those culpable remained open and independent from the very beginning, alternative scenarios were developed which might explain the course of events. In these scenarios, also all the scenarios about the alleged course of events that were communicated by the different conflict parties were included. The JIM's fact-finding department then was tasked to gather facts related to the different scenarios.

The JIM worked from two locations. In New York were the political and legal department, finances, administration and logistics; in The Hague departments were established for information gathering, for analysis and information validation, a translation service and an evidence and data archive.

For information gathering, the JIM used all sources accessible to it. It needed support to this end and contacted more than 30 UN Member States with specific questions. The JIM also worked with non-governmental organisations, interviewed a large number of eyewitnesses, and engaged with forensic institutes, subject matter experts, and the network of OPCW Designated Laboratories. The goal of investigation was to collect facts that could be evaluated and confirmed by the analysis department of the JIM and become evidence. The JIM was looking for evidence to confirm: date and time of the chemical weapons incident, the location of the release of the chemical warfare agent, the munitions type and the chemical composition of the agent, the method of dissemination, the medical consequences observed in exposed individuals, and the countermeasures taken by local relief forces where applicable.

### JIM Investigation 2017

In September 2016, an incident happened in the village of Umm Hawsh northeast of Aleppo, in an area that was under the control of rebel forces, which involved mustard agent – this was the first incident for which the JIM initiated an investigation in 2017. The house of a woman was hit by a mortar round. The grenade left behind an oily brown liquid. The house resident called a neighbour to help her wash off the liquid. Both women suffered severe mustard agent poisoning as a consequence, and were taken into medical care. In November, a specialised NBC unit of the Russian army arrived at the location. The unit secured a mortar shell filled with mustard agent at a roadside. In December and January, the OPCW FFM conducted a visit to Syria and received in this context samples that the Russian special unit had collected, and it investigated the mortar shell and collected blood samples from the two mustard agent victims. The JIM investigated the incident starting from the report of the OPCW FFM<sup>4</sup> and could demonstrate in its seventh report<sup>5</sup> that the so-called Islamic State had been responsible for the mustard gas attack in Umm Hawsh.

The second chemical weapons attack investigated by the JIM was the incident in Khan Shaykhun on 4 April 2017. As a result of the release of Sarin between 5.30 and 7.00 a.m., in accordance with the OPCW FFM approximately 100 persons died and 200 were injured. The official JIM investigation commenced immediately after the publication of the OPCW FFM report on Khan Shaykhun<sup>6</sup> at the end of June.

Shortly after the incident, different explanations about the course of events were already circulating in the media. Both the Syrian government and the rebels were blamed for the Sarin attack. There was intensive political commentary in the media about the work of the OPCW FFM as well as the JIM. In the context of the publication and discussion of the sixth JIM report<sup>7</sup> in the Security Council on

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4 OPCW S/1491/2017

5 UN S/2017/904

6 OPCW S/1510/2017

7 UN S/2017/552

7 July, the Head of the JIM therefore used his press conference to request that the JIM investigation not be unnecessarily politicised.

In the framework of its investigation activities of the incident in Khan Shaykhun, which lasted from the end of June to October 2017, the JIM was able to draw the following conclusions:

- On 4 April 2017, airplanes dropped munitions over Khan Shaykhun between 06.30 and 07.00, and a Syrian aircraft was near the air-space over Khan Shaykhun at this point in time;
- The crater from which the Sarin dispersed into the city was created by a bomb hitting the ground with high velocity on the morning of 4 April;
- A large number of people were exposed to Sarin between 06.30 and 07.00;
- The large number of victims and the identification of intact Sarin in samples, which allegedly were collected from the crater as late as 10 days after the attack, indicated that probably, a large amount of Sarin had been released. This is consistent with the dissemination of Sarin by means of a chemical weapons bomb;
- The symptoms of the victims, their medical treatment and the generally observed scale of the incident is consistent with what would be expected in a mass poisoning incident;
- The Sarin found in the samples from Khan Shaykhun had been manufactured, with a high probability, using a precursor chemical from the former Syrian chemical weapons stockpile;
- Certain inconsistencies that the JIM had observed and described in the Annex to its seventh report did not in its view invalidate the above conclusions.

Based on these findings, the JIM attributed responsibility for the release of Sarin in Khan Shaykhun on 4 April to the Syrian government, and presented these results in its seventh report on 7 November to the UN Security Council. Except for Bolivia and Russia, all members of the Security Council supported the JIM report. Despite several attempts to modify draft resolutions, a request for an extension of the JIM mandate beyond 16 November 2017 failed as a result of Russia's veto in the Security Council.

### **Postscript**

At the end of January 2018, Russia tabled a draft resolution in the Security Council that aimed at establishing a new investigative mechanism with reduced competences. This mechanism was to be set up under the name United Nations Independent Mechanism of Investigation (UNIMI). The US responded to the Russian text with an alternative proposal. During January and February 2018, news in the media appeared more frequently again about chemical weapons attacks in Syria, and the OPCW FFM has been conducting investigations to confirm or refute these allegations, within the capabilities of the organisation. For the time being, however, there is no mechanism to evaluate who bears responsibility of the chemical weapons uses, and to bring the already identified parties to account.



Decontamination training of the US army during an exercise in Yeoncheon, South Korea in 2013

# Decontamination of Chemical Warfare Agents



**Military incidents in the recent past and in particular the use of chemical weapons in Syria have shown that swift and effective decontamination of highly toxic substances is important in order to keep the damage to humans and the environment to a minimum. This is equally true for response organisations which potentially may come into contact with toxic chemicals. Spiez Laboratory has developed a small-scale method that allows determining the efficacy of a decontamination agent against chemical warfare agents.**

Decontamination is the process of removing or neutralising toxic chemicals from humans, materials or the environment. To achieve this, there are two types of decontamination: physical and chemical. The first includes all removal methods that do not involve a chemical reaction. Its goal is to remove the contamination from surfaces. This method utilises mechanical working principles such as absorption or rinsing, without destroying or decomposing the substance in the process. Chemical decontamination, on the other hand, includes all methods that detoxify a substance by means of a chemical reaction. These may be hydrolysis, elimination, oxidation or enzymatic reactions.

The ideal decontamination system would be a combination of physical and chemical processes, i.e. an efficient and universal surface cleaning combined with a fast decomposition of all types of contaminating chemicals,

*Dr. Anna Gerber,  
Fausto Guidetti,  
Michael Arnold,  
Dr. Christophe Curtly*



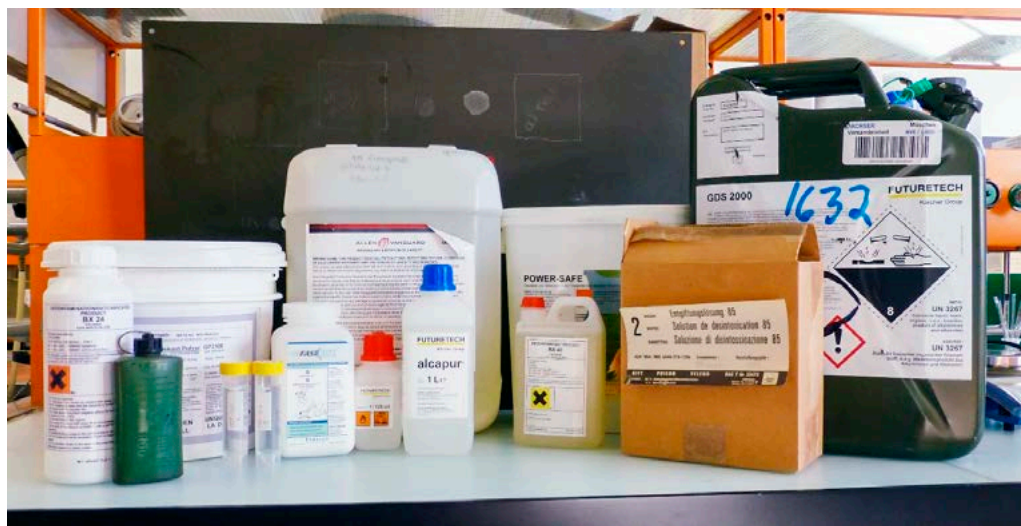


Figure 1: A selection of decontamination agents tested at Spiez Laboratory.

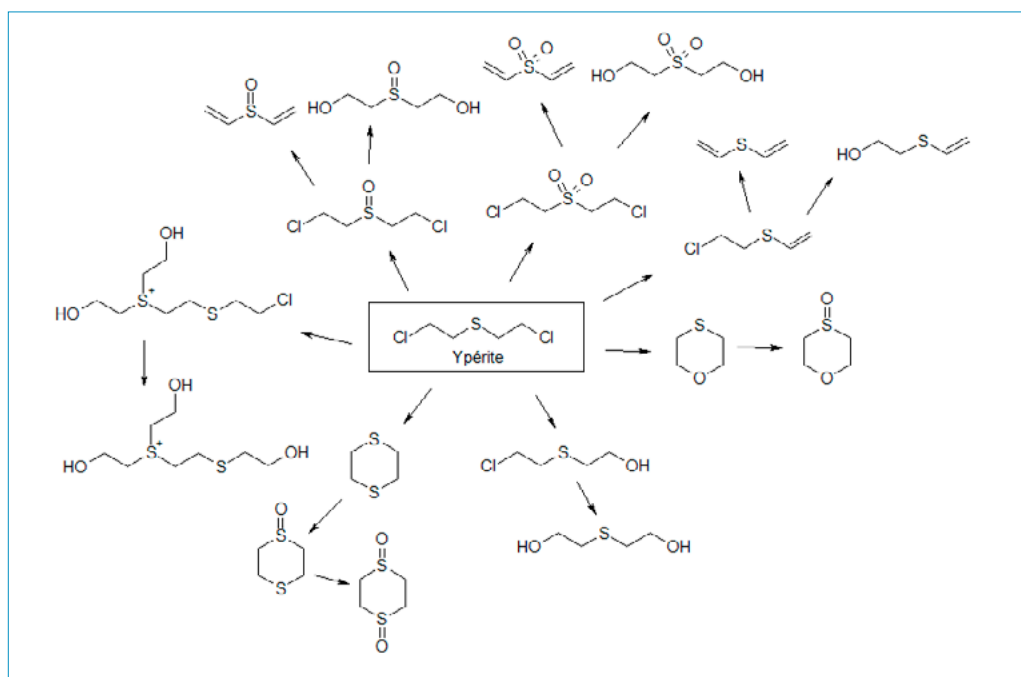


Figure 2: A selection of degradation products that may be formed in the decontamination of sulphur mustard.

and without endangering humans and the environment. Such a decontamination system remains a utopia. Several commercially available products are efficient in decomposing substances over short periods of time yet at the same time they damage material and – if insufficiently protected – also humans. Other decontaminants may be harmless for humans and the environment but take a long time to act, and react only with specific substances. Promising new technologies such as nanoparticles, photochemical and catalytic applications, or metal-organic compounds might contribute to achieving this goal in the future. Another trend would be a universal decontamination agent that can be used in a gentle manner against chemical as well as biological threats. This is part of contemporary research; some products with such properties are gradually appearing on the market. Their utility in the field however has yet to be examined.

