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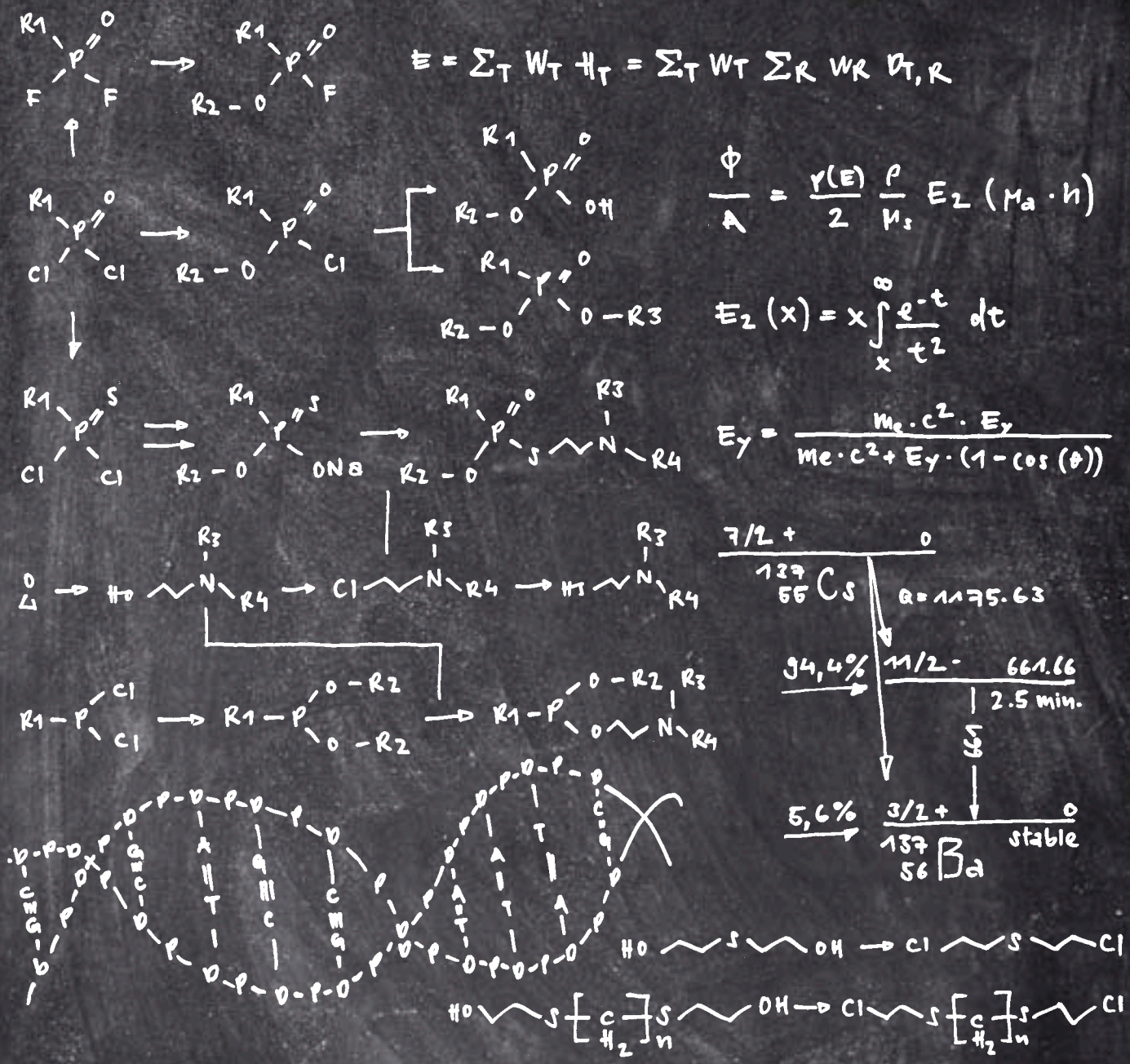
SPIEZ LABORATORY

Annual Report 2011

SPIEZ LABORATORY



Production
Dr. Andreas B. Bucher

Design/Layout
Electronic Media Center EMC, 3003 Berne

Editor
Federal Department of Defence, Civil Protection and Sports DDPS,
Federal Office for Civil Protection FOCP
SPIEZ LABORATORY
CH-3700 Spiez
Tel. +41 33 228 14 00
Fax +41 33 228 14 02
laborspiez@babs.admin.ch
www.labor-spiez.ch

Dear reader,

After the major earthquake and the Tsunami of 11 March 2011, core meltdown occurred in three reactors of the Fukushima Daiichi nuclear power plant. Large amounts of radioactive substances were released and extensive areas had to be evacuated. This disaster occupied our physics section in particular, whose collaborators provided information on the background and the possible consequences of the accident via all Swiss media channels. The clean-up in Japan will take a long time, decontamination efforts will be difficult and expensive (page 6).

In the summer of 2011, the entire SPIEZ LABORATORY was shaken by the unexpected death of Dr. Martin Schütz, head of the biology section. Martin died on 7 August at the age of 47, after short but severe illness. With him the laboratory has not only lost an outstanding biologist and brilliant colleague, but especially a great friend and person. Martin did his PhD in microbiology at the University of Bern and specialised at the Federal Office for Agriculture in microbiological aspects of wine cultivation. In 1995, he changed over to SPIEZ LABORATORY. Under his direction the biology section grew from two employees to a staff of 15 scientists today. Martin also served as UN weapons inspector in Iraq. But primarily he was initiator and the driving force for the construction of the first and only biological laboratory for human pathogenic forms in Switzerland with the highest safety level. This laboratory will be opened for scientific use in 2012 and will keep our memory of Martin alive during our daily work.

Martin Schütz
1964 - 2011

In select areas our chemistry section is becoming a central actor in Swiss disarmament and arms control policy: In the autumn of 2011 this specialist section organised an international workshop with high ranking participants on the subject of incapacitating chemical agents. These substances could become a problem for international arms control in the coming years. With this event, SPIEZ LABORATORY has significantly contributed to basic technical issues in the debate on incapacitating agents being clarified early on (page 24).

Dr. Marc Cadisch
Head of SPIEZ LABORATORY

Among other activities, the NBC protection technology section has been occupied with the elaboration of a handbook for individual personal equipment. Practical experience has shown that there is wide interest in understandable information on individual personal equipment not only among military, but also among civil protection and private industrial security personnel. The handbook provides the most important information on the use of protective equipment and assists in important procurement decisions (page 40).

Generally the year 2011 was characterised by our international focus. SPIEZ LABORATORY has stated its goal not only to strengthen its expertise, but also its efficiency through the development of specific international agreements: In accordance with a framework agreement signed in June 2011, we assist the International Committee of the Red Cross ICRC in establishing technical and operational capabilities to deal with nuclear, radiological, biological and chemical incidents the organization may face. The analytical expertise of the laboratory is on standby for support on short notice and may involve the dispatchment of experts to assist the ICRC in the field. Furthermore, we are part of the ALMERA network of the International Atomic Energy Agency (IAEA) since the end of 2011. ALMERA – the Analytical Laboratories for the Measurement of Environmental Radioactivity – consists of over 100 special laboratories capable of reliably measuring environmental samples when radioactivity is released. Our participation in this network indicates the quality of our activities in the field of radioactivity.

These new obligations augment the already extensive international activities of our staff. These include for instance the operation of a designated laboratory in connection with the Chemical Weapons Conventions (page 32) as well as operations relating to post-conflict assistance and disaster relief. We seek to develop this engagement further if possible, because an intact international network is one of the basic prerequisites for successful and efficient NBC protection.

After Fukushima

Managing a nuclear power plant accident involving the release of radioactivity in Switzerland

Dr. Emmanuel Egger

The accident in the nuclear power plants of Fukushima Daiichi raises the question, how Switzerland would deal with such an incident. For introducing adequate measures to protect the population it is important to gain an overview of the extent and type of contamination as quickly as possible. And in order to optimise decontamination measures, knowledge of the detailed distribution of contaminants is necessary. Gaining such information poses a challenge for the Emergency Organisation Radioactivity.

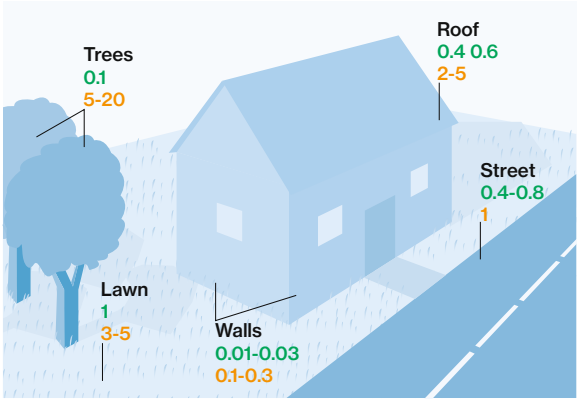
Aerial radiometry would be used in Switzerland to gain a preliminary overview of the extent of ground contamination. A Swiss Air Force helicopter is available for such a purpose. It can be equipped with corresponding measuring instruments and flies about 100 m above the ground across the contaminated area. Both the extent of contaminated area and its local dose rate as well as existing nuclides can be determined in this way. The Japanese authorities obviously did not have this means at their disposal so that finally a measuring team of the US Department of Energy took over this task and provided preliminary measurements (see Figure 1). Other measuring methods can also be applied to obtain more detailed information on contaminant distribution.

In Switzerland the measures that would have to be prescribed in such a case are regulated in the conception of measures to be taken in function of the dose DMK of the ordinance on the organisation of interventions in the case of ABC and natural events . For the protection of the population evacuation or protected residence (i.e. staying in the house, the cellar or the shelter) is prescribed. In addition, as a precaution, harvesting, grazing, hunting and fishing are prohibited on the downwind side of the incident to prevent the sale and consumption of contaminated food.

Once the inhabitants have evacuated from the contaminated area when contamination is severe, the question arises how this area can be decontaminated so that people are able to return to their houses. For optimising decontamination measures, the distribution of contaminants must be precisely known. Contamination depends greatly on whether the weather was

Steel tanks, each with a capacity of 100 or 120 tons, to be used to store radioactive wastewater that is accumulating at the Fukushima Daiichi nuclear power plant. (Reuters)

Figure 2:
Relative ^{137}Cs contamination of surfaces (green: wet deposition, orange: dry deposition)



dry or it rained when the radioactive cloud passed by (see Figure 2).

As evident from Figure 2, grass areas, roads and roofs are especially contaminated in the event of rain, while in dry weather primarily trees, grass areas and roofs and to a lesser extent roads are affected. The season also plays an essential role. Thus in autumn trees and especially their leaves would be contaminated. A few weeks later, these would fall in accordance with the season and contamination would be transferred to the ground.

For establishing the three-dimensional distribution of contamination, it is necessary to send measuring teams into the contaminated area. Measurement vehicles are best suited for such a purpose. But if the contaminated area attains a size of several 1000 km², it may be necessary to send measuring teams on foot with simple local dose rate measuring instruments into the contaminated area – especially where contamination is minor. Soil samples have to be taken and analysed in the laboratory to establish the precise composition of soil contamination. Here an important task has been assigned to SPIEZ LABORATORY and NBC Defence Lab 1 of the armed forces.