Spiez Laboratory undertakes the testing of products that are available on the market with regard to their efficiency in the degradation of chemical warfare agents (Figure 1). There is a wide range of types and forms of decontamination agents but mostly, they can be categorised as powders, liquids and foams.

Powder decontamination agents are used to decontaminate small surface areas; they remove chemicals by physical and/or chemical processes. Foams and liquids find their application in the decontamination of large surface areas such as building facades or vehicles.

Decontamination agents differ not only in their consistency, but also in their chemical composition. Depending on the active ingredient of the particular decontamination agent, different degradation products are formed, which

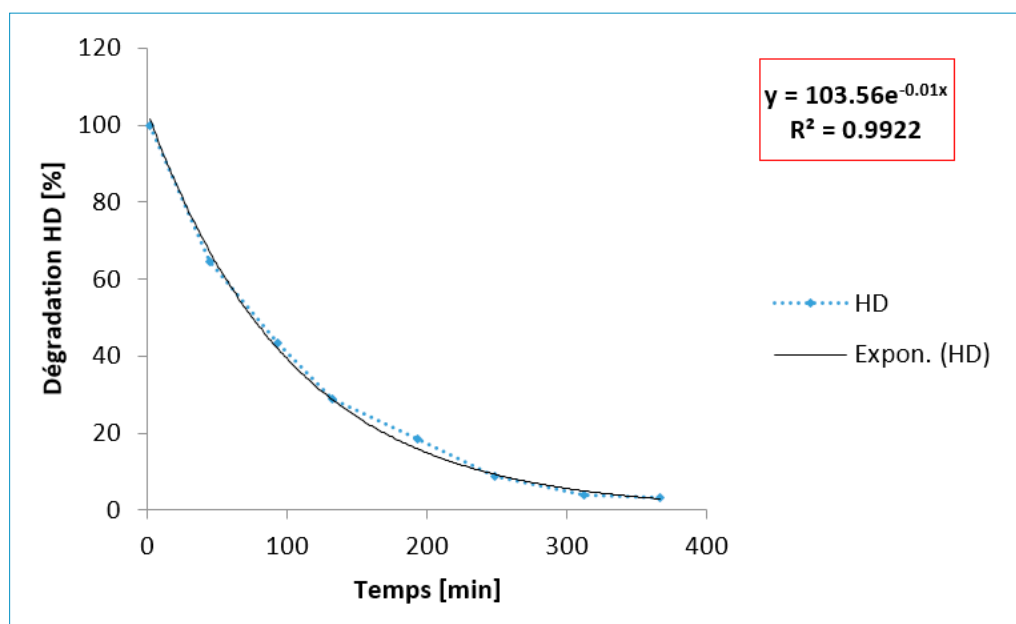


Figure 3: The degradation of sulfur mustard (HD) in a decontamination agent.

complicates the decontamination process. The improvement of the knowledge of detoxification reactions, and the characterisation of degradation products that could be potentially toxic, remain challenges. Figure 2 shows some possible degradation products that may be formed in the decontamination of sulfur mustard.

In the “Organic Chemistry, Detection, Decontamination Branch” we have developed a method that allows us to determine at small scale in the laboratory the efficiency of a decontamination agent against chemical warfare agents. The focus is on the speed of the degradation of the chemical agent, and in a subsequent step the degradation products, too, are being analysed.

In the experiment, the chemical warfare agent and the decontamination agent are mixed, and at discrete time intervals samples are taken and analysed using nuclear magnetic resonance spectroscopy (NMR) or gas chromatography coupled with mass spectrometry (GC-MS). The data allow determining the degradation kinetics (Figure 3).

Using these parameters, the efficiency of different decontamination agents for a particular substance can be compared and a suitable decontamination agent can be selected with respect to a particular substance or scenario.

In the case of the use of chemical warfare agents in urban areas there are further ques-

tions: Does one have to decontaminate all walls and floors or is it sufficient to air the rooms? How long does it take after contamination (and perhaps decontamination) until safe access to these rooms can be guaranteed? In order to find answers to these questions, Spiez Laboratory in collaboration with the Military Research Institute (MRI) in Brno (CZ) has been investigating since 2011 the interaction between chemical warfare agents in the gas phase and different construction and protective materials. In particular, experiments are being conducted to determine the amounts of the contaminating substance that penetrate into the materials, the amounts that will subsequently be released, and whether the materials had a decontaminating effect. Thanks to these tests we are better positioned to assess situations and to provide more precise information more quickly.

The tested materials were selected in relation to their abundance in Swiss buildings (sandstone, concrete, wood, etc.); the protective materials tested were those used by the Swiss Army as well as by Spiez Laboratory. As contaminating agent, the nerve agent Sarin was selected because it is both highly toxic and volatile. Experiments with other chemical warfare agents are under way.

Figure 4 shows the total amount of Sarin that is taken up by the materials (purple), and the residual amount of Sarin still found in the material after one hour (blue), and after 24 hours (orange). It is clearly visible that the

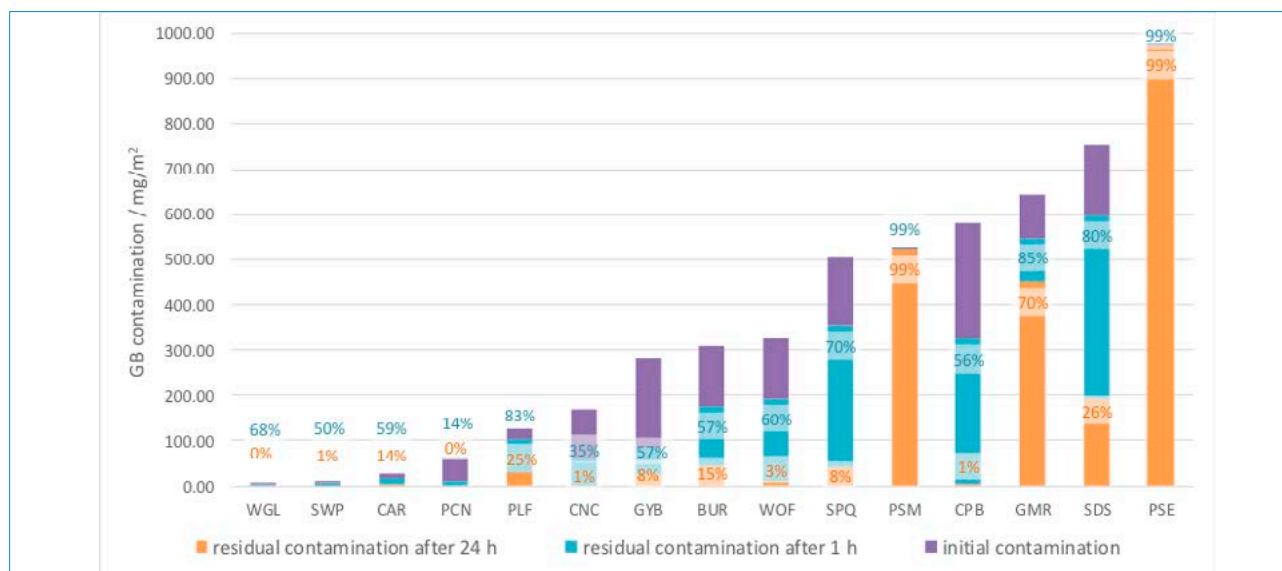


Figure 4: Materials contaminated with gaseous Sarin.

WGL: window glass, SWP: stone slab, CAR: paint resistant to chemicals, PCN: painted concrete, PLF: plastic floor, CNC: rough concrete, GYB: plaster, BUR: butyl rubber, WOF: lacquered wood, SPQ: sealed parquet floor, PSM: military protective suit, CPB: fitted carpet, GMR: respirator rubber, SDS: sandstone, PSE: C-EEVBS protective suit

more porous the material, the more Sarin it takes up. The two protective suits (PSM and PSE) take up large amounts of substance but these amounts are fixed in the fabric by activated carbon.

All information gathered from our decontamination projects, we can use internally, for example to equip our Chemical Safety Laboratory or the C-EEVBS<sup>1</sup> with new decontamination agents; on the other hand, it helps us to offer support to other institutions such as the Swiss Army. Currently, we are providing advice to armasuisse<sup>2</sup>, in the procurement of a new decontamination agent for the Swiss Army.

<sup>1</sup> C-EEVBS: the chemistry specialists of the EEVBS Emergency Response Team.

<sup>2</sup> armasuisse: the Federal Office for Defence Procurement within the Federal Department of Defence, Civil Protection and Sport (DDPS).





OPCW headquarters in The Hague, NL

## 20 Years OPCW



**On 29 April 2017, the Organisation for the Prohibition of Chemical Weapons (OPCW) celebrated the entry into force of the Chemical Weapons Convention and thus its twentieth anniversary. This convention is seen as the most effective disarmament agreement worldwide. 192 States or 98 per cent of the global population have ratified the treaty. The CWC prohibits the development, production, stockpiling and use of chemical weapons. Thanks to this regulatory framework, over the past 20 years an entire category of weapons of mass destruction has been eliminated almost completely under the supervision of the OPCW. For this achievement, the OPCW received the Nobel Peace Prize in 2013.**

The Chemical Weapons Convention (CWC) was opened for signature in Paris on 13 January 1993. Switzerland signed it already the next day, and about one year later, it deposited its instrument of ratification. The OPCW has the mandate to enforce the implementation of the CWC, including by conducting on-site inspections to ensure that no chemical substances are being secretly diverted for development or the production of chemical weapons.

To date, the specialists of the OPCW have verified the destruction of some 70 000 tonnes of chemical warfare agents declared by the possessor States Russia, USA, Albania, South Korea, India, Iraq, Libya and Syria. Thus, by April 2017 – 20 years after the establishment of the OPCW – all possessor states had eliminated their stockpiles except for Russia and the USA: Russia destroyed the last of its stocks in September 2017, and for the USA, the target date is 2023. In addition to these verification measures, the inspectors also have been training for the special case – an emergency which had not occurred in those 20 years – that a Member State was accused of

*Dr. Beat Schmidt*



OPCW-inspectors walking in the desert in Libya

working with chemical weapons (Challenge Inspection) or suspected of actually having used them (Investigation of Alleged Use). All these measures do not merely aim at ensuring the implementation of the CWC and building mutual confidence, but they act as deterrent to prevent states from aiming for new chemical weapons programmes.