Decontamination measures can be taken, once contaminant distribution is known. In Table 1 possible measures are listed for decontaminating grass areas as well as their efficacy, i.e. the percentage of radioactive contamination that can be removed by applying the respective method. In Table 2 methods are listed for decontaminating roads, in Table 3 such for houses. [1]

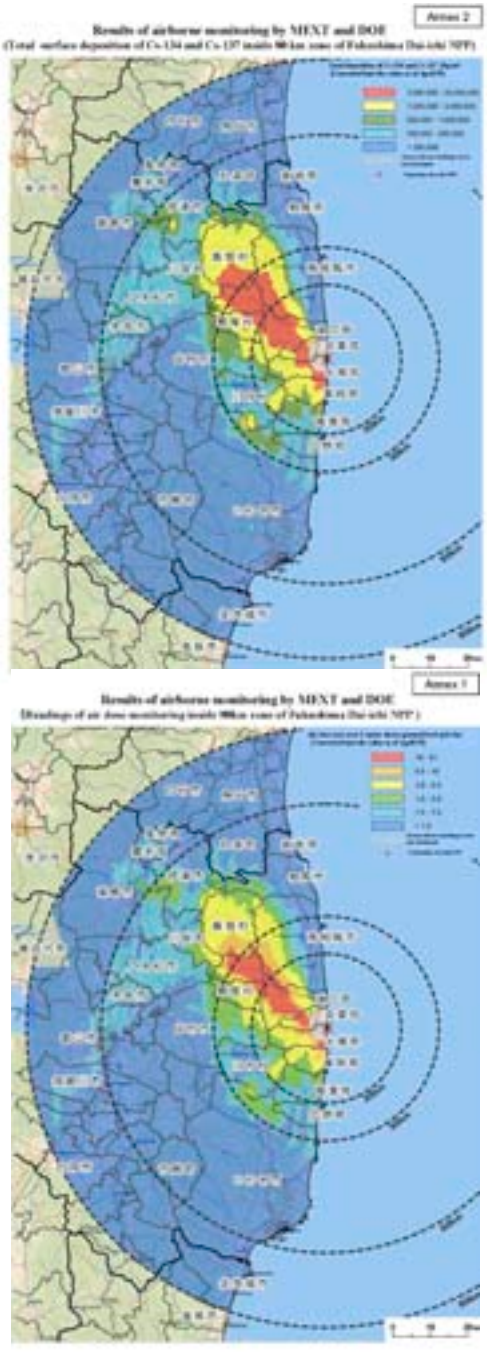


Figure 1:
Preliminary measurements by the US Department of Energy

[1] EURANOS, Generic Handbook for Assisting in the Management of Contaminated Inhabited Areas in Europe following a Radiological Emergency V1.0, May 2007, obtained at http://www.euranos.fzk.de/Products/EURANOS_InhabitedHandbook_Version1.0.zip

Decontamination method	Efficacy
Cut grass, mow lawn	50–90 %
Remove plants and shrubs	50–90 %
Remove top layer of soil (1cm)	65–90 %
Remove top layer of soil (5 cm, mechanically)	90–95 %
Remove top layer of soil (5 cm, manually)	90–95 %

Table 1: Decontamination methods for grass und soil und their efficacy

Decontamination method	Efficacy
Rinse with fire hose	50–75 %
Water jet clean	65–85 %
Hoover	50–65 %
Remove and replace surface	100 %

Table 2: Decontamination methods for roads and their efficacy

According to the Swiss Radiological Protection Ordinance the directive value for ground contamination with ^{137}Cs is 30 000 Bq/m². As evident from Figure 1, after the Fukushima incidents, approx. 1000 km² were contaminated with more than 600 000 Bq/m² i.e. a 20-fold directive value and approx. 300 km² with more than 3 000 000 Bq/m² or a 100-fold directive value. In the most severely contaminated regions methods that enable up to 90 % decontamination will not be sufficient for inhabitants to return. The only method to re-establish the normal situation here is to destroy the buildings, remove the top layer of the ground, cut down the trees and dispose of everything as radioactive waste. Therefore, it must be expected that dealing with such an incident will be very expensive.

Decontamination method	Efficacy
Destroy and dispose of building	100 %
Rinse with fire hose	25 %
Brush off roofs	50–85 %
Sand blast walls	75–90 %
Water jet clean walls and roofs (cold water)	35–80 %
Water jet clean roofs with hot water	50–85 %
Replace roofs	100 %
Treat walls with ammonium nitrate	25–50 %
Grind off wooden walls	35–60 %

Table 3: Decontamination of buildings and efficacy of method

Workers remove topsoil from the playground of Kaoru Elementary School in Koriyama, Fukushima Prefecture. (Reuters)

International Exercise for In-Situ Gamma Ray Spectrometry

François Byrde

This international exercise for measuring ground radioactivity (ISIGamma 2011 Davos) was organised by SPIEZ LABORATORY, the Swiss Federal Nuclear Safety Inspectorate (ENSI) and the Federal Office of Public Health (FOPH). 24 teams from 9 European countries participated in this exercise in Davos from 19 to 23 September.

In-situ gamma ray spectrometry is a technique for determining the level of ground radioactivity on site, i.e. without taking samples and subsequently analysing in the laboratory; it is possible to obtain rapid results with a simple infrastructure.

This measuring technique established itself after the Chernobyl accident. Early groups used instruments that were poorly suited for outdoor use. The search for more adequate equipment became a priority and international exercises were regularly organised after 1990 and largely contributed to the development of today's method. Several dates should be mentioned with respect to the development of in-situ gamma ray spectrometry:

- 1960 First papers on in-situ method by H.L. Beck
- 1994 Report 53 of ICRU
- 1995 Use of portable computers
- 2005 Electrically cooled detectors

Objective of exercise

For the 2011 exercise, we have set our priorities on the following points:

- Classical in-situ: Measurement will be exclusively ground contamination.
- Exchange of information between participants: The measuring program must not be overloaded.
- Balance of radioactive decay chains: The Dischma valley has sites with greatly elevated uranium radionuclide levels and notorious deviations from their decay chain equilibrium.
- Calibration check: Calibrated point sources have made it possible to check the calibration of the detectors.
- Checking of methods and references used: Participants had to fill in a comprehensive questionnaire concerning the description of instruments, the method of analysis and the results obtained.

The date of the exercise was chosen with regard to avoiding holidays, harvest and cold periods.

Measurement in Klosters

Measurement in Davos

Sequence
After a preparatory phase of almost one year (site designation and measurement of the sites, form preparation , registration of participants and administrative work), the exercise began with severe snowfall resulting in:

- Change of measuring site (danger of avalanches)
- Clearing of retained sites
- Reorganisation of the programme.

During the exercise, groups of 4 to 5 measuring teams were formed and led by the following organisation:

- Administration and office: 3 persons
- Measuring site: 1 person per site
- Escort of groups: 2 persons per group.

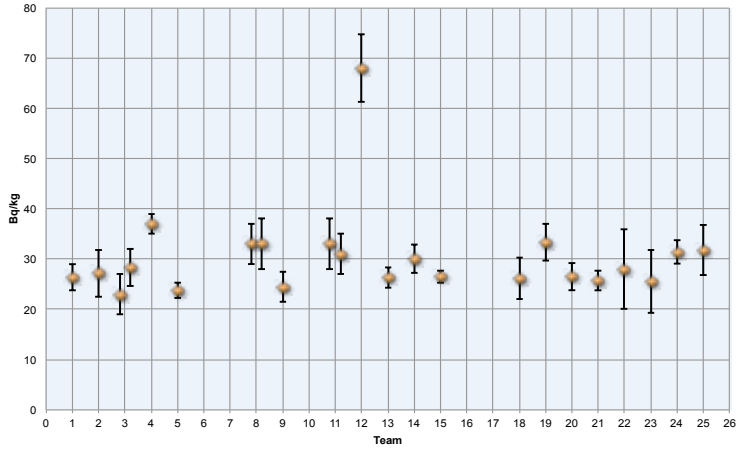
This redundancy in leadership allowed the organisers to hold discussions with the participants.

The first day was dedicated to various presentations (in-situ inspection, ISO standardisation project, features of interest in the Dischma valley) as well as measurement at the Klosters site below the snow line.

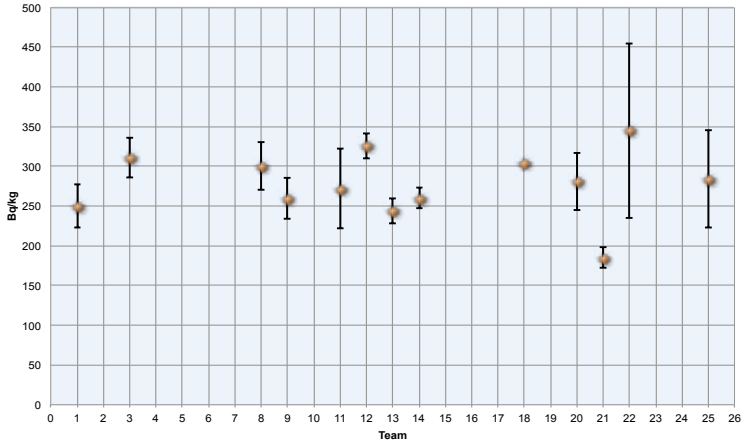
Over the following two days, the groups worked in rotation at the various sites (3 per day), one of their duties being to report their results on the same evening.

Despite the extreme temperatures, no major problem with the instruments was reported to the organisation.

Site 7 - Klosters: 214Bi (238U-Chain) concentrations in soil



Site 2 - Boden Sertigtal: ^{40}K concentrations in soil



Results

The results handed in by the participants are being studied and the final report will be published no sooner than spring 2012.

A preliminary evaluation has shown wide agreement of the results. After complete evaluation of the results, we will be able to examine certain points in detail, such as:

1. Taking the effect of snow cover on the analysis into account
2. Knowing where to find the analytical parameters
3. Ability to compute the dose
4. Understanding the radioactive decay chains

Equipment of the EEVBS, the DDPS task force

Conclusions

Although the exercise was not based on a contamination scenario, it became evident that only some of the equipment used in Davos would be adequate for such operations, such as the equipment used by the EEVBS, the DDPS task force.

It might be a good idea to organise an exercise with a contamination scenario. The use of marker agents would allow us to determine which teams are capable of working in a contaminated environment, and would show inadequate equipment.

It should be noted that international standardisation projects exist for task teams.

Notably at the instigation of Germany, standards concerning in-situ spectrometry exist or are being elaborated (DIN, ISO, IEC).

Without denying the advantages of their use, we observe that more and more users have a good understanding of the standards, but a poor understanding of the method.

In-situ spectrometry is one of the analytical methods SPIEZ LABORATORY has to master. In offering its services, the SL has not only contributed to this measuring campaign, but also enabled seven of its employees to consolidate their expertise in and practical use of this technique.

Inadequate equipment for contaminated areas

Work for the Comprehensive Nuclear Test Ban Treaty

Dr. Christoph Wirz

The Nuclear Test Ban Treaty prohibits any kind of nuclear weapons explosion, whether for civilian or for military purposes. Assistance to this end is also prohibited. SPIEZ LABORATORY uses its expertise in the field of treaty verification, in particular by participating in activities to build up on site inspection capability.

In the Comprehensive Test Ban Treaty (CTBT) the treaty parties undertake not to carry out any nuclear explosion, and to prevent those in their sphere of influence, to refrain from any participation in a nuclear explosion and not to encourage let alone support other parties in such efforts. States that possess no nuclear weapons have already stated their renouncement of nuclear weapons in the Treaty on the Non-Proliferation of Nuclear Weapons, NPT. In this respect the CTBT has no effect on them. The CTBT, however, is intended to prevent the further development of nuclear weapons in states that possess them.

182 states have signed this treaty, while 156 have ratified it (as of January 2012). The treaty is not yet in force however. It will not be applicable until it has been ratified by 44 so-called Annex 2 states, listed in the treaty. These 44 states posses nuclear power or research reactors and were listed on the 1994 and 1995 inventories of the International Atomic Energy Agency. Outstanding are Egypt, China, Iran, Israel and the USA, who, although they have signed the treaty, have not ratified it yet, as well as India, North Korea and Pakistan, who have not yet signed it.