An important incentive for developing and transition countries to join the CWC was the prospect that they would be able to reap benefits in the civilian uses of chemistry. An example is the Associate Programme, which has made possible for more than 400 young scientists from 118 countries to benefit from a three-month professional training in a modern chemical enterprise.

#### **Syria and the OPCW**

The most difficult period for the OPCW has been the repeated and at times massive uses of chemical weapons in the Syrian civil war. In August 2013, an attack with chemical weapons in a suburb of Damascus claimed 1400 human lives. The samples collected by the UN Investigation Mission – some of which were analysed by Spiez Laboratory – confirmed that the nerve agent Sarin was used. Consequently, under pressure from the USA and with mediation by Russia, Syria had to accede to the CWC. This resulted in a disarmament process without precedence, which lasted until January 2016 and which involved the destruction of 1300 tonnes of chemical warfare agents and precursor materials of the Syrian regime outside of Syria, with international help and under supervision of the OPCW. Simultaneously, under the most difficult security conditions, the OPCW supervised the destruction of

25 of the 27 Syrian CW production facilities. The Syrian declaration contained a number of inconsistencies, contradictions and gaps, and thus the completeness of the disarmament process could not be demonstrated. The OPCW undertook huge efforts to verify the Syrian declaration. Despite the fact that inspectors were at times subjected to severe accusations, they recorded these discrepancies in their reports. With similar determination, the OPCW collected facts regarding alleged cases of chemical weapons uses in Syria (and Iraq) and reported objectively about their findings. The massive attack on Khan Shaykhun on 4 April 2017, which resulted in almost 100 fatalities, has shown that this is not an easy task. The OPCW inspectors focus on the question which chemical weapons were used, not who conducted the attack. To this end, the OPCW collected and analysed information from all sources available. This professional and impartial attitude is recognised by all sides.

#### **Switzerland and the OPCW**

Switzerland enjoys an excellent reputation at the OPCW, given that it has supported the organisation significantly in many areas. In particular, Switzerland is promoting a strong verification regime, so that any violation of the Convention (Article I of the CWC) can be determined unequivocally. To this end, Spiez Laboratory has made available several thousand reference-data of chemical warfare agents and relevant chemical compounds to the OPCW, free of charge, and it has supplied reference chemicals. As a Designated Laboratory of the OPCW, the support of Spiez for several international missions, in particular in Syria, has been crucial. Apprentice OPCW inspectors



were given the chance to train in practice, by conducting trial inspections at the Schedule 1 facility. Every year, Spiez invites a scientist from a developing country for an internship that lasts several months. Furthermore, Switzerland offers international OPCW courses; the chemists trained in Spiez can transfer their acquired expertise to their home countries. Switzerland insists that the newest developments in science and technology are evaluated constantly to understand their impact on the disarmament treaties in time. In this regard, Switzerland has succeeded in placing its experts in the advisory bodies of the OPCW, such as Dr. Christophe Curty or Stefan Mogl of Spiez Laboratory in the 25-member Scientific Advisory Board of the OPCW.

### Switzerland and the Chemical Weapons Convention

From the very beginning, Switzerland has fully implemented the CWC and has constantly adapted its legislative basis, for example the Chemicals Control Ordinance<sup>1</sup> in October 2013. As a highly industrialised country with an

important chemical sector, Switzerland has declared 44 enterprises to the OPCW, of which 38 plant sites are viable to receive inspections (as of August 2017). Consequently, 94 OPCW inspections were conducted in Switzerland (that is approximately five per year).

The CWC obligates every State Party to establish a National Authority to implement the CWC. In Switzerland, the Department for Security Policy of the Federal Department of Foreign Affairs chairs this authority. Members of this body also include the State Secretariat for Economic Affairs, the International Relations Defence (IB V) and Spiez Laboratory. The National Authority maintains a website<sup>2</sup> with information for industry and trade. In international negotiations, Switzerland promotes in particular strong verification and the comprehensive implementation of the CWC by the States Parties.

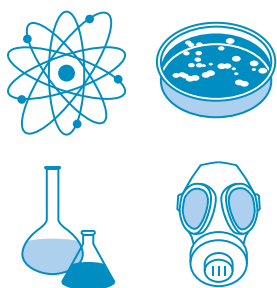
### History of chemical weapons – important milestones

1675	Treaty of Strasbourg	First international agreement on the limitation of the use of chemical weapons, in this case poisoned munitions
1874	Convention concerning the Laws and Customs of the Land	Prohibition of the use of poisons and poisoned weapons and of the use of weapons/projectiles that cause unnecessary suffering
1899/ 1907	The Hague Conventions on Land Warfare	Prohibition of the use of asphyxiating or poisoning gases
1915– 1918	1 <sup>st</sup> World War	90.000 fatalities caused by chemical weapons
1925	Geneva Protocol	Prohibition of the use of chemical weapons in war but no prohibition of their development
1930ies	China and Abyssinia	Uses of chemical weapons in China and Abyssinia
1972	Biological Weapons Convention	Comprehensive prohibition but no verification mechanism; obligation to continue negotiations on chemical weapons
1980ies	Iran Iraq War	Use of chemical weapons by Iraq against Iran and its own civilian population
1993	Chemical Weapons Convention	Opened for signature on 13 January 1993
1997	Establishment of the OPCW	29. April 2017 – The Chemical Weapons Convention enters into force and the OPCW commences its work
2007	10 <sup>th</sup> Anniversary of the CWC	182 States Parties, 2500 tonnes of chemical weapons destroyed
2013–	Syrian Civil War	Use of chemical weapons by belligerents, including against the civilian population
2013	Nobel Peace Prize	OPCW receives the Nobel Peace Prize for its efforts to eliminate chemical weapons worldwide.

1 SR 946.202.21

2 <http://www.labor-spiez.ch/de/the/cw/index.htm>





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## Collective Protection in Buildings

**The collective CBRE protection in buildings aims at the protection of persons in aboveground structures against the effects of chemical, biological, radiological and explosive substances (CBRE). Spiez Laboratory has been working out conceptual principles for the selection and evaluation of concrete protection measures to this end. The methodology was validated in practice, and in the case of four civilian buildings, Spiez Laboratory has undertaken the hazard and risk analyses. The methods for the evaluation of hazards and the assessment of risks can be applied successfully and result in practical recommendations for cost effective protective measures.**

Underground protective structures offer good protection against the effects of weapons. When needed, they will be occupied as a precautionary measure. Incidents as well as attacks by terrorists or extremists however, most of the time take place with no or only short advance warning. In such cases, moving into emergency shelters is often not possible. As a complement to the classical shelter construction there is therefore a need for appropriate and practical concepts for collective protection of persons in aboveground buildings.

The National Risk Analysis of the Federal Office for Civil Protection FOCP covers the hazards and risks caused by NBC disasters as well as natural hazards at the national level. With regard to CBRE collective protection in buildings, the focus is on the specific analysis and evaluation of protective measures for a particular building (figure 1).

### Hazard and risk analysis

To undertake hazard and risk analyses, reference scenarios are used that describe the possible CBRE hazards for persons in buildings. These scenarios are based on the *Hazard*

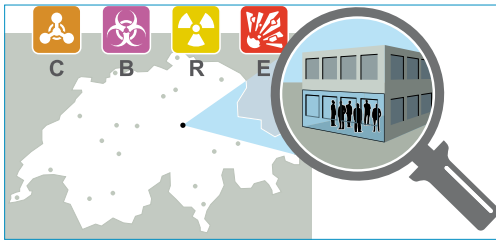


Figure 1: The focus of CBRE collective protection in buildings is on the individual assessment of buildings

*Catalogue and the Reference Scenarios CBRN* issued by the Federal Office of Civil Protection.

The hazards and risks for persons in buildings are determined based on these reference scenarios. The approach taken for the development of such object-specific and situational hazard and risk analyses is shown in figure 2.

The relevance of the reference scenarios is assessed using the object-specific *hazard analysis*. In case of scenarios assessed as not being relevant, no further analysis is required. Reference scenarios that have been assessed as relevant can be adapted with regard to the CBRE substances and their amounts considered. The plausibility and magnitude of the reference scenarios are assessed using object-specific *risk analysis*. Scenarios that lack plausibility can be discarded. The risks of possible incidents are situationally adapted by taking account of the structural characteristics, utilisation and operation of the building and the actual hazard potential present.

### Risks of the reference scenarios

The risks considered in the reference scenarios are set out in the conceptual principles. They have been developed using the methodology that was developed by the Federal Office for Civil Protection in the framework of the National Risk Analysis. Because these CBRE risks are individual risks related to single buildings, they are much smaller for most scenarios than those associated with disasters and emergencies in Switzerland.

In contrast to statistically recorded accident events, scenarios that have a terrorism or extremism background can be described only with difficulty using frequentist statistics. Therefore, for such scenarios subjective probabilities and the related frequencies are estimated, from which is derived the plausibility of the occurrence of the scenario. For the qualitative risk estimation, six classes with regard to both plausibility and damage are defined, as shown in figure 3:

As a semi-quantitative support for the estimation of the *plausibility* of the scenarios, the probabilities are given for the occurrence of a

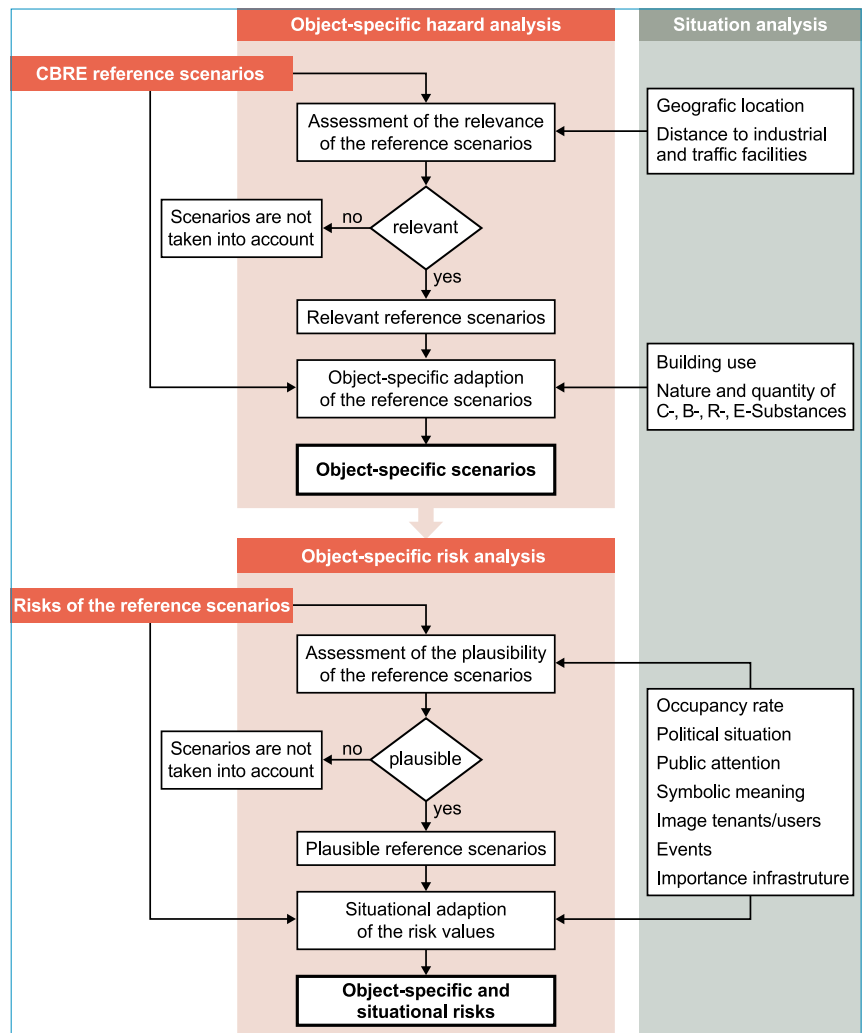


Figure 2: Approach to the object specific hazard and risk analysis

scenario related to a building over a period of 20 years, as well as the corresponding return period of the scenario.

The estimation of the *damage* is done primarily based on the expected personal and financial damage. The estimation of the financial losses takes into account the damage to property, consequential losses and reputational damage, as well as losses due to business interruptions.

The representation of the risks in matrix format with plausibility and damage enables a visualised comparison of different risks. Figure 4 shows the risks of the CBRE reference scenarios.

### Protective measures

Figure 5 provides an overview of the protective measures that can be adopted for CBRE collective protection in buildings:

The *design and arrangement* of buildings relates to the most advantageous array of sensitive building elements such as ventilation

Plausibility		Likelihood of the scenario within 20 years		Return period of the scenario
P5	Relatively plausible	likely	≥ 10%	< 200 years
P4	Rather implausible	relatively likely	≈ 5%	200 - 1'000 years
P3	Implausible	rather unlikely	≈ 1%	1'000 - 5'000 years
P2	Very implausible	unlikely	≈ 0.2%	5'000 - 20'000 years
P1	Extremely implausible	highly unlikely	≈ 0.05%	20'000 - 100'000 years
P0	Hardly imaginable	extremely unlikely	< 0.01%	> 100'000 years

Damage		Personal damage	Monetary damages incl. consequential and reputational damages
D0	Very low	No personal damage	< 100'000 CHF
D1	Low	1 - 20 Injured	100'000 - 750'000 CHF
D2	Medium	1 Fatality / 10 - 50 Injured	750'000 - 5 Mio CHF
D3	High	2 - 9 Fatalities / ≈ 100 Injured	5 Mio - 50 Mio CHF
D4	Very high	10 - 50 Fatalities / ≈ 500 Injured	50 Mio - 500 Mio CHF
D5	Disastrous	> 50 Fatalities / > 500 Injured	> 500 Mio CHF

Figure 3: Plausibility and damage classes of CBRE scenarios

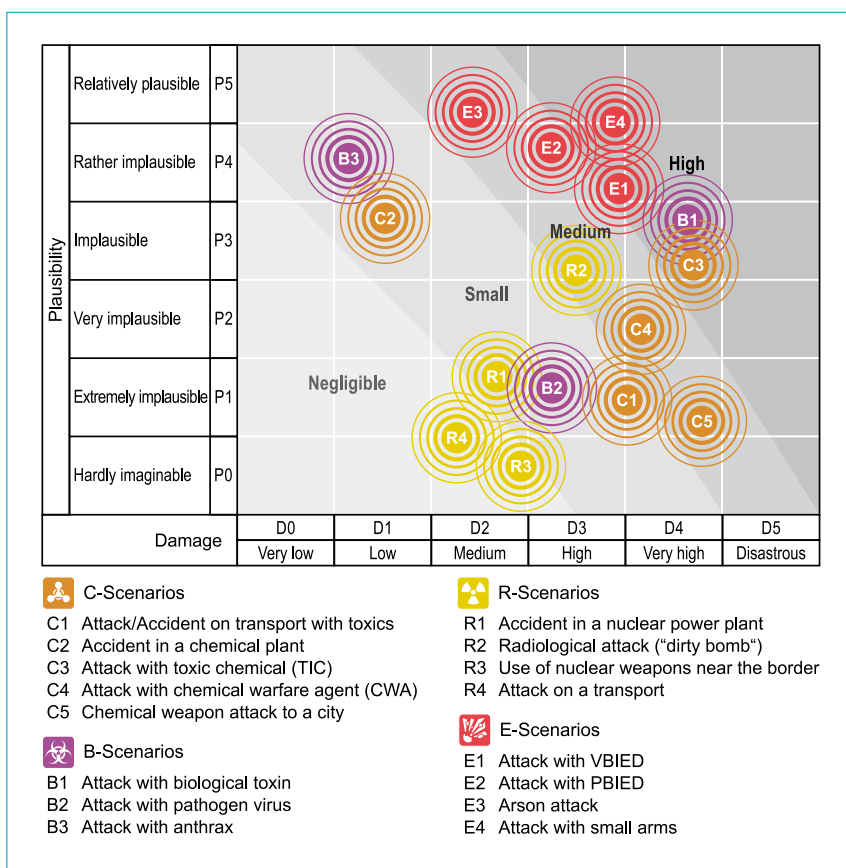


Figure 4: Risks of the CBRE reference scenarios

openings, the creation of stand-off distances or the construction of shelters.

With regard to the *building services*, hazards caused by toxic gases, aerosols and ionising radiation are relevant. Important in this context are the detection of hazardous substances, the processing of the detector signals and the use of filtering systems.

*Security* measures are technical/organisational measures such as controls, surveillance and guarding which prevent an incident. *Safety* measures include measures that reduce the impact of an incident. They include alert systems, evacuation as well as fire protection. *Construction measures* include amongst others perimeter protection. By limiting access to a building, incidents can be prevented. With a sufficient distance between perimeter and building, the effects of an incident (e.g. the impact of explosions) can be reduced. Construction measures and hardening that improve the robustness of buildings or structural building elements are typical examples for construction measures that reduce the consequences of incidents.

### Cost effective planning of measures

The conceptual principles of CBRE collective protection in buildings describe a risk-oriented approach for the assessment of protective measures that is based on marginal costs. In this approach, the expenses required for the measures are contrasted with their effectiveness. The relation between costs of the measures and achievable risk reduction quantifies their efficiency. For cost effective measures, the expenses for the protective measures are less than the costs incurred by the risks.

Protective measures reduce risks, which is associated with decreasing risk costs. An increased expenditure for protective measures does however lead to increases safety costs. Expenses for protective measures are at an optimum when the total costs of safety costs and risk costs are minimal (figure 6).

### Validation of the conceptual principles

The applicability of the principals for collective CBRE protection in buildings were validated in practice by Spiez Laboratory. To this end, the object-specific CBRE hazards and risks were evaluated for four different buildings and facilities. The buildings selected for this validation project differed profoundly with regard to size, geographical location, utilization and occupancy rate. The methods for hazard and risk analysis as well as the evaluation of the protective measures were applied to an office building, a bus depot of a public transport enterprise of a city, the datacentre of a bank, and



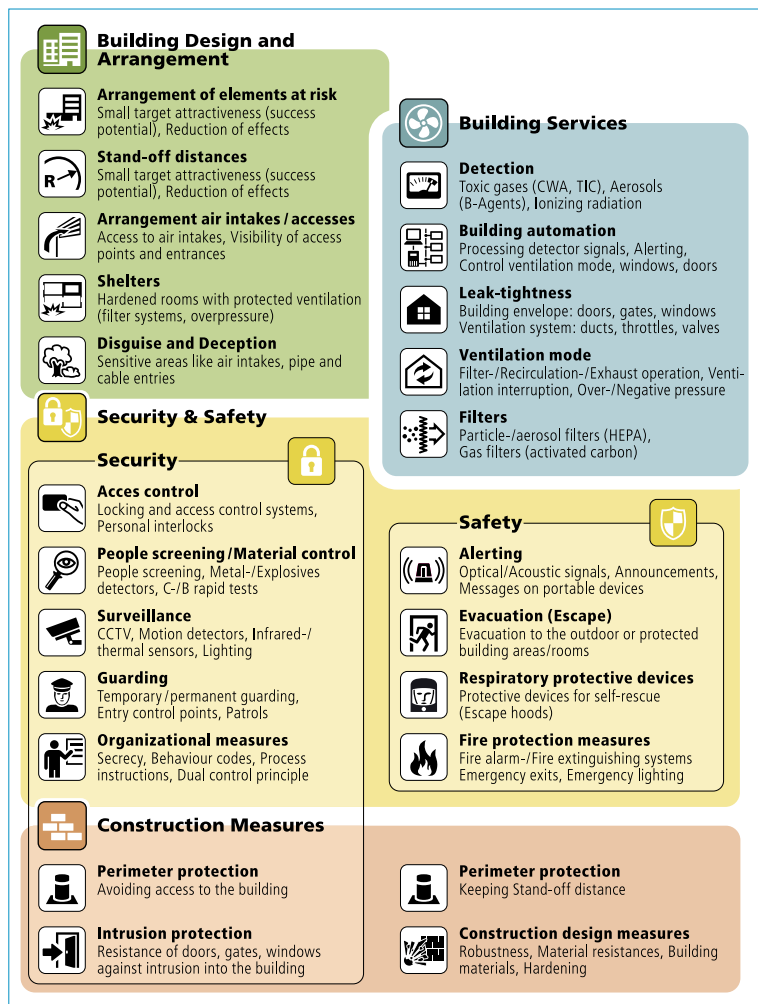


Figure 5: Technical areas and protective measures

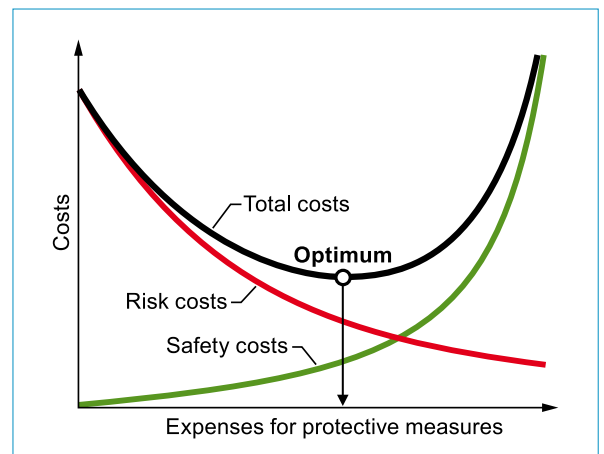


Figure 6: Optimum expenses for protective measures at minimum total cost (schematic)

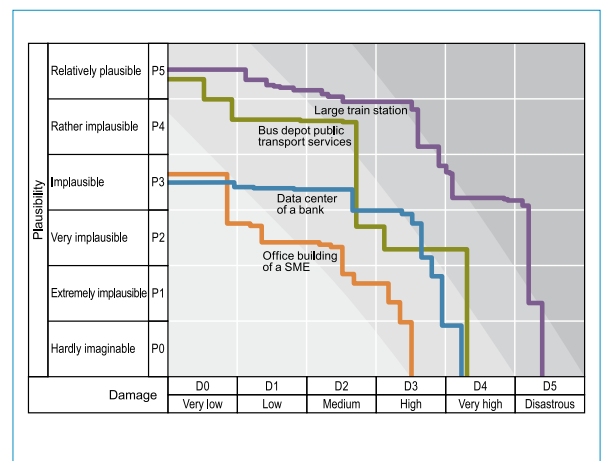


Figure 7: Risk profiles (cumulative curves) of the objects studied in the validation project

a large railway station. The analyses were conducted with the help of specialists who contributed their particular competencies to the resolution of the different problems. Stakeholders who were familiar with the building, security experts and facility managers as well as external risk experts and CBRE specialists participated in the expert groups (Delphi survey).