It must also be possible to verify compliance with the treaty. The Comprehensive Nuclear-Test Ban Treaty Organization (CTBTO) is responsible for this. The Preparatory Commission of the CTBTO based in Vienna is currently working on a global verification system. It consists of a world-wide monitoring network for remote detection of nuclear explosions whether atmospheric, underground or submarine. It consists of:

- 170 seismological stations
- 11 hydro-acoustic stations (sound wave monitoring of the oceans)
- 60 infrasound recording stations for measur-

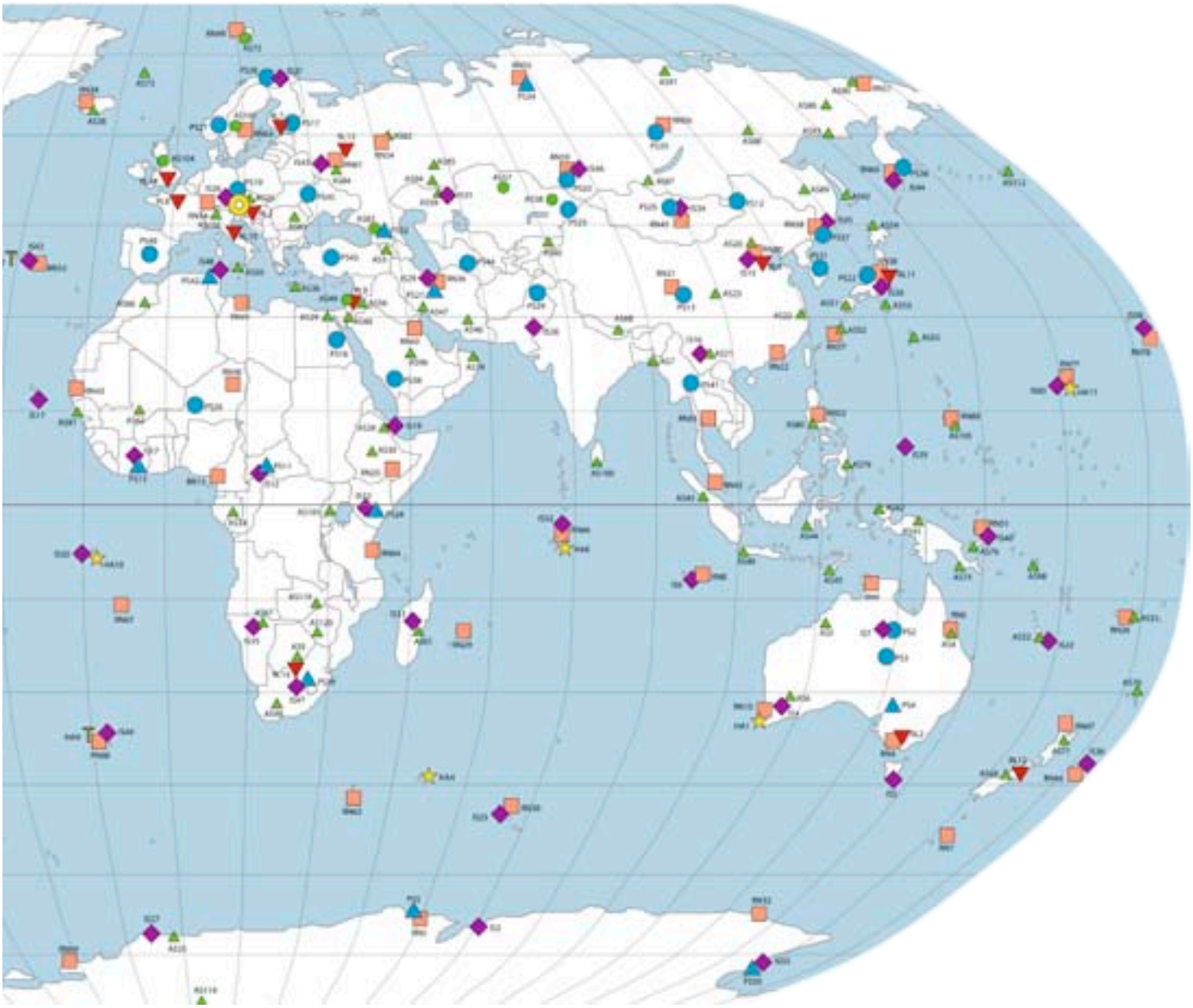
ing minute variations in air pressure
- 80 stations with radionuclide detectors for detecting special radioactive particles or for measuring the concentration of radioactive noble gases, in addition 16 radionuclide laboratories.

At the end of 2011, 260 measuring stations were already fully installed and certified. Data are constantly sent to the international data centre in Vienna for evaluation. The stations are distributed over the entire world and are often located in remote regions with extreme climate conditions. Functional requirements on the measuring instruments are therefore quite severe.

Once the treaty goes into effect, a member state will be able to demand an inspection in another state, especially if the monitoring system detects suspicious activity. This is precisely regulated in the treaty. In such a case what is called on site inspection could be adopted, in order to clarify, whether a suspicious incident was a nuclear explosion. The perpetrator of treaty violation should also be determined by the inspection team. The freedom of operation of the inspection team is, however, limited with regard to measuring methods, analytical instruments and the duration of inspection. Furthermore, for financial reasons it will be impossible to permanently have an inspection task force on location. Therefore, the CTBTO carries out training courses in the various measuring methods for experts from all member states, in order to raise a well trained pool of specialists that would enable staffing of an efficient inspection team that is adapted to the requirements.

Switzerland acceded to the treaty in 1996 and ratified it in 1999. The Arms Control and Disarmament Section of the DFA represents Switzerland in Vienna and coordinates the various services of Switzerland in accordance with the treaty. These include:
- The DAVOX seismic station in the Dischma valley near Davos, which is operated by the seismological service of the ETH Zurich. Pressure waves registered here are relayed by satellite link to Vienna.
- The Climate and Environment Division of the University of Bern carries out extremely precise measurements of the 37Ar nuclide. Its natural concentration is very low. An increased level would be a strong indication of an underground atomic bomb test.
- SPIEZ LABORATORY experts are engaged in the field of on-site inspection. They participate in radioactivity measuring exercises

such as that in Chernobyl a few years ago (emphasis on sampling and decontamination). An extensive inspection exercise is planned for the end of 2014 where, if possible, all procedures and measuring methods are to be tested. The host nation will be determined at the end of June 2012 from the candidate countries which are Hungary, the Ukraine or Jordan. In a second training cycle the CTBTO will be doubling the size of its inspector pool over the next two years. About 50 participants from more than 40 countries participate in this training. An employee of SPIEZ LABORATORY also attends. In 2011, the training blocks were conducted at a military training area in Hungary and at the new Equipment Storage and Maintenance Facility (ESMF) to the south of Vienna. Apart from these activities, SPIEZ LABORATORY was, as already in the previous years, engaged in 2011 with elaborating the «On-site inspection operational manual» in working group B of the CTBTO.



CTBTO global monitoring system

●	Seismic primary array (PS)	T	Hydroacoustic (T-phase) station (HA)
▲	Seismic primary three-component station (PS)	◆	Infrasound station (IS)
●	Seismic auxiliary array (AS)	■	Radionuclide station (RN)
▲	Seismic auxiliary three-component station (AS)	▼	Radionuclide laboratory (RL)
★	Hydroacoustic (hydrophone) station (HA)	◎	International Data Centre, CTBTO PrepCom, Vienna

Ricinus communis with inflorescence and mature seeds

Ricinine as Marker Substance in Cases of Ricin Poisoning

Marc-André Avondet

For terrorist-related use the ricin protein is extracted from the seeds with an aqueous buffer solution. Through this process the alkaloid ricinine is also dissolved, which, contrary to ricin, is chemically extremely stable, and can be detected for several days even in clinical samples. Furthermore, ricin is only present in the Ricinus communis plant. For these reasons the detection of the relatively small ricinine molecule as marker substance provides an excellent procedure for rapid assessment of inspection samples and is recommended by the US Centers for Disease Control and Prevention (CDC) [1].

Despite its moderate suitability as weapon of mass destruction (instable protein, low oral toxicity [2]) ricin is listed as a Schedule 1 compound in the Chemical Weapons Convention. Even in the latest version of the Bio and Toxine Weapons Convention ricin appears in the respective lists. For this reason the analysis at SPIEZ LABORATORY of ricin from terrorist-related inspection samples are of great importance. The focus here is on immunological procedures (ELISA & LFA) and mass spectrometric methods (MALDI-TOF MS & LC-MS/MS).

Ricinine [3, 4] is also an ingredient of the Ricinus communis plant and belongs to the group of pyridine alkaloids. Together with the highly toxic ricin protein it is responsible for the plant's toxicity. Ricinine is present in all parts of the plant and serves as an insecticide among other things. In humans it attacks the liver and kidneys and can also cause serious poisoning. The seeds contain approximately 0.2 % of the alkaloid. Unlike ricin, ricinine cannot be inactivated by heat treatment.

Therefore, in order to use press cake residues from castor oil production as animal feed, ricinine would have to be removed after ricin inactivation in an elaborate extraction process. Ricinine can be synthesised by cyclisation of 1,1-dicyano-4-(N,N-dimethylamino)-2-methoxy-1,3-butadien and subsequent methylation. In the plant it is produced biosynthetically via nicotinamide as intermediate.

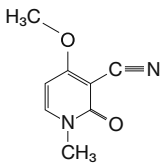
[1] D. L. Ashley: Preparing for Chemical Terrorism – Response at the Centers for Disease Control (CDC) and Prevention; PowerPoint Presentation. http://acscinf.org/docs/meetings/230nm/presentations/230nm78.pdf

[2] Ricin as a weapon of mass terror – separating fact from fiction. Schep LJ, Temple WA, Butt GA, Beasley MD. Environ Int. 2009 Nov;35(8): 1267-71. Epub 2009 Sep 19. Review.

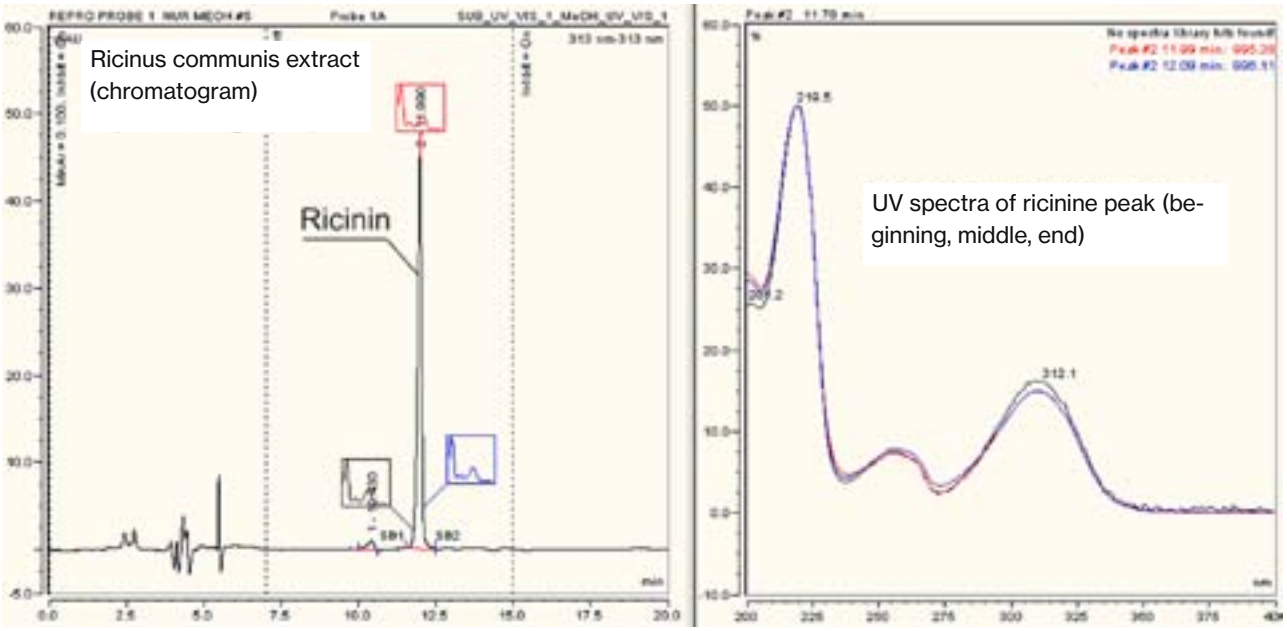
[3] Wikipedia; Freie Enzyklopädie (http://de.wikipedia.org/wiki/Ricinin).

[4] Hagers Enzyklopädie der Arzneistoffe und Drogen; Springer Medizin Verlag, Heidelberg, 2008.

3-D Structure of ricin glycoprotein

Stuctural formula of Ricinine	
Name (IUPAC)	4-Methoxy-1-methyl-2-oxo-1,2-dihydropyridin-3-carbonitril
Molecular formula	$C_8H_8N_2O_2$
CAS-Nummer	524-40-3
PubChem	10666
Molar mass	164.16 g mol^{-1}
Melting point	200–201 °C
Solubility	soluble in water, methanol and ethanol
LD_{50}	19-20 mg kg^{-1} (mouse, oral)

Ricin is a water soluble protein with a high molecular weight from the seed of ricinus communis (castor oil plant). It is a powerful inhibitor of eukaryotic protein synthesis and one of the most toxic proteins that exist in nature. If an organism assimilates the poison, it kills infested cells by inactivating protein synthesis.



Chromatogram of an extract from Ricinus communis with verification of ricinine peak purity.

Analysis of ricinine

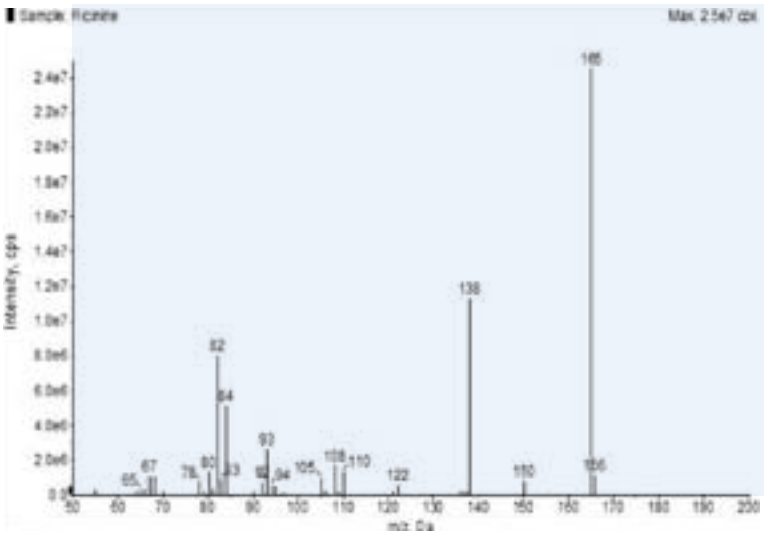
Ricinine in Ricinus communis and its extracts is routinely analysed and quantified in the toxinology group of the biology section through liquid chromatography and UV detection with the photodiode array detector (HPLC-DAD) [5]. For this purpose samples are exhaustively extracted with methanol and extracts are purified by solid phase extraction (C18 SPE). A «terrorist powder» was made on the basis of the castor oil plant according to a recipe available on the internet and analysed for ricinine content.

Because the applied HPLC-DAD method is stretched beyond its selective capacity with clinical samples such as blood or urine, alternative methods such as LC MS/MS have to be used. The presence of ricinine in complex matrices can be confirmed by LC MS/MS using the Multiple Reaction Monitoring (MRM) method. A very high degree of specificity and sensitivity can be achieved with MRM. The first quadrupole filter (Q1) of a triple quadrupole mass spectrometer allows to preselect the precursor ion of interest (usually [M+H]+) with subsequent fragmentation in the pressurized collision cell (Q2). With the quadrupole filter Q3 a specific fragment ion is selected. A fragment ion of high abundance is used for quantitation and a second (qualifier) to confirm the detection of ricinine. This is achieved through comparison of their relative MRM signal areas for sample and standard. For a positive identification the area ratios should usually lay within a ±20 % range.

Quantitation was carried out in MRM mode using the external standard method. For this purpose the quantifier transition was used.
An Agilent Series 1200 HPLC and a 3200 QTrap mass spectrometer from Applied Biosystems/MDS Sciex with ESI source was used. The ricinine standard was obtained from the French company Latoxan (L 6012; purity = 98 %). For further information on measuring conditions and parameters see [6].

[5]Bericht LABOR SPIEZ 2010-01 Quantitative HPLC-Bestimmung von Ricinin in Ricinus communis und daraus hergestellten Extrakten (Werner Arnold; 04.02.2010)

[6] Prüfbericht LABOR SPIEZ Nr. OA-2011-04 vom 07.09.2011



750 ppb ricinine. Enhanced product ion spectrum (MS/MS). Collision energy: 40

Analysis of genuine samples

Since 1980 there have repeatedly been cases of dog poisoning in Germany with so-called bio fertiliser [7]. Such biological fertiliser consists of a mixture of bone or horn meal and castor oil press cake. Due to no or insufficient inactivation of the ricin contained in the castor cake the dogs were poisoned by eating the bio fertiliser and several animals died as a result. The cause of these poisoning cases was no or insufficient inactivation of the ricin contained in the press cake from castor oil production. Surprisingly such poisonings reoccurred in 2010, the reason again being bio fertiliser with a clearly too high ricin content.

Subsequently, the Robert Koch-Institute analysed the ricin content of bio fertiliser samples, finding that their content clearly exceeded the legally prescribed limit. An autopsy of a dog revealed symptoms of hemorrhagic enteritis typical for oral ricin poisoning. Unfortunately, no ricin could be found in the clinical samples (kidneys, liver, stomach and blood) because it could no longer be detected after intake by affected cells.

At SPIEZ LABORATORY urine samples of the dead dog were examined for ricinine with HPLC-DAD. Because of the ambiguous results, LC MS/MS measurements were carried out in collaboration with the colleagues from the analytical chemistry group which led to a definitely positive detection of ricinine. Thus, the chain of evidence indicating that the poisoned dogs had been in contact with the bio fertiliser could be closed.

[7] Ricinus communis Intoxications in Human and Veterinary Medicine – A Summary of Real Cases; Sylvia Worbs et al; Toxins 2011, 3(10), 1332-1372

Monitoring of Human Pathogenic Viruses in Mosquitoes

Dr. Olivier Engler, Dr. Christian Beuret and Dr. Marc Strasser

Because of changes in climate, the habitat of many mosquito species have shifted and species that may serve as vectors for new emerging infectious diseases have established themselves in Switzerland. Due to increased travel exotic pathogens are frequently transported from endemic regions to Switzerland. Under suitable conditions some of these pathogens could be spread by mosquito species. Other viruses are brought into Europe trough animals such as migratory birds and are transmitted to human and animals through local mosquito populations. To better understand the dissemination of viral pathogens in Europe, SPIEZ LABORATORY contributes to the microbiological surveillance of potential mosquito vectors.

Amongst other factors climatic changes have lead to shifts in the habitat of mosquito species worldwide. For Switzerland of central importance are the immigration of the Asian tiger mosquito *(aedes albopictus)* from Italy to the Ticino and the import of the Asian bush mosquito *(aedes japonicus)* north of the Alps. In endemic regions the tiger mosquito may transfer the Chikungunya virus and is also considered to be the vector for the widely disseminated Dengue virus.

Both Chikungunya and Dengue viruses do not normally occur in Europe, can however be introduced to Europe by infected travellers returning to Europe. Accordingly, it is suspected that the outbreak of Chikungunya viruses in 2007 in northern Italy originated from a patient who was infected by the virus during his stay on the Indian subcontinent. Furthermore, the first known case of Dengue viruses being transmitted by resident Asian tiger mosquitoes was in 2010 in the south of France. The tiger mosquito is continuously spreading in the Ticino and in greatly affected areas could attain a critical density for autochthonous infections. North of the Alps, the Asian tiger mosquito has only been found in isolated cases; it is assumed that these individuals were introduced by vehicles coming from the south. In the north of Switzerland a second exotic mosquito type has spread in recent years; the Asian bush mosquito. It is likely that this mosquito species is also a candidate for carrying a variety of exotic viruses
The danger originating from West Nile viruses has been known for quite a while. In recent

years West Nile viruses have spread across the North American continent and caused more than 30 000 clinical infections. In Europe West Nile viruses that in most cases are carried to Europe by migratory birds and transferred by various mosquito species to birds, horses and humans, sporadically give rise to small outbreaks. In the summer of 2010, there was an outbreak in Greece with more than 200 suspected or confirmed infections and 35 deaths. In 2011, the West Nile virus was detected in patients in Italy, Hungary, Albania and Macedonia. It is yet unclear, whether and how the virus will continue to spread.

In collaboration with the Istituto Cantonale di Microbiologia (ICM) in Bellinzona and the Fondazione Bolle di Magadino, the Biology section of the SPIEZ LABORATORY has launched a three-year project to examine indigenous and immigrant mosquito species for viral human pathogens. Due to the latest development in Europe and the spreading of corresponding vectors in Switzerland, the three virus types, West Nile virus (WNV), Dengue virus (DENV) and the Chikungunya virus (CHIKV), have been taxed as particular relevant.

In 2010, a pilot study was carried out in the Ticino to establish the methods, and in 2011, the first results were obtained. For this purpose, mosquito traps were regularly set up for several months at select locations. Tiger mosquitoes were caught in the border region around Chiasso. They have propagated most in this region. Other mosquitoes (Aedes vexans) were caught in the natural reserve area of Bolle di Magadino and in the immediate vicinity of residential areas *(Culex pipiens)*. At the end of the year, the mosquitoes were sent for examination to SPIEZ LABORATORY and were examined in collaboration with the ICM Bellinzona in 2011. The genetic material (RNA and DNA) was extracted from the mosquito pools and tested with a series of microbiological methods (quantitative real-time RT-PCR and generic PCR protocols) for traces of West Nile, Chikungunya, Dengue and other viruses. Genetic traces of viruses could be detected in more than 10 % of the mosquitoes pooled. Detailed analysis of viral sequences, however, showed that most of the viral species were harmless to humans and animals.

In several of the Culex mosquito pools the Usutu virus was found which is closely related to the West Nile virus. This virus is currently being propagated in cell cultures to enable better characterisation. By applying whole genome sequencing the entire genome of the Utsu virus is to be deciphered in order to establish its origin. Usutu viruses became known in connection with extensive bird deaths. For immune healthy persons the Usutu viruses do not constitute a risk, but can – as recently two cases in Italy have shown – give rise to severe infection in persons with a weakened immune system. As experience over the past years has shown, the epidemiological situation in Europe is rapidly changing due to climate change, the migration of birds and greatly increased travel. In particular due to the spread of West Nil viruses in Europe, but also due to the appearance of new viruses, the surveillance of mosquitoes in the Ticino is to be continued and extended to further areas in Switzerland.