The results of the four risk analyses are shown in figure 7, in a comparative manner. As is common in safety engineering, the risk profiles of the buildings are shown as so-called cumulative curves. A comparison of the risks shows that the highest risks are present at the railway station whilst the lowest ones are found for the office building. The comparatively large risks at the railway station result from the large public exposure as well as the general vulnerabilities related to the operation of a railway station. For all buildings, the E scenarios (attacks with explosives or small arms) contribute the largest share of the overall risk whilst the risk contribution of C scenarios is generally small. The risks associated with an attack using a radiological bomb ("dirty

bomb") are significant for the railway station as well as the bus depot. The analysis of the datacentre yielded small risks because security and safety measures are already implemented and because the bank operates a redundant datacentre.

The validation of the conceptual principles for the CBRE collective protection in buildings was able to demonstrate that the methodology for the conduct of hazard and risk analyses can be used for very different objects and, consequently, that the evaluation of cost effective protection measures is possible.



Figure 2: Test material contaminated with  $4 \times 4 \mu\text{l}$  sulfur mustard



## Materials Testing for future NBC protective suits of the Army

Thomas Friedrich

**As part of the evaluation of the individual Next Generation NBC protective suit of the Swiss Army, Spiez Laboratory has undertaken a series of materials tests of material samples from five vendors. To get an overall picture of the performance of today's materials for protective suits, properties were measured such as breakthrough time for liquid chemical warfare agent, resistance against mechanical stress, as well as properties that affect the wearing comfort of the suits. These measurements supported the Federal Office for Defence Procurement in its pre-selection from the products in offer.**

In collaboration with the textile specialists of armasuisse, Spiez Laboratory has developed a test plan that covers the relevant materials properties. Subsequently, test specimens were punched out of the sample materials supplied by the vendors. *Figure 1* shows how the test specimens were removed. To this end, all 1650 pieces had to be punched out in exact alignment with the run of the thread, both in machine direction (thread) and cross direction (weft). This is necessary because woven textiles show different strength values in both directions as a result of different denier and thread tally.

One of the most important property of the tested materials is the breakthrough time for liquid chemical warfare agents. In Switzerland, only Spiez Laboratory is allowed to conduct these measurements. In this test,  $4 \times 4 \mu\text{l}$  pure sulphur mustard (HD) was applied to the outside of the sample materials (shown in *figure 2*). The breakthrough time was then measured as the time until the agent could be detected on the inside of the materials.

The resulting breakthrough times are shown in *figure 3* (normalised). The materials A through D provide a considerably longer protection as compared to products E and F. If one takes the standard deviations of the measurements into account, the large variation of the results of product A becomes apparent. This has been interpreted as indicating the inhomogeneity of that material, which could result in performance gaps in field uses.

Wearing comfort is another aspect of the selection of an NBC protective suit. The body temperature must not exceed a certain level to maintain a high operational capability. Material properties that affect the wearing comfort include the area density, the resistance against the dissipation of body heat and water vapour, as well as air permeability. *Figure 4* shows the normalised air permeability values measured by the Textile Laboratory of armasuisse. The reference material as well as products A through E are air permeable systems while product F is non-permeable for air. The air permeability values for the products A through E are higher than those of the reference material, which indicates a higher wearing comfort.

The resistance to mechanical stress, too, is an important factor. Tensile strength, ultimate elongation, abrasion resistance as well as tear resistance (the force needed to enlarge an already existing tear in the fabric) have been tested in destructive material testing. Given that the tear resistance is seen as more important than the other mechanical properties, the aging investigations were carried out using this property. The test set-up is shown in *figure 5*.

After procurement of new suits, these must remain fit for purpose even after many years in storage. In order to study the aging behaviour the materials were subjected to an accelerated aging by warm air storage at 80 degrees celsius. *Figure 6* shows the arrangement of the test specimens in the heating cabinet.

The aging method aims at testing the thermally induced oxidation of the synthetic fibres and the consequential decrease in mechanical strength. The test conditions are aggravated in comparison to real-life conditions, which provides valuable evidence regarding the long-term stability of the materials.

*Figure 7* shows the changes in the tear resistance of the C-protective layer (weaker direction) depending on the simulated age. In new conditions, product A has the highest tear resistance, which also decreases the fastest with age compared to the other products. However, the overall level remains higher than with the other products. Product F has

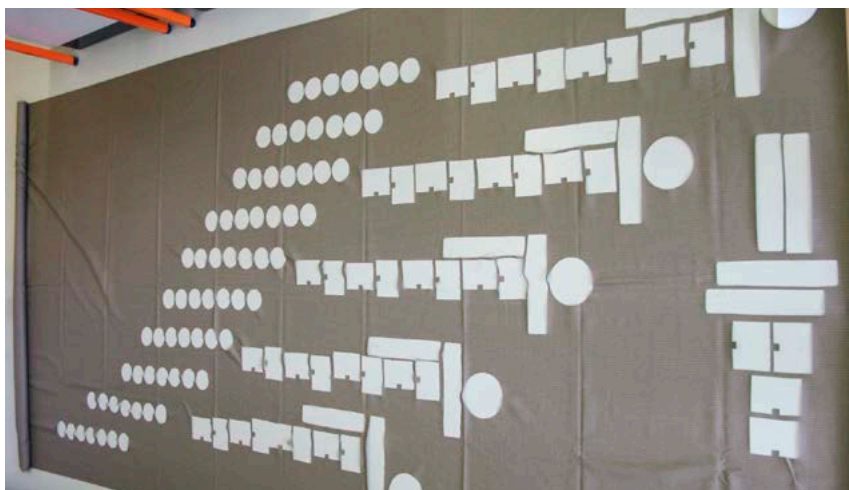


Figure 1: Punching out standard test specimens

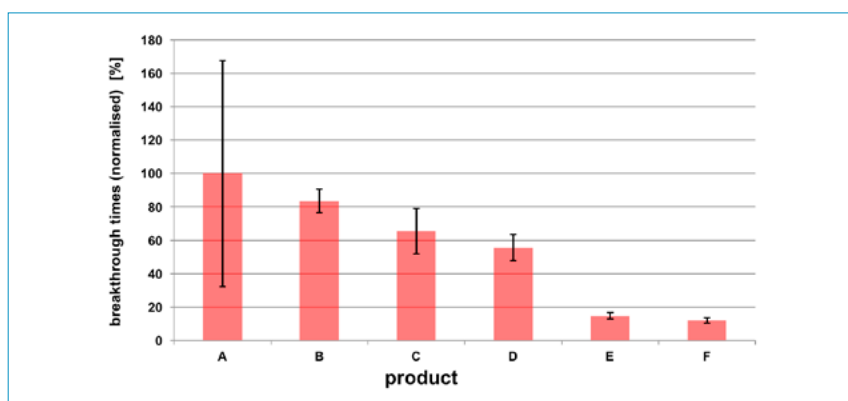


Figure 3: Breakthrough times

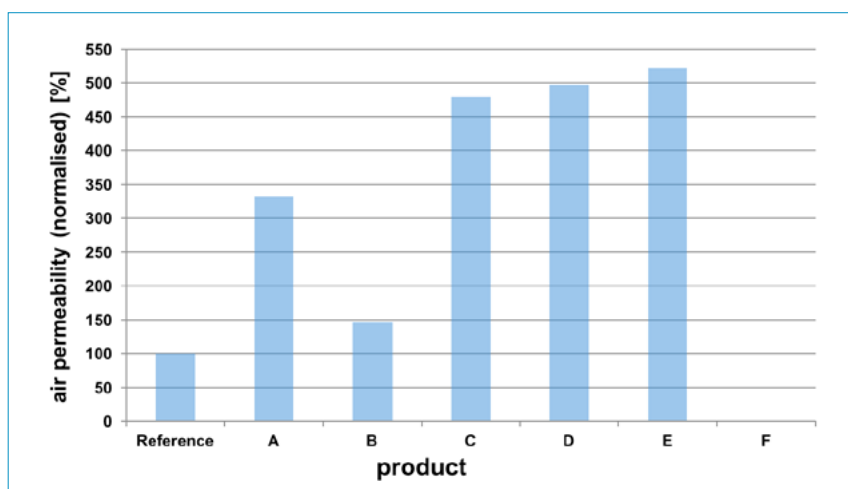


Figure 4: Air permeability data





Figure 5: Tear resistance tests



Figure 6: Accelerated artificial aging

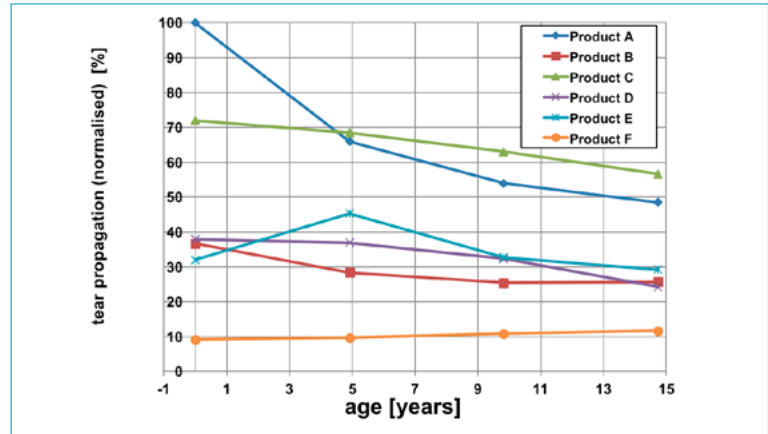


Figure 7: Changes in tear resistance by aging

the lowest tear resistance, and it changes little with age.