Workshop on Incapacitating Chemical Agents (ICA)*

Stefan Mogl

*Executive summary of the conference report.

This technical workshop follows in line with a number of activities Switzerland has undertaken in the past to address uncertainties pertaining to the status of ICA under the Chemical Weapons Convention (CWC). The event was organized by SPIEZ LABORATORY together with VERIFIN, the Finnish Institute for the Implementation for the CWC. The objective was to bring together policy and technical experts to discuss some of the underlying technical questions that may influence policy discussion on ICA.

What are ICA? – ICA are toxic chemicals that typically act on the Central Nervous System (CNS). They are different from Riot Control Agents (RCA) – whose main target is the peripheral nervous system. There is no need for a scientific definition for ICA because such a definition would not affect their treatment as toxic chemicals under the CWC. Because some ICA are more potent than Nerve Agents, the question was posed whether such materials should even be considered for law enforcement purposes.

How could ICA be detected? – The procedures for the analysis of chemicals relevant to the CWC cannot be transferred as such to the analysis and identification of ICA , which are likely to be more drug-like substances. Similarly, the procedures used at toxicology laboratories are not aimed at detecting ICA. The expertise reflected in both fields however may serve as starting point for suitable methods. High resolution mass spectrometry is a promising analytical technique to support the screening of ICA type substances.

ICA (will) typically act on the CNS, but any effect is dose dependent, and any agent – including ICA – will have side effects. Furthermore, there is significant variability in the effects due to individual susceptibility. The understanding of the enormous complexity of cellular biology, molecular biology, biochemistry and physiology of the brain is far from complete. Whilst there has been a large increase in knowledge regarding the existence of neurotransmitters, a detailed understanding of their actions is limited to a small number of them. Furthermore, over-dosing is a typical occurrence when an agent is used in the field, as is known to happen during field use of RCA. There are just too many variables to ascertain that a use would be «safe» in all circumstances.

It is the way a substance is used that makes it an ICA and not its intrinsic properties. Which risks and how much risk is acceptable is a cru-

cial question in the safety debate on ICA. Outside of medically controlled circumstances – for field use of an agent – the issue of safety is much more complex than just ensuring a wide therapeutic window. Where is the cut-off point for an "acceptable number" of casualties – and, what does this mean for litigation in relation to such casualties? Who would be responsible for risk assessment over the development, deployment and how and when to use ICAs? It is far from clear that such risk assessment would be performed thoroughly. The development of any sort of weapon intended for law enforcement purposes therefore must happen in a transparent manner under public scrutiny – there is no place for secrecy.

Confrontations of large groups of people that lead to possible violence seem to be on the rise and some saw using ICA for law enforcement purposes as a permitted activity – because of a need for a range of response capabilities between «persuasion» and «lethal force». Whilst RCA are in use for a variety of different scenarios – and the CWC contains a functional definition for RCA – it remained unclear what benefits could be gained from the use of ICA.

Science and technology have not changed fundamentally since entry into force of the CWC, when ICA were also discussed but perceptions in relation to armed conflict versus law enforcement may be different today. In light of new roles taken on by military forces in the form of peacekeeping operations and similar scenarios, where is the borderline between law enforcement and combat use, which legal framework(s) would apply? What would the impact be on the CWC, if ICA were accepted in law enforcement and possibly incorporated into military structure for «military operations other than war»? If there is no clear view on what is permitted, then the risk is, that ICAs could be introduced (more) into such scenarios. The types and quantities aspects of agents as well as delivery systems that might be developed for disseminating such agents are critical to the debate in the context of the CWC – in particular to the prohibition of the development and possession of chemical weapons and the prohibition of any military preparations for their use. Furthermore, if ICA were to be developed, stockpiled and used, proliferation will be unavoidable.

The CWC has been a success story and is not about to collapse on account of the ICA issue. But ICA may well represent a first step onto a slippery slope at the end of which countries may start re-arming with a new generation of chemical weapons, more developed than the ones currently being destroyed.

Despite all the ideas presented on the ICA problem, there is a risk of going round in circles – breaking out is the key challenge. Any future debate must include all key stakeholders, in particular the law enforcement community. Law enforcement organisations in the traditional sense have yet to take a public stance on whether they see a need or justification for ICA. Progress on CBW arms control and disarmament is notoriously slow. Extensive and active NGO interest and campaigning however have been critical factors in securing action in the past. It therefore is highly desirable to engage these communities in further discussions on ICA on the way ahead. One possible approach might be to see the establishment of a process similar to the Meetings of Experts in the framework of the BWC, where the main aim was to «promote common understandings». Most importantly though, the debate needs to broaden out to include other states, who thus far have shown little interest in the issue. We hope therefore that this report will assist future deliberations.

A full copy can be ordered at: laborspiez@babs.admin.ch

Microreactor Technology in Warfare Agent Chemistry

Andreas Zaugg

Even though the use of microreactors for synthesising classical chemical warfare agents or other compounds scheduled in the CWC (Chemical Weapons Convention) has not been published to date, the new technology has attracted the attention of the organic chemistry group of SPIEZ LABORATORY. Studies of the group show that in a few areas and sectors of chemistry, microreactors can provide a good alternative to the batch procedure. In classic warfare chemistry however, the technology can bring no benefits, since many reactions produce a solid are thus entirely unsuitable for microreactors.

Microreactor-Sytems

Round bottom glass flasks have always been used in organic chemistry for conducting reactions. Processes ranging from one milligram to several grams and reactive volumes from less than one millilitre to several litres are absolutely common. Much time and energy is spent in searching for the best reaction parameters. Later, further problems arise during up-scaling, requiring further refinement and adjustment of the reaction parameters. Because of these recurring problems, micro structured continuous flow reactors have experienced fast-paced development since the 1990s [1]. These reactors consist of minute channels embedded in a flat body of material (chip, Fig. 1) or of shaped ducts (Fig. 2) such as tubes of chemically resistant material. Glass, PTFE (Teflon), stainless steel or Hastelloy is used most frequently.

The minute dimensions of the channels in the range of 10 – 1000 µm form an extremely large surface to volume ratio from which chemical reactions benefit due to optimum heat exchange and good mixing. The rapid heat exchange and efficient mixing greatly enhance the process, which often results in better selectivity, yield and purity [2]. The system particularly brings advantages when synthesising instable or highly reactive (explosive) substances as the product can on the one hand be processed further after formation and, on the other, always only small amounts are present in the reactor for a short time.

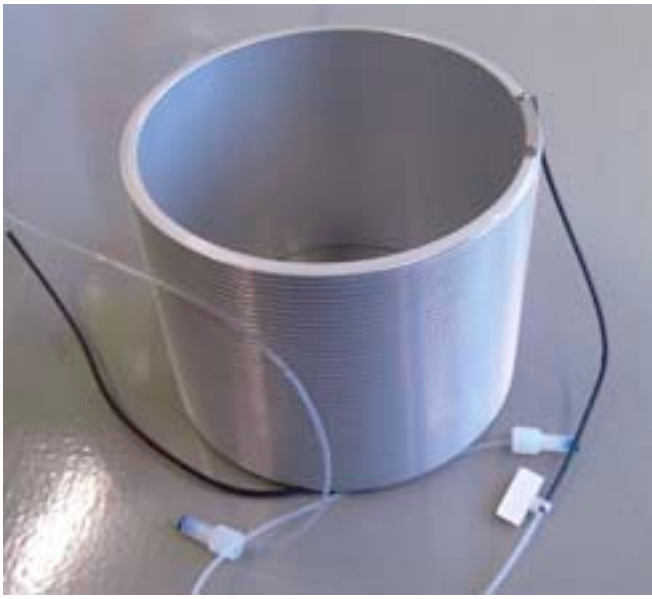
For about ten years now, microreactors have been commercially obtainable in a variety of forms. Specialised firms even manufacture reactors to order. But apart from reactors entire systems can also be purchased as compact devices (Fig. 3). Along with the necessary pumps they also contain heatable holders for the reactors, fraction collectors and pressure

[1] L. Rumi, «Beitrag zur Grünen Chemie: Anwendung der Mikroreaktor Technologie und neuartiger heterogener Palladium(0)-Katalysatoren auf Grafitoxid-Basis' doctoral thesis, 2009, Faculty for Chemistry, Pharmacy and Earth Sciences at the Albert-Ludwigs University in Freiburg, Germany.

[2] K. Geyer, «Fabrication and Use of Microreactors for Synthetic Organic Chemistry», Doctoral Thesis; 2009, ETH no. 18148, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland.

Fig. 1: Glass mixer chip

Fig. 2: Teflon coil reactor Uniqsis



systems for conducting reactions above the boiling point of the solvents used, similar to the microwave system. In most cases the devices are computer controlled so that a reaction sequence can be carried out under various parameters (optimising the reaction). Continuously, new peripheral equipment is being added to the market such as liquid handlers or diagnosis programmes.

What is known as flow chemistry has also found its way into industry. Here the advantages of smaller dimensions as opposed to batch processes are especially to be made use of. In implementing this technology from laboratory dimension to major dimension scaling up (enlargement of the microreactors) is preferred over scaling out (microreactors in parallel). Because of this and due to its supposedly novel capabilities this new technology, has been discussed in international export control forums (Australia Group, Scientific Advisory Board of the OPCW). For this reason microreactor technology attracted also the attention of SPIEZ LABORATORY, although the use of microreactors for synthesising classical chemical warfare agents or other compounds scheduled in the CWC (Chemical Weapons Convention) has not been published to date.

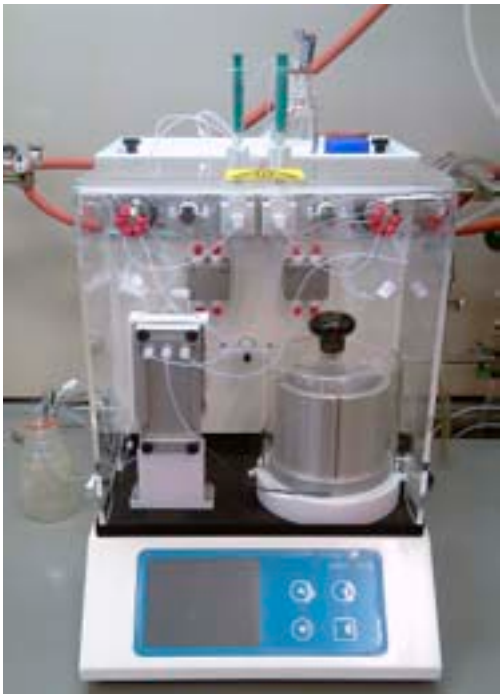


Fig. 3: FlowSyn™ Uniqsis

Statistics
Often new technologies are praised as the solution to all problems. That was the case when microwave technology was introduced and it is now similar with microreactor technology. Since 1950, microwave technology is used for organic synthesis and has doubtlessly brought advantages in many areas. But it has not revolutionised organic chemistry. A comparison of the publications and patents of both subjects should reveal the overall picture.

Figure 4 shows that the number of scientific publications relating to microreactors has continuously grown over the last ten years until 2005 and from then on has remained at a relatively stable, more modest level. The number of patents shows a similar development.
In contrast, the already well known microwave technology is the object of a multitude of publications and patents (Figure 5), whose number is still increasing [3].

A second theoretical approach for categorising microreactor technology regarding usability for the synthesis of chemical warfare agents and related substances can be made with the help of an evaluation of the standard syntheses used by the organic chemistry group of SPIEZ LABORATORY. Standard procedures are grouped according to the following criteria gleaned from literature and their suitability for microreactors evaluated.

- Reactions that function fairly well with batch processing, but have a reaction time of >60 min combined with a reaction temperature of 100 °C or more, can be carried out with the microreactor system with difficulty only or not at all.
- Reactions that produce solids during synthesis cannot be carried out with microreactor technology. Precipitations cause blockage of the system and immediate termination of the reaction process.

On the basis of analysis of 81 different reactions relating to warfare agent chemistry such as esterification, chlorination, fluorination, cyanidation, sulphuration, amidation, hydrolysis and oxidation, the standard processes of SPIEZ LABORATORY were rated as compatible or incompatible with microreactors (Figure 6).

So, after applying the selected criteria, microreactor technology can only be applied to 25 % of the standard reaction procedures used by our organic chemistry group. 75 %, of which 66 % due to solids and 9 % due to bad kinetics, are incompatible with microreactor technology. A similar result has been published [4]. Thus, after evaluating 86 different industrial processes, the Lonza company in Visp came to the conclusion that 63 % of these processes could not be carried out with microreactor technology.

[3] C. O. Kappe, D. Dallinger, S. S. Murphree, «Practical Microwave Synthesis for Organic Chemists», ed. WILEY-VCH, 2009.

[4] D. M. Roberge, L. Ducry, N. Bieler, P. Cretton, B. Zimmermann, «Microreactor Technology: A Revolution for the Fine Chemical and Pharmaceutical Industries?», Chem. Eng. Technol., 28 (3), 2005.

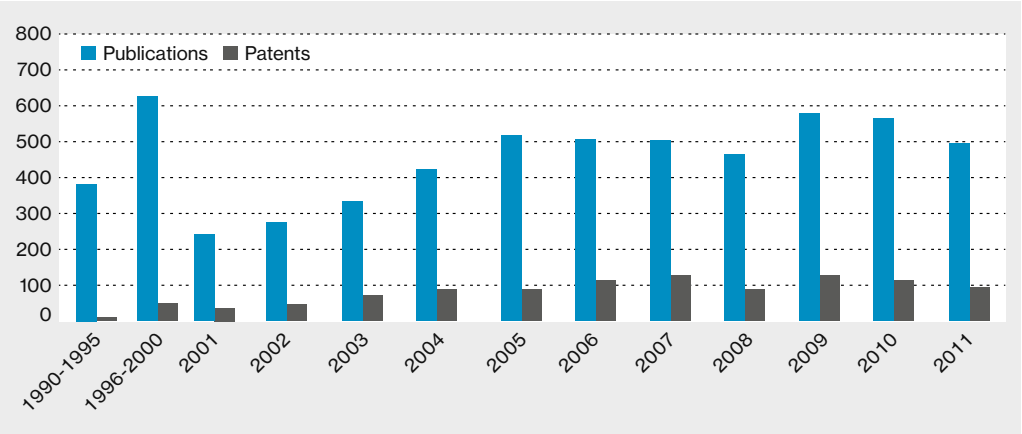


Fig. 4: Number of publications and patents relating to microreactors (2001 until today and 5-year summary 1990 – 2000). Source: SciFinder, full text search «microreactor» on 14 February 2012.

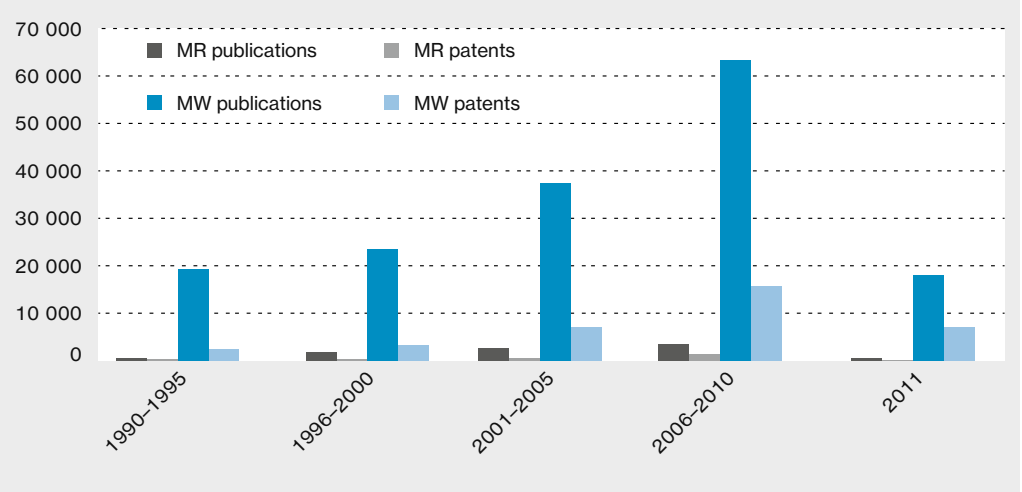


Fig. 5: Comparison of number of publications and patents relating to microwave and microreactor technology. Source: SciFinder, full text search «microwave» (MW) and «microreactor» (MR) on 14 February 2012.

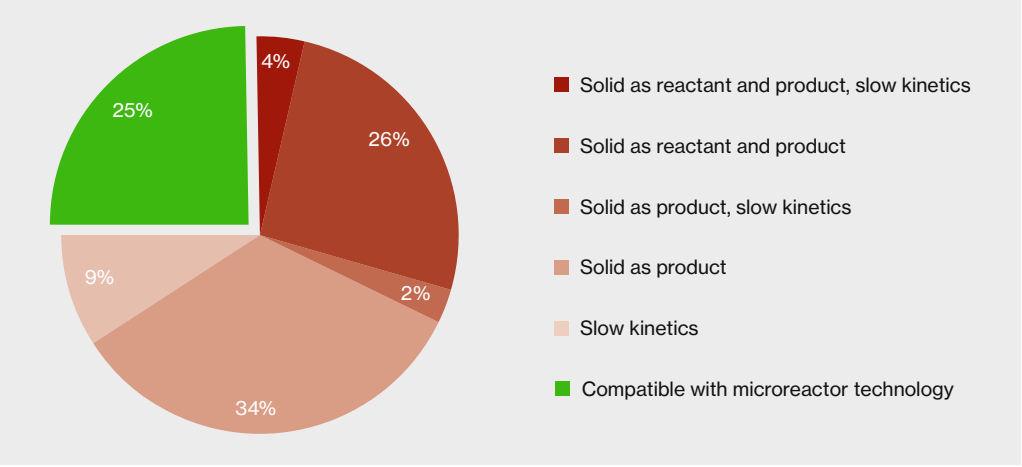


Fig. 6: Evaluation of 81 batch methods routinely used in chemical warfare chemistry by the SPIEZ LABORATORY regarding the suitability for microreactor technology. The percentage of microreactor compatible reactions is shown in green (25 %), that of incompatible reactions in red (75 %).

Application examples

The examples of bis(2-chloroethyl)sulphide, methylphosphonyl dichloride and bis(2-chloroethyl)ethylamine synthesis clearly show that microreactor technology is unsuitable for reactions that produce solids or reactions with bad kinetics.

Bis(2-chloroethyl)sulphide can be synthesised according to conventional batch methods with a yield of 95 % and a GC/MS purity of 99 % without difficulty and relatively fast (Figure 7). After a reaction time of 1 h with a 350 ml standard lab sulfonation flask, the amount obtained is about 30 g.

The same reaction and method also works very well in a microreactor system. With a yield of 92 % and a GC/MS purity of 97 %, the values obtained are practically equally good as with the conventional batch procedure.

For synthesising methylphosphonyl dichloride the two ester groups are substituted with one chlorine atom each in two subsequent steps, but without isolating the intermediary product (Figure 8). From the kinetic point of view (approx. 15 min), the first step does not provide a great obstacle for the batch process. For the second step to the end product, however, considerably more energy has to be invested. In the batch process 105 °C are required for 3 h. A yield of 80 % and a ^{31}P-NMR purity of 98 % can be achieved with this method.
In a microreactor system the first step is achieved to 96 % within a retention time of 4 min and at a temperature of 100 °C. But the second step could never be fully accomplished. In the best case only 35 % of the end product could be detected.

Bis(2-chloroethyl)ethylamine can be obtained with the batch method after 2 h with a 87 % yield and a ^{1}H-NMR purity of >95 % (Figure 9). The product always appears in the form of its hydrochloride salt.

Efforts to synthesise bis(2-chloroethyl)ethylamine according to this method in a microreactor system were unsuccessful. After less than 20 sec., the ensuing hydrochloride salt of the product precipitated, blocking the reactor. An immediate increase in pressure resulted and the device shut off automatically.

Conclusion

Microreactor technology is currently regarded as promising new technology with a broad range of possible applications in organic chemistry. However, our experience shows that this technology, like many others, cannot solve all problems. Certainly, in a few areas and sectors of chemistry microreactor technology will provide a good or even better alternative to the batch procedure. In classic warfare chemistry, however, it can bring no benefits when compared to the conventional batch procedure, as many reactions produce a solid, the main criterion excluding microreactor technology. Furthermore, an individual setup has to be developed for practically every batch procedure that is to be replaced with microreactor technology, requiring much effort and time.

Of course it must not be forgotten that new developments in this technology could make it possible to conduct further reactions with this system.

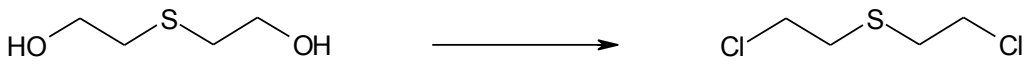


Fig. 7: Schematic presentation of bis(2-chloroethyl) sulphide synthesis.

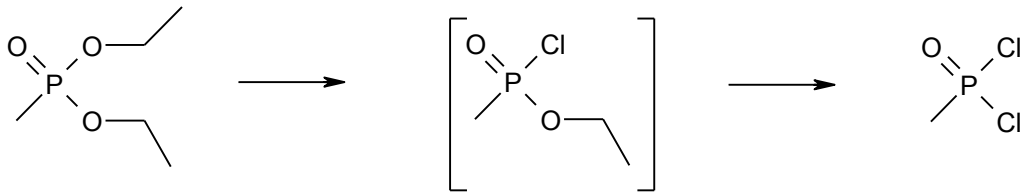
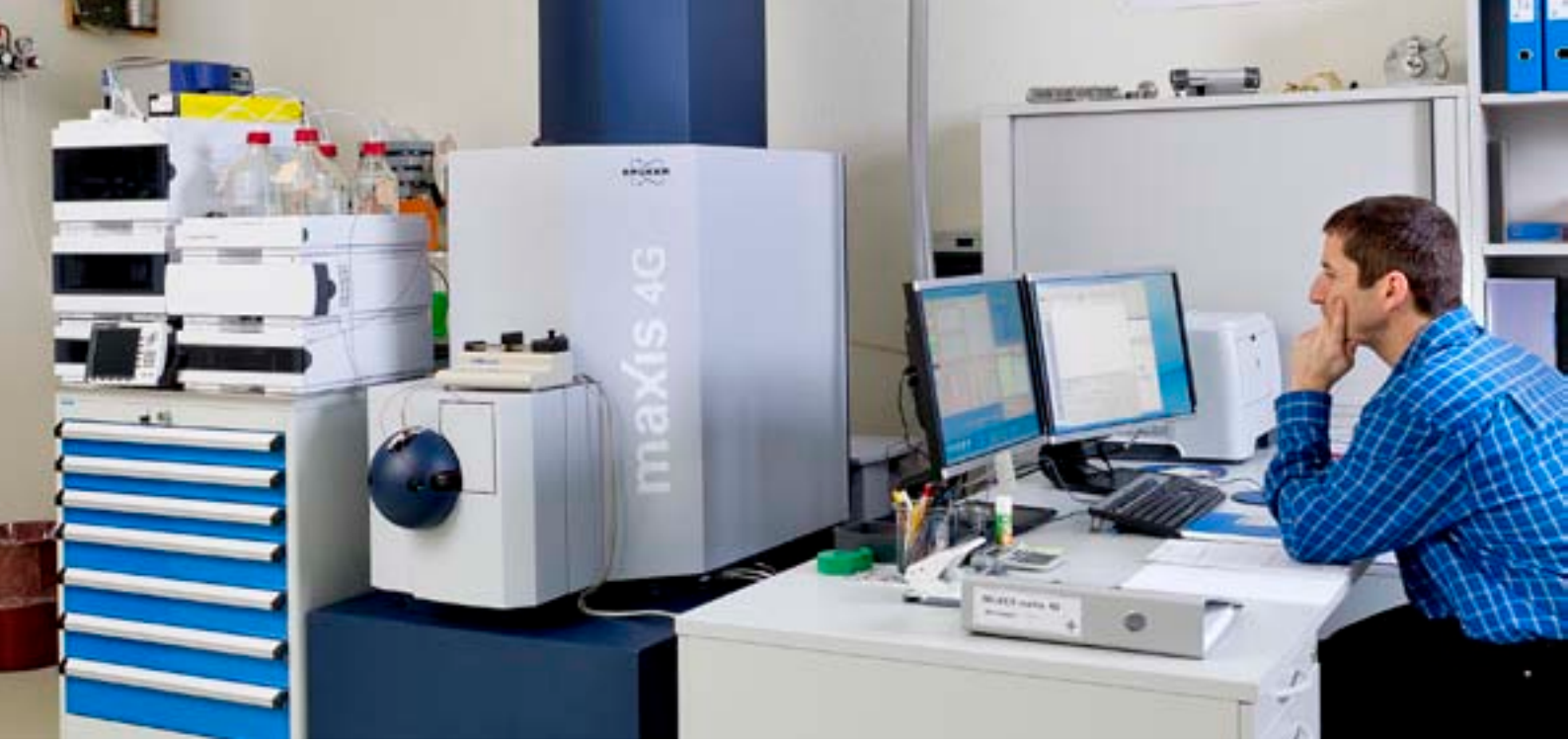