### Summary

The results of the material tests lead to the conclusion that the use of the available materials in chemical protective suits would result in a higher wearing comfort if compared to a reference material, because they are lighter in weight, thinner, and show higher air permeability. However, an extended protection time in case of contamination with liquid C agent (mustard agent, HD) cannot be expected. Subsequently to the materials tests using material samples, whole suits will be tested in an integral manner, that is to say the protective performance of the entire system will be tested.

The comprehensive testing capabilities of the Materials Testing Branch of the Spiez Laboratory are offered globally as services for clients from private industry as well as other interested institutions and organisations.

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Figure 1: Left: MIST Chamber with wind simulation and moving mannequin at Spiez Laboratory, right: location of the 33 samplers.

# New application for the evaluation of protective equipment against CBRN threats



**A decade ago, the Incident Response and Individual Protection Branch of Spiez Laboratory built a testing chamber for the simulation of contaminated field conditions to evaluate the efficiency of CBRN personal protective equipment (PPE). Since then, over a hundred tests have been performed. A pioneering passive sampling method using silicone-based SBSE (Stir Bar Sorptive Extraction) was developed, allowing for the local monitoring of skin exposure under the PPE.**

Dermal absorption is one of the major routes of entry for toxic substances into the body. Therefore, the performance of full protective ensembles (that include a suit with head-piece, boots, gloves and gas mask or respiratory unit) against chemical gases and vapors as well as biological agents must be carefully evaluated and quantified.

The integral testing of the performance of CBRN protective ensembles consists of a mannequin or a test person wearing the PPE following a specific exercise protocol during a few hours in a contaminated chamber. The concentration of the contaminant, either a gas or an aerosol, is precisely monitored and controlled. A set of ventilators simulates wind speed. This method, based on the so-called MIST (Man-in-simulation test) program launched about 3 decades ago in the US [1], was first implemented during the early 2000s at Spiez Laboratory.

*Dr. César Metzger  
Dr. Gilles Richner*

### **Skin sensitivity and local effect**

Inhomogeneous skin sensitivity and dermal permeability toward toxic agents over the entire body surface require the performance of CBRN ensembles to be assessed both globally as a system as well as locally for any given part of the body. Local protection factors, PFs, are defined as the ratio between external dosage (e.g. dosage of an unprotected body) and the local inside dosages. In the case of blister agents, the local physiological protection dosage factors are given by weighing the local PFs against the local sensitivity of the skin. For nerve agents, a systemic physiological protection dosage factor (or overall protection factor) is given by the geometric mean of local PFs, weighted by the corresponding local dermal permeability. It is therefore crucial to measure the chemical exposure locally but over the entire body surface. The realistic monitoring of chemical and biological exposure at various skin sites is one major challenge of the MIST program.

### **Measuring exposition dose**

Several techniques are available to monitor the exposure of workers to toxic gas in contaminated environments. One can distinguish real-time monitoring from deferred analysis (when the exposure dose is determined after the exposition), as well as passive from active sampling. For the latter, gas is actively pumped into a detector. Most of these methods are, however, not suited for the purpose of the MIST program as they are either destructive (pumping systems change the local concentration of contaminant), not sensitive enough (such as passive dosimeters), or too invasive (e.g. bulky portable electronic monitoring system). The Natick RDEC Center (USA) developed passive samplers [1] for the MIST program to adsorb specifically Methyl salicylate, a well-established simulant for chemical warfare agents. The so-called Natick samplers are small flat bags, about 5 cm<sup>2</sup>, filled with a precise amount of Tenax® powder (Buchem B.V., Netherlands), a common chemical adsorbent. The bags are then attached to the skin of the test person. After exposition, the contaminated Tenax® powder is removed from the bags. The contaminants are extracted with a solvent and finally quantitatively analyzed by chromatography.

Protection factors against biological agents are typically determined with simulated solid or liquid aerosol contamination of various size and composition. A classical method of esti-

imating skin exposure to solid aerosols is the fluorometric analysis with fluorescent-tagged silica powder. The skin regions exposed to aerosol are subsequently revealed under UV illumination.

Fluorometric analysis allows for fast qualitative assessment of the aerosol deposition pattern on the skin and the visual evidence is easy to convey to an unversed public. However, to provide quantitative results, sample skin locations are washed with swaps and further analysed [2].

Aerosol spectrometers and particle counters, such as Portacount® (TSI Inc., U.S.), are common devices for quantitative testing of aerosol filters and fit-testing of protective masks, respectively. Both methods are, however, not suited for ensemble testing, as these are too intrusive and have technical limitations for multiple sampling points.

### **Novel analytical method with Twister®**

After a few initial tests in the early 2000s with Natick samplers, Spiez Laboratory quickly noticed several drawbacks of this labor intensive and time-consuming analysis, and set out to develop a new sampling technique using silicone as absorptive material. The commercially available silicone-coated extraction bars Twister® (Gertsel, Germany), were then evaluated and showed promising early results.

Professor P. J. Sandra invented the Stir Bar Sorptive Extraction (SBSE) technique, patented under the name Twister®, during the 1990s in Belgium [3]. The aim of the technique was to extract organic compounds from aqueous matrix and it is now well-established in the chromatography community. Twister® are up to 20 mm long magnetic stir bars with a 0.5 to 1 mm thick silicone coating. The chemical analysis is easily performed by automated thermal desorption followed by gas chromatography, requiring minimal labor time.

In the method developed by Spiez Laboratory, 33 Twister® are maintained in carefully chosen locations by a tight-fit cotton suit worn directly on the naked skin of the test person. Therefore, Twister® absorb contaminants realistically at the very location where the effect of exposure would take place. Importantly, the change of humidity and temperature, due to perspiration and body heat generation at the sampling location barely affects Twister® sorption behavior. With the appropriate calibration





Figure 2 right: Twister® and left: Thermal desorption unit coupled with GC-MS at Spiez Laboratory

procedure, the local protection factors are determined over a scale up to four orders of magnitude, allowing the assessment of all types of gas protection suits. The method, initially designed for measuring exposure to Methyl salicylate, also shows good performance with the liquid aerosol Di-Ethyl-Hexyl-Sebacat (DEHS). Currently, a new thermal desorption method for DEHS is being investigated in Spiez.

This unconventional application of silicone-coated bars by Spiez Laboratory for the assessment of personal protective ensembles against gaseous and aerosol challenges shows great versatility and many advantages such as:

- An easy-to-handle, passive sampling methodology
- A highly automatised analytic system
- A unique sampling methodology for both gas and liquid aerosol challenges with mannequin or test person

This method recently received additional recognition outside of the CBRN community. Indeed, Spiez Laboratory was invited to present this innovative sampling method at the international SBSE Technical Meeting in September 2017 in Paris, in the presence of topmost SBSE-experts and the SBSE-inventors themselves. CBRN laboratories throughout the world also have shown interest in the implementation of this method in their own testing facilities. Interestingly, nowadays, other analytical laboratories use Twister® for air sampling of organic compounds.

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Tiffany Portmann  
Vanina Prumatt  
Eileen Trenkler  
Julian Remund  
Carole Schärer

## MASTERSTUDIES

Tim Gelmi  
Andreas Wenger

## UNIVERSITY GRADUATES INTERNSHIP

Guy Doerfel  
Nicolas Sambiagio

## DOCTORATES

Joyce Akello  
Andreas Biemann  
Stephen Jenkinson  
Nicole Liechti  
Samuel Lüdin  
Corinne Oechslin

## POST-DOCTORATES

Dr. Nicole Lenz

## FELLOWSHIP IAEA

Manjola Shyti  
Miha Trdin

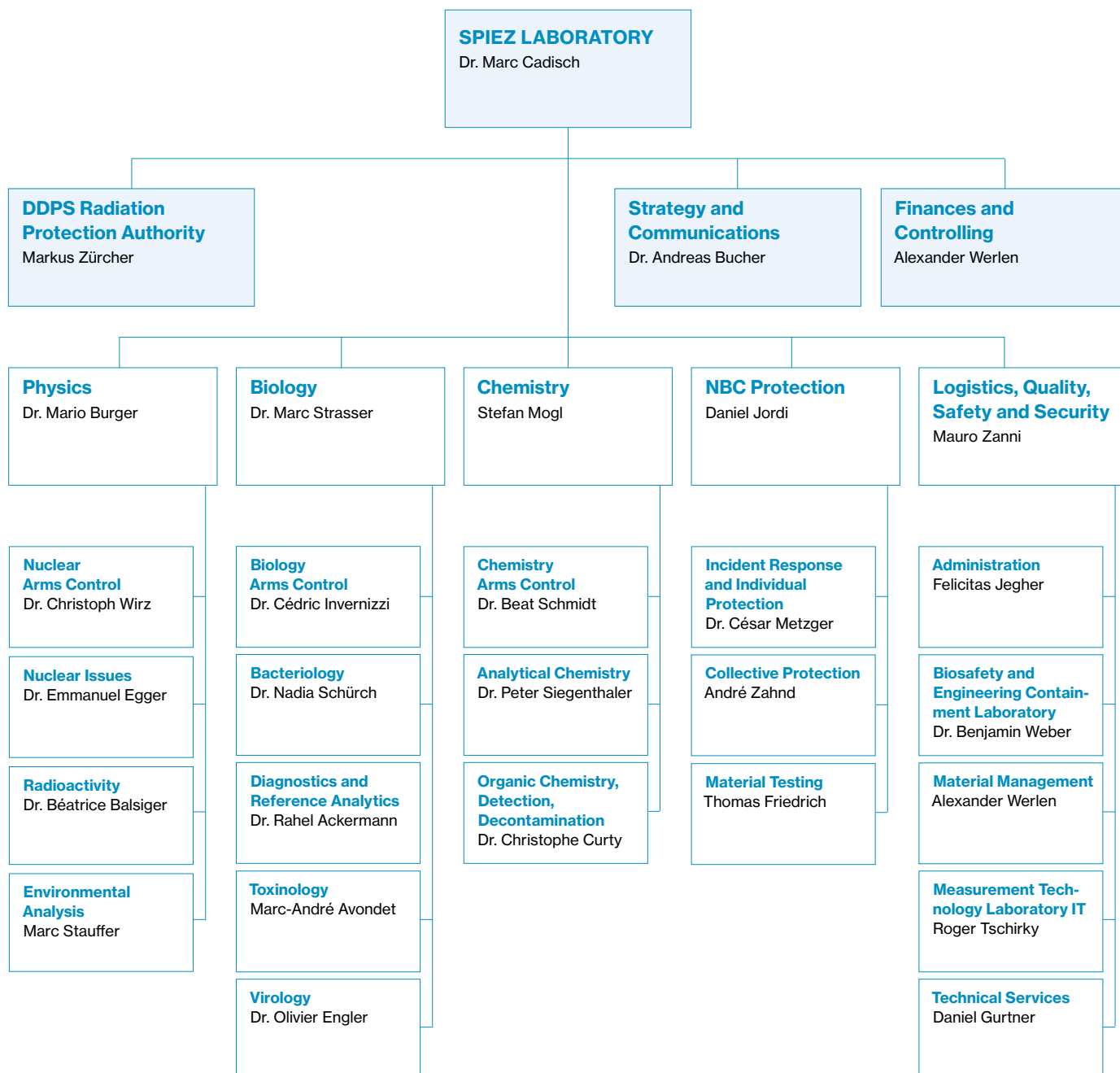
## INTERNSHIP OPCW

Thierry Mawete Dani

## Notes

<sup>1)</sup> Deputy Director SPIEZ LABORATORY

# Organisation



Status:  
2017



# Accredited Activities

## ISO/IEC 17025 accredited laboratories

STS 0019	Testing laboratory for the analysis of samples for chemical warfare agents and related compounds
STS 0022	Testing laboratory for adsorbents and respiratory protection filters
STS 0028	Testing laboratory for the determination of radionuclide concentration
STS 0036	Testing laboratory for polymers and rubber, and for the protection performance of polymers, rubber and textiles against chemical warfare agents
STS 0054	Testing laboratory for the detection of biological agents
STS 0055	Testing laboratory for NBC protection material, shelter equipment and shelter installations
STS 0101	Testing laboratory for the determination of main and trace elements and selected air-pollutants