Fig. 8: Schematic presentation of methylphosphonyl dichloride synthesis.

Fig. 9: Schematic presentation of bis(2-chloroethyl) ethylamine synthesis.



Method Development in the Analytical Chemistry Group

Dr. Peter Siegenthaler

One of the most important challenges for the work of the Analytical Chemistry group is the continuous development of methods for reliable detection of chemical warfare agents (CWA) and CWA-related compounds in difficult matrices, e.g. in decontamination solutions: due to their complex composition and aggressive chemical characteristics as well as the large number of acidic, alkaline and neutral agent degradation products, these solutions constitute a particular challenge to analysts.

Liquid decontamination samples can be screened directly without sample preparation through nuclear magnetic resonance spectrometry (NMR) for the presence of phosphorous nerve agent degradation products (31-P-NMR). In the 1-H-NMR spectra of non-phosphorous degradation products, however, some of the substance signals are masked by signals from components of the decontamination solution. What is known as the LC-SPE technique can provide a remedy, where the relevant compounds are separated from the interfering matrix components by high performance liquid chromatography (LC, HPLC), concentrated through solid phase extraction (SPE), eluted and analysed either directly through NMR or with gas chromatographic methods (GC) after derivatisation. Alternatively, samples can be directly examined after neutralisation and/or dilution with liquid chromatography - mass spectrometry (LC-MS). With this technique it is difficult to separate compounds that elute early and their signals are often suppressed by co-eluting matrix components. The preparation of samples for GC analysis is particularly laborious; the general approach is to specifically extract the relevant compounds out of the matrix or to remove the interfering decontamination components prior to derivatisation and analysis and then to apply a suitable derivatisation procedure.

In collaboration with our Finnish research partner VERIFIN (Finnish Institute for Verification of the Chemical Weapons Convention) various projects on processing and analysing difficult matrices were worked on in 2011 and the results were documented in several reports. Furthermore, in December 2011, after two years of intensive work by VERIFIN and various institutes from all over the world the new 2011 edition on «Recommended Operating Procedures for Analysis in the Verification of Chemical Dis-

maXis 4G Q-TOF LC-MS System by Bruker Daltonics

OPCW designated laboratory

The Analytical Chemistry group of SPIEZ LABORATORY runs an accredited laboratory for the analysis of chemical warfare agents (CWA) and related compounds in different types of samples. The laboratory uses state of the art instrumentation and procedures for sample preparation and analysis of a wide variety of sample types as well as databases and reference standards for confirmation of identification.

For the Swiss authorities as well as international organisations samples from all over the world are tested for the presence of CWA and other toxic chemicals. The group must demonstrate its expertise regularly in international proficiency tests. In the spring of 2011, the Analytical Chemistry group again obtained the maximum rating of A in the 29th Proficiency Test of the Organisation for the Prohibition of Chemical Weapons (OPCW), thus ensuring its status as designated laboratory of the OPCW for another year.

As OPCW designated laboratory SPIEZ LABORATORY may receive samples from inspections for analysis and supports the organisation in proficiency testing. Furthermore, it contributes to the development of the OPCW Central Analytical Database (OCAD). The OCAD is the reference database for inspectors and contains an extensive collection of mass spectra, retention indexes as well as infrared and magnetic nuclear resonance spectra. The submission of analytical data for the expansion of the OCAD has a long tradition at SPIEZ LABORATORY. In 2011, 170 mass spectra and 170 retention indexes data were contributed to the OPCW and the submission of new sets of data was offered. To date, SPIEZ LABORATORY has provided about 60 percent of the approximately 10 000 entries in the OCAD.

armament» could be published. These so-called «blue books» contain a collection of standardised operating procedures for the testing of different samples for the presence of chemical warfare agents and related compounds. The first version of blue books was published in 1989 and was updated last in 1994. For the 2011 edition the staff of the Analytical Chemistry group contributed to all sections of the book as chapter coordinator, authors or reviewers.

Development of instrument park and extension of databases

In 2011, we were able to procure a maXis 4G Q-TOF LC-MS system from Bruker Daltonics which has opened new possibilities for verification analysis: Due to its high resolution (60 000) and mass precision (< 2ppm) the time of flight mass analyser (TOF) allows specific and sensitive screening for known compounds and simplifies the identification of unknown compounds by predicting their molecular formula.

Furthermore, the in-house database was extended in 2011 with electron impact (EI) mass spectra and retention indexes for chemical warfare agents (CWA) and other CWA-related-compounds with data from own synthesis and measuring campaigns as well as data from other sources. The CWA database currently contains about 9700 EI mass spectra. On the basis of commercially available databases, a screening database was compiled with more than 23 000 EI mass spectra from drugs, pesticides and other toxic chemicals. In addition, the LC-MS database with electrospray mass spectra and the database with NMR spectra are continuously being extended.

Testing of High Performance Particulate Matter Filters

Andres Wittwer

When NBC agents are released, the elimination of particulate matter from the air (aerosol) is crucial for respiratory protection. Due to progress in biotechnology and pharmacy, the hazard potential of aerosols is even gaining in importance in comparison with gaseous compounds. In 2011, SPIEZ LABORATORY was able to take an updated test stand into operation which fully satisfies the technical measuring requirements of the relevant EN 1822 standard for classifying and testing HEPA and ULPA filters. Furthermore, the full separation characteristic is measured in each test, which enhances its quality and informative value.

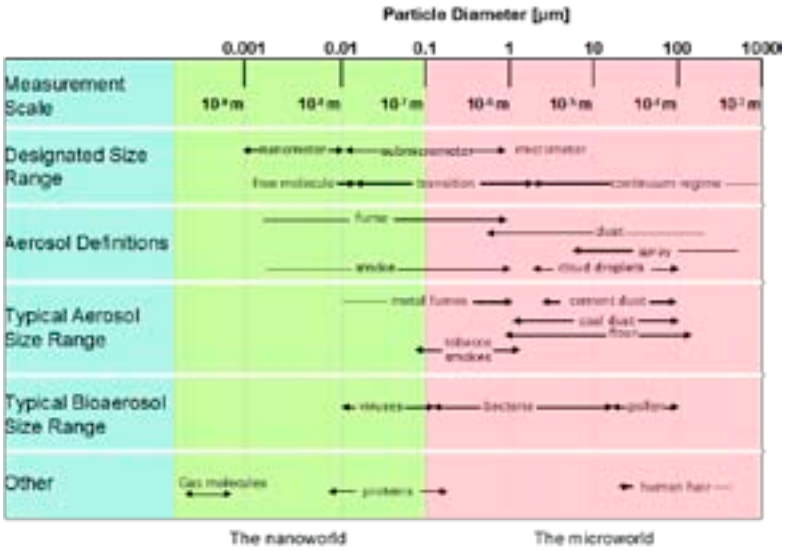
Upon a release of chemical, biological or radiological agents, the separation of particulate matter from the air (aerosol) is crucial for respiratory protection.

Progress in biotechnology and pharmacy leads to new production options for biological agents and increasingly effective chemical substances that interact even more specifically with biological processes. These substances possess such a molecular size, that they can be dispersed in the air in aerosol form only, like biological germs. Thus the hazard potential of aerosols tends to gain in importance as compared to gaseous compounds. High efficiency particulate and ultra low penetration air filters (HEPA and ULPA), based on «glass fibre papers» with fibre diameters in the micrometer range have been used in respiratory protection and ventilation technology for a long time. Upcoming nano fibre technology offers new possibilities.

For physical reasons, different separation mechanisms take effect depending on particle size. In the transition zone between diffusion and mass inertia effects, a separation minimum (or penetration maximum) of aerosol filters is observed for a certain particle size (most penetrating particle size, MPPS for short). Its position is characteristic for filter material and flow conditions and commonly lies in the range of 0.1 to 0.3 micrometers (µm). Due to similar physical laws, the centre of particle size distribution for stable harmful aerosols in the atmosphere often lies in the same size range.

Clear and strict metrological requirements for characterising and testing such filters follow

Overall view of the «Mammut 3» test stand with filter being tested (NBC protection filter for 150 m³/h filtered air for shelters)



Overall view of aerosol particle sizes and origin

from the facts above:

- Testing must be carried out within the range of MPPS, that means
- sensitivity and size resolution capacity within the respective range
- generation of a corresponding test aerosol
- Use of particle counting methods to achieve sufficient detection sensitivity, (ULPA filter: separation efficiency at least 99.9995 %).

Already in the early 1990s, SPIEZ LABORATORY was able to begin operating a test stand that principally* met the requirements mentioned above and was thus capable of routinely testing and imposing the respective requirements for NBC filters (* with external proof that the MMPS really were in the range of 0.1 to 0.3 µm).

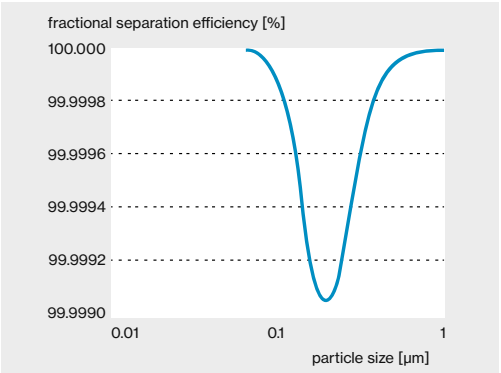
EN 1822, today's relevant standard for the classification and testing of HEPA and ULPA filters, came into force in 1998 with considerable technical support from SPIEZ LABORATORY.