## Round Robin tests October 2016–September 2017

Accredited laboratory	Number	Type and partner
<b>STS 0019</b> Chemical analysis/verification	0	Due to successfully concluded OPCW analysis assignments, Spiez Laboratory was released from participation in the proficiency tests and has been able to ensure the OPCW designation for another year.
<b>STS 0022</b> Adsorbents and respiratory protection filters	1	Aerosol retention rate of HEPA filters, WIS Munster
<b>STS 0028</b> Radionuclides	8	<ul style="list-style-type: none"> <li>– PT IAEA TEL 2017–04 ALMERA</li> <li>– IAEA Pu reference material: radiochemistry, ICP-MS</li> <li>– IAEA ConvEx-3: Gamma</li> <li>– IRSN: Tc-99: radiochemistry, ICP-MS</li> <li>– NMRoRo: U/Pu with ICP-MS-MC</li> <li>– IRA/FOPH: gamma in water</li> <li>– IRA/FOPH: gamma in pulver</li> <li>– IRA/FOPH: tritium in water</li> </ul>
<b>STS 0036</b> Polymers and rubber	5	DRRR – German reference office for proficiency testing and reference materials
<b>STS 0054</b> Biological toxins	2	<ul style="list-style-type: none"> <li>– Ricin exercise OPCW 01.2017</li> <li>– Comparative measurements (LS/RKI)</li> </ul>
Medical biochemistry	0	
Diagnostics of bacteria – drinking water	5	Public Health England
Diagnostics of bacteria – molecular biology	3	<ul style="list-style-type: none"> <li>– INSTAND Round Robin 11.2016</li> <li>– EMERGE Round Robin 06.2016</li> <li>– INSTAND Round Robin 06.2017</li> </ul>
Diagnostics of viruses – molecular biology	2	<ul style="list-style-type: none"> <li>– INSTAND Round Robin DENV PCR 09.2017</li> <li>– INSTAND Round Robin WNV PCR 09.2017</li> </ul>
Diagnostics of viruses – serology	2	<ul style="list-style-type: none"> <li>– INSTAND Round Robin FSMEV Serology 11.2016</li> <li>– INSTAND Round Robin FSMEV Serology 06.2017</li> </ul>
<b>STS 0055</b> Ventilation	0	
Air blast effects	0	
Ground shock effects	0	
<b>STS 0101</b> Main and trace elements	3	Ielab Alicante
	1	ISE Wageningen
Air pollutants	0	

# Presentations

Our scientists attend and actively contribute to conferences and offer their input at training courses dealing with NBC protection issues. Below are some of the presentations, given by our specialists during 2017

Date	Subject
24.01.2017	Dr. César Metzger: Impulsvortrag Nationaler ABC-Schutz – 10 Jahre später, Eidgenössische Kommission für ABC Schutz, Bern
03.02.2017	Dr. Cédric Invernizzi: Misuse of biological research: do we need to be concerned? LS2 Annual Meeting – Parallel Symposia IV: SCNAT Forum for Genetic Research, Zurich
07.02.2017	Dr. Marc Cadisch: National Laboratory Systems, Geneva Centre for Security Policy, Geneva
08.02.2017	Dr. José Corcho: Radiochemical separation of cesium and measurement by other techniques, IAEA Workshop and Proficiency Test on determination of low activity radiocaesium in freshwater, Vienna, AT
21.03.2017	Dr. Anna Gerber: Chemical and Biological Weapons: Current and Future Challenges, 8 <sup>th</sup> SISPAT, Singapore, SG
22.03.2017	Dr. Cédric Invernizzi: Session on CBRN Verification and Forensics, 8 <sup>th</sup> SISPAT, Singapore, SG
28.04.2017	Markus Zürcher: Radonmessungen ALC-M, Kaderrapport 01/17 ALC-M, Andermatt
03.05.2017	Dr. Cédric Invernizzi: DEFTECH-Event zu CRISPR-Cas9, armasuisse W+T, Thun
09.05.2017	Dr. César Metzger: Investigations avec risques chimiques, biologiques et radiologiques, Institut Suisse de Police & Université de Lausanne, Lausanne
23.05.2017	Dr. Peter Siegenthaler: OPCW BioPT-2: Spiez Laboratory Findings, Auswertemeeting 2 <sup>nd</sup> OPCW Biomedical Proficiency Test, The Hague, NL
15.06.2017	Dr. Benjamin Weber: Peracetic Acid (PAA) as a Decontaminant and Its Use in a Chemical Shower, IVBW18, Santo Antônio, BR
26.06.2017	Dr. José Corcho: Sampling techniques for water and sediment, IAEA Regional Workshop on Sampling Procedures for Water and Sediment Sample, Kozloduy, BG
27.06.2017	Dr. José Corcho: Enrichment methods, IAEA Regional Workshop on Sampling Procedures for Water and Sediment Sample, Kozloduy, BG
28.08.2017	Andreas Biemann: Synthetic Approach to Protein S Organophosphorus Chemical Warfare Agent Bioadducts, Swiss Summer School 2017, Villars sur Ollon
18.09.2017	Dr. Gilles Richner: SBSE applied to evaluate personal protective equipment against NBC threats, 4th Stir Bar Sorptive Extraction Technical Meeting, Paris, F
19.09.2017	Daniel Jordi: Prüfung Persönlicher Schutzausrüstung und Konzeptioneller CBRNe Schutz von Hochbauten, 13. Europäischer Katastrophenschutzkongress, Berlin, D
04.10.2017	Dr. Cédric Invernizzi: One Study, Two Paths: The Dual-Use Dilemma in the Life Sciences, ETH Zurich, Zurich
17.10.2017	Andreas Schorer: Einsatz des Agilent 7200 GC/Q-TOF in der Analyse von Chemischen Kampfstoffen, Agilent Technologies in Waldbronn, DE
19.10.2017	Dr. Marc Cadisch: Aktuelles aus dem Labor Spiez, 14. Bevölkerungsschutzkonferenz 2017, Basel
19.10.2017	Tim Johan Gelmi: Comparison of different methylation methods for acids related to the Chemical Weapons Convention (CWC) in diverse matrices with/without background, École des sciences criminelles, Université de Lausanne, Lausanne
25.10.2017	Dr. Cédric Invernizzi: UNSGM Designated Laboratories Workshop, UNSGM Workshop, Berlin, DE
25.10.2017	Dr. César Metzger: Neue Herausforderungen im ABC-Schutz, 2. Informationsanlass SVS, Bern
06.11.2017	Dr. Peter Siegenthaler: Aufgaben und analytische Möglichkeiten des Verifikationslabors für C-Kampfstoffe im LABOR SPIEZ, GERSTEL GmbH in Mülheim, DE
16.11.2017	Dr. Cédric Invernizzi: Dual-Use, Briefing for BSOs of the University Hospital Zurich, Zurich
28.11.2017	Dr. José Corcho: Environmental radioactivity monitoring, Setting up a programme, IAEA national training course, Marshall Islands, MH
04.12.2017	Dr. José Corcho: Environmental sampling, Theoretical Basis, IAEA national training course, Marshall Islands, MH
19.12.2017	Dr. Martin Schär: Analytische Chemie im Labor Spiez: Einführung in die Methodik zur Detektion und Identifikation von Chemischen Kampfstoffen, ZHAW, Wädenswil

# Publications

The list is not exhaustive. Some of the reports are classified



## Physics Division

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Astner Markus, Burger Mario

**Products of in Situ Corrosion of Depleted Uranium Ammunition in Bosnia and Herzegovina Soils**

Environmental Science & Technology

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Corcho José

**A Comparison and Validation of Pb-210 Chronologies of Deep Sediment Cores from the Southern Gulf of Mexico**

Gulf of Mexico Research Initiative, University of South Florida

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Egger Emmanuel, Zürcher Markus

**Schwerpunktkontrolle am Zollamt Chiasso**

LN 2017-01 EGM/ZMS

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Ossola Jasmin

**Validierung der Gadoliniumbestimmung mit dem ICP-Massenspektrometer NexION 300D**

LN 2017-02 OSJA

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Pignolet André

**Validierung der Quecksilber-Messungen in Luft mittels autoCOLLECT und DMA-80**

LN 2017-02 PAN

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Pignolet André

**Validierung der Bodenaufbereitungsgeräte Backenbrecher BB 50, Planeten-Monomühle, Pulverisette 6, Schlagkreuzmühle SK 300**

LN 2017-03 PAN

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Sahli Hans, Rölli Stefan, Corcho José

**Determination of Tc-99 in environmental samples and depleted uranium penetrators using ICP-MS**

J Radioanal Nucl Chem

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Sahli Hans, Rölli Stefan, Balsiger Béatrice, Corcho José e.a.

**A procedure for the sequential determination of radionuclides in soil and sediment samples**

J Radioanal Nucl Chem

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Stauffer Marc

**Ringversuchsergebnisse 2016 der Prüfstelle STS 0101**

LN 2017-01 STM

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Stauffer Marc, Pignolet André, Corcho José

**Persistent Mercury Contamination in Shooting Range Soils: The Legacy from Former Primers**

Bull Environ Contam Toxicol, 2017 Jan; 98 (1):14-21

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Rölli Stefan

**Isotopenverdünnungsanalyse von schwach an- und abgereichertem Uran**

LN 2017-01 ROF



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Röllin Stefan

**Validierung der Methode zur Bestimmung von Isotopenverhältnissen gemäss Vorschrift L 028 59 mit dem Neptune ICP-MS**

LN 2017-02 ROF

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Röllin Stefan

**Isotopenverhältnisanalyse in Bleiprobe mit dem Neptune MC-ICP-MS**

LN 2017-03 ROF

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Trdin Miha

**Determination of radium-226 in water: Micro-coprecipitation as barium (radium) sulfate**

LN 2017-01 TRDM

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Trdin Miha

**Procedure for determination of Radium-226 and Radium-228 in water**

LN 2017-03 TRDM

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Trdin Miha, Shyti Manjola

**Determination of Radium-226 and Radium-228 in water by gamma spectrometry micro-coprecipitation as lead (radium) sulfate**

LN 2017-02 TRDM, SHMA

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von Gunten Cédric

**Methodenbeschrieb der Quecksilberspeziation mittels LC-ICP-MS**

LN 2017-01 VGCE

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von Gunten Cédric

**Inaktive Nukleare Forensik – Spuren- und Isotopenverhältnisanalyse in Bleiprobe**

LN 2017-02 VGCE

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von Gunten Cédric

**Validierung der Hg-Speziation mittels LC-ICP-MS auf dem NexION 300D**

LN 2017-03 VGCE

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von Gunten Cédric

**Validierung der Schwefel- und Phosphorbestimmung mit dem ICP-Massenspektrometer NexION 300 D mittels Reaktionszelle**

LN 2017-04 VGCE

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von Gunten Cédric

**Validierung der massenspektrometrischen Osmium-Bestimmung in Wasserproben mittels Isotopenverdünnung**

LN 2017-05 VGCE

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Wirz Christoph, Mosimann Nina

**Teppich mit Thorium**

LN 2017-01 WIC-SNIN

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Wirz Christoph

**Atmosphärische Ausbreitungsrechnung in urbanem Gebiet**

LN 2017-01 WIC

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Wirz Christoph

**Nordkoreas Atomprogramm Technische Sicht aus Spiez**

LN 2017-02 WIC

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Wirz Christoph

**CTBTO Radioaktivitätsmessungen und Atmosphärische Rückrechnungen**

LN 2017-04 WIC

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Stauffer Marc, Pignolet André, von Gunten Cédric, Ossola Jasmin

**Fehlereinträge durch die Probenahme und die Feldanalytik (XRF) bei der Untersuchung militärischer Altlasten**  
LS 2017-02

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von Gunten Cédric

**Störungen der handheld-Röntgenfluoreszenz-Spektrometrie durch partikuläres Blei**  
LS 2017-10

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## Biology Division

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Ackermann Rahel, Beuret Christian, Oechslin Corinne

**Prevalence of tick-borne pathogens in questing Ixodes ricinus ticks in urban and suburban areas of Switzerland**  
Parasites & Vectors, 2017 Nov 9;10(1):558

---

Ackermann Rahel, Beuret Christian, Oechslin Corinne

**Prevalence of tick-borne pathogens in questing Ixodes ricinus ticks in urban and suburban areas of Switzerland**  
Parasites & Vectors, 2017 Nov 9; 10 (1):558

---

Ackermann Rahel

**Fatal Outcome of European Tick-borne Encephalitis after Vaccine Failure**  
Front Neurol. 2017 Apr 3; 8:119

---

Ackermann Rahel

**Le Centre national de référence pour les maladies transmises par les tiques (CNRT/NRZK)**  
Pipette – Swiss Laboratory Medicine, 2017 Oct;5: 8-9

---

Ackermann Rahel

**Etablierung eines Serumneutralisationstests (SNT) zum Nachweis neutralisierender Antikörper gegen Tick-borne Encephalitis Virus (TBEV).**  
Laborbericht März 2017

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Avondet Marc-André, Jenkinson Stephen

**Embryonic Stem Cell-Derived Neurons Grown on Multi-Electrode Arrays as a Novel In vitro Bioassay for the Detection of Clostridium botulinum Neurotoxins**  
Front Pharmacol. 2017 Feb 23; 8:73

---

Beuret Christian

**Development and evaluation of a bioinformatics approach for designing molecular assays for viral detection**  
PLoS One. 2017 May 25; 12(5):e0178195

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Beuret Christian

**Susceptibility to Mycobacterium ulcerans Disease (Buruli ulcer) Is Associated with IFNG and iNOS Gene Polymorphisms**  
Front Microbiol. 2017 Oct 4; 8:1903

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Jenkinson Stephen (Marc Avondet)

**Development of an In Vitro Stem Cell-Based Bioassay for the Detection of Clostridium Botulinum Neurotoxins**  
PhD Thesis 2017 Universität Bern

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Invernizzi Cédric

**CRISPR and the Hype Cycle**  
Defence Future Technologies – What we see on the horizon (Publisher: armasuisse W+T)

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Portmann Jasmine

**Different features of V 2 T and NK cells in fatal and non-fatal human Ebola infections**  
PLoS Negl Trop Dis. 2017 May 30; 11(5):e0005645

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Rothenberger Sylvia, Engler Olivier

**Neue Strategien zur Entwicklung antiviraler Medikamente gegen Hantaviren**

Pipette – Swiss Laboratory Medicine. 2017 Oct; 5:8-9

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Thomann Susanne, Schürch Nadia

**Structural Insights into the Mode of Action of the Peptide Antibiotic Copsin**

Biochemistry, 2017 Sep 19; 56 (37):4992-5001

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Wenger Andreas (Marc Avondet)

**Bioanalytische Charakterisierung des Pflanzentoxins Abrin**

Masterarbeit 2017 ZHAW Wädenswil

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Strasser Marc

**The contribution of the European high containment laboratories during the 2014-2015 Ebola Virus Disease emergency**

Clin Microbiol Infect, 2017; 23;2; 58-60

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Wenger Andreas (Marc Avondet)

**Bioanalytische Charakterisierung des Pflanzentoxins Abrin**

Masterarbeit 2017 ZHAW Wädenswil

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Züst Roland

**Early endonuclease-mediated evasion of RNA sensing ensures efficient coronavirus replication**

PLoS Pathog. 2017 Feb 3; 13(2): e1006195

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## Chemistry Division

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Arnold Michael

**Prüfung des toxic chemicals detection kit (K.D.T.C) des Herstellers NBC Sys**

LN 2017-01 ARND

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Arnold Michael

**Prüfung der Agri-Screen Tickets zum Nachweis von C-Kampfstoffen des Herstellers Neogen**

LN 2017-02 ARND

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Bielmann Andreas, Curty Christophe, Christian Bochet

**Solid-Phase Synthesis of the Aged-Nonapeptide-Nerve-Agent Adduct of Butyrylcholinesterase as Reference Materials for Analytical Verification**

Helv. Chim. Acta, 100: e1700198. doi: 10.1002/hlca.201700198

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Gelmi Tim Johan

**Comparison of different methylation methods for acids related to the Chemical Weapons Convention (CWC) in diverse matrices with/without background**

MA-2017-01-Gelmi-Tim (Masterarbeit), École des sciences criminelles, Université de Lausanne

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Gerber Anna Barbara

**Auswertung der Dokumentation über die Dekontaminationsprodukte BX 24, SX 34, BX 40, BX 29 und BX 30 der Firma Cristanini**

LN 2017-01 GERA

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Guidetti Fausto

**Überprüfung von C-Nachweisgeräten – 2016**

LN 2017-01 GIF

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Guidetti Fausto, Gerber Anna Barbara

**Überprüfung von C-Nachweisgeräten – 2017**

LN 2017-01 GIF/GERA

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Kurzo Roland, Guidetti Fausto

**Evaluierung eines Ersatzes für das Toximeter II**

LN 2017-01 KURO/GIF

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Menzi Benjamin

**Entgiftung, Kontrolle und Entsorgung von Abfällen aus dem C-Sicherheitslabor**

LN 2017-01 MEN

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Sambiagio Nicolas

**Solid-phase peptide synthesis approach to investigate the formation of Glutathione – Sulfur Mustard adducts**

Master Thesis, 2017, Ecole polytechnique fédérale de Lausanne, Spiez Laboratory, Switzerland

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Siegenthaler Peter, Clare Thomas, Meier Urs

**Anleitung zur Bestimmung des extrahierbaren 1,4-Diazabicyclo-[2.2.2]octan (TEDA) auf imprägnierter Aktivkohle mit GC-FID und NMR**

LN 2017-01-SIG

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Schorer Andreas, Gfeller Leonie, Trenkler Eileen, Siegenthaler Peter

**Vergleich der Wiederfindungsraten von CWC-relevanten Verbindungen in Wasserproben mit verschiedenen Extraktionstechniken**

LN 2017-02-ANDRS/SIG

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Trenkler Eileen, Schorer Andreas, Siegenthaler Peter

**Bestimmung der Wiederfindung von Ethyl methylphosphonsäure und Bis(2-diisopropylaminoethyl)disulfid bei der apolaren und polaren Extraktion von Wipe-Samples**

LN 2017-03-TEIL/ANDRS/SIG

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Meier Urs

**Eignung einer Fluorophenylkolonne zur Auftrennung von CWÜ relevanten Sulfiden und Sulfoxiden**

LN 2017-04-MRU

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Meier Urs

**Anwendung von der DOSY NMR Technik zur Analyse von Umweltproben mit CWÜ relevanten Verbindungen**

LN 2017-05-MRU

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Siegenthaler Peter, Dutoit Jean-Claude, Meier Urs, Schär Martin

**Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament, Blue Book 2017 Edition**

University of Helsinki, Finland, 2017 (Editor: Vanninen Paula)

ISBN 978-951-51-3917-7 (PDF)

ISBN 978-951-51-3916-0 (paperback)



## NBC Protection Division

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Richner Gilles

**Effekt von Feuchte und Transpiration auf die Schutzwirkung vom semi-permeablen C-Schutzanzug der C-EEVBS – Stand der Kenntnisse**

LN-2017-01 GRIC

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Wittwer Andres

**Abschätzung der Messunsicherheit für den Strömungswiderstand von Staubschutzmedien**

LN 2017-01 WITA

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Wittwer Andres

**Sorptionsprüfung von grossen Filtern mit Cyclohexan**

LS-2017-08 Validierungsbericht

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Zahnd André

**CBRE-Kollektivschutz in Hochbauten – Grundlagen, Abgrenzungen und Zielsetzungen (Teil 1)**

Version 4.1, Ausgabe vom 11.09.2017, 24 Seiten

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Zahnd André

**CBRE-Kollektivschutz in Hochbauten – Gefährdungen und Risiken, Grundsätze der Massnahmenplanung (Teil 2)**

Version 4.1, Ausgabe vom 11.09.2017, 40 Seiten



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