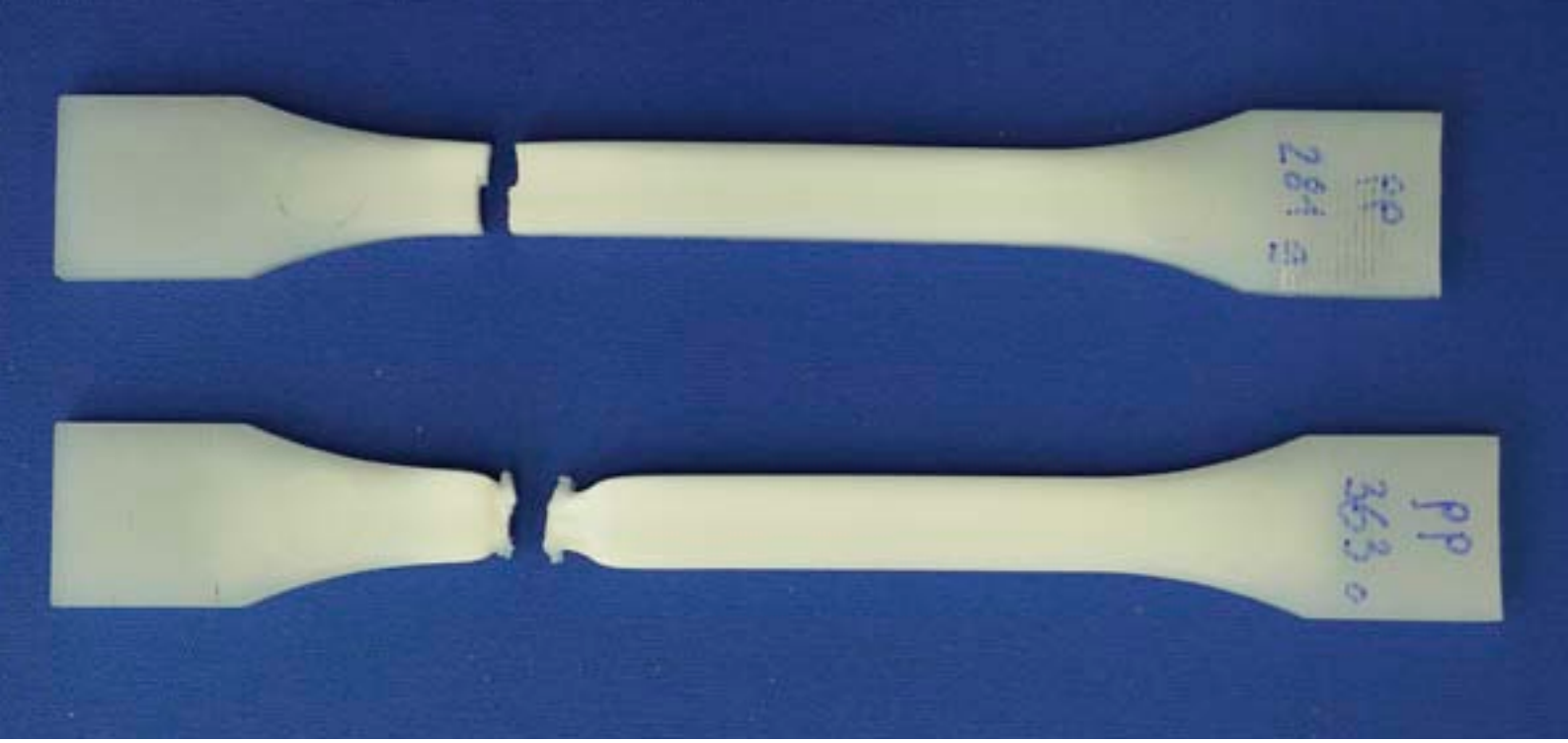
Last year, we were finally able to put into operation the metrologically updated and optimised test stand with a highly sensitive aerosol spectrometer originally developed for atmospheric research.

Now, the metrological requirements of EN 1822 can be fully met. The degree of separation in dependence to particle size (separation characteristic) is measured in each test; this in a short measurement time, avoiding an excessive load (alteration) of the filter with test aerosol.

Comparison of alterations in the separation characteristic to that of the pure filtration medium makes it easier to identify the causes of a problem (such as lesions in the media, non-uniform face flow velocity, leaking probe downstream of filter) than before on the basis of mean value and flow resistance only. And, finally, the efficiency curve allows the assessment of effective filter separation performance for a particular harmful aerosol if its particle size distribution is known.

Fractional separation efficiency curve (filter efficiency in dependence of particle size)





Processing Test for Plastic Parts by Determining Molecular Mass

Thomas Friedrich

In a study the STS 036 testing laboratory has compared four test methods for determining molecular weight. The purpose was to find out which method would be most suitable for a daily test routine to verify the optimal processing quality for thermoplastic form parts and to detect possible material damages. Among other things, these procedures are based on the fact that the viscosity of plastic melts and solutions decrease with diminishing molecular weight.

Thermal and mechanical stressing of thermoplastics during processing (extrusion, injection moulding etc.) may cleave molecules and thus result in a reduction of mean molecular weight. This causes a change/decline in material properties as these depend significantly on mean molecular weight. Therefore, as a quality assurance measure the processing quality of plastic parts must be checked.

With the flow test device the plastic is melted in a heated cylinder. The melt is pressed out of a nozzle by a piston under constant force. The flow rate of the plastic is a measure for its viscosity and thus an indirect indication of its molecular weight (Fig.1)

In the case of the rotational rheometer the plastic material is melted between two plates. Through cyclic rotation of one of the plates in both directions the plastic melt is exposed to changing shear forces. Viscosity, and thus indirectly molecular weight, is determined from the measured angles of rotation and torques as well as the plate distance. (Fig. 2)

In the case of solution viscometry a diluted solution is made of a plastic sample with a suitable solvent. The flow times are measured in sequence for the solution as compared to the pure solvent for passing through a glass capillary. From these values the viscosity number is computed which again provides an indirect measure for the molecular weight of the plastic material. (Fig. 3)

In the case of gel permeation chromatography GPC the diluted solution of plastic flows through a separation column packed with porous material. Statistically, the smaller plastic molecules find more pores where they can stay for a certain time than the large molecules. Therefore, the large molecules appear first at the exit of the separation column, followed by the medium and the small molecules. Through prior calibra-

Fracture images of material after tensile impact tests: Damaged (top) and optimal (bottom)

Fig. 1: Flow test device

Fig. 2:
Rotational rheometer

tion with plastic materials of known molecular weight, the distribution of the molecular weight of the material being tested can be determined. (Fig. 4)

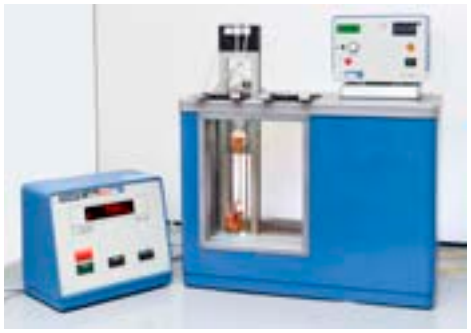
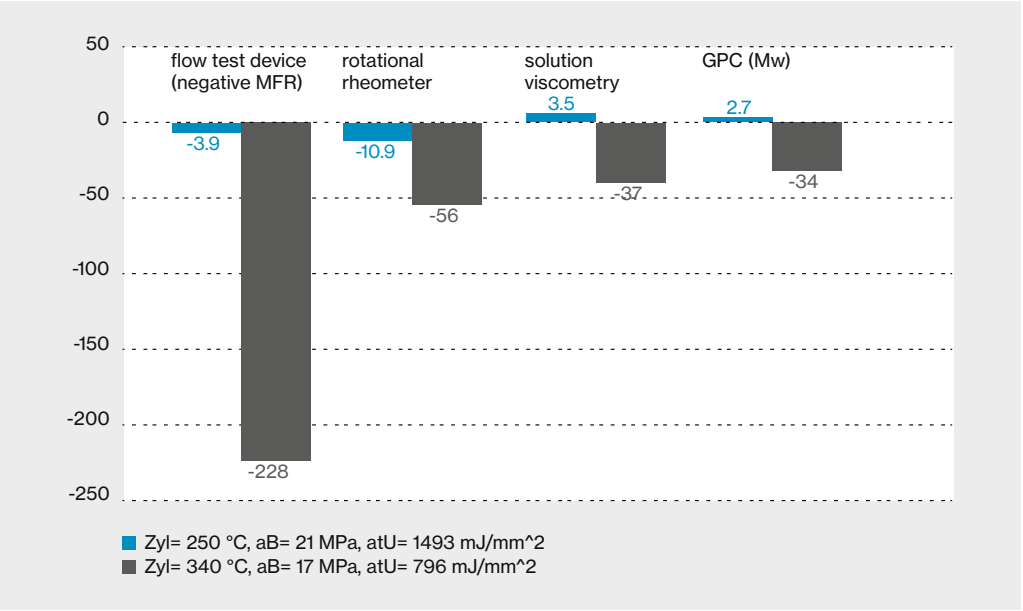


Fig. 3:
Solution viscometry

Fig. 4: Gel permeation chromatography GPC

Fig 5: Measuring results on polypropylene PP



Measuring Results and Interpretations

The basic findings of the study can be exemplified with the measuring results of one of six tested materials. The length of the bars in figure 5 illustrates the change measured in the material for each test method. So a long bar means that the respective method responds sensitively to changes. A short bar means that the method either confirms that the processing was optimal or that the former is not sensitive enough to detect even small differences.

With carefully processed material none of the investigated test methods is able to detect a change (blue bars). This confirms that the material really has been processed optimally. But for material that had been deliberately processed at higher temperatures it was possible to clearly show that changes had taken place (red bars). Damage to the material can be detected by far the most clearly with the flow test device.

That we are really dealing with damage to the material is confirmed by additionally measuring its mechanical properties. Breaking stress is reduced by 20 % and tensile impact strength by 50 %. The material has become brittle through the high temperature at which it has been processed. This is also clearly evident from the fracture images of the material after tensile impact tests. (see image on page 36)

Conclusion

Melt viscometry with the flow test device has proven to be more sensitive than three other methods for all investigated materials and thus the most reliable, fastest and least expensive method for determining molecular weight. While this method makes it possible to prove optimum processing quality, it also allows detection of even small changes in the material caused by processing.

Processing Test for Plastic Parts of NBC Protection Equipment

In technical equipment for NBC protection plastics are often used in the form of films, fabrics, form parts etc. In order to ensure that these plastic parts fulfil their protective function in an emergency, their material properties also have to be verified. For this purpose the material testing group of SPIEZ LABORATORY operates a testing laboratory (STS 036) that is accredited according to ISO 17025, which focuses on the analysis of polymers, i.e. thermoplastics, thermoplastic elastomers, thermosets and elastomers. As a speciality, resistance tests against chemical warfare agents are offered for plastics, elastomers and textiles.

Personal NBC Protection Equipment Handbook

Dr. Patrick Wick

The individual protection team analyses and tests the function of NBC protective materials. The expertise entailed is based on complex physical, chemical and physiological relationships that are understood by a limited circle of experts only. In contrast, however, practical experience (armed forces, civil protection, industrial plant security), shows that demand for clear information on personal NBC protection equipment is widespread. A handbook elaborated by SPIEZ LABORATORY serves to close this gap.*

There is a great variety of personal protective equipment (PPE) products. Material requirements, tests and designation are standardised. Although the field of personal NBC protection equipment is but one sector, it comprises approx. 75 different standards and a great number and variety of products. This handbook should provide an overview by providing understandable and practical information. SPIEZ LABORATORY with its specialist section for NBC protection technology has extensive expertise and wishes to address elements of both civil protection and private industrial plant security involving personal NBC protection equipment.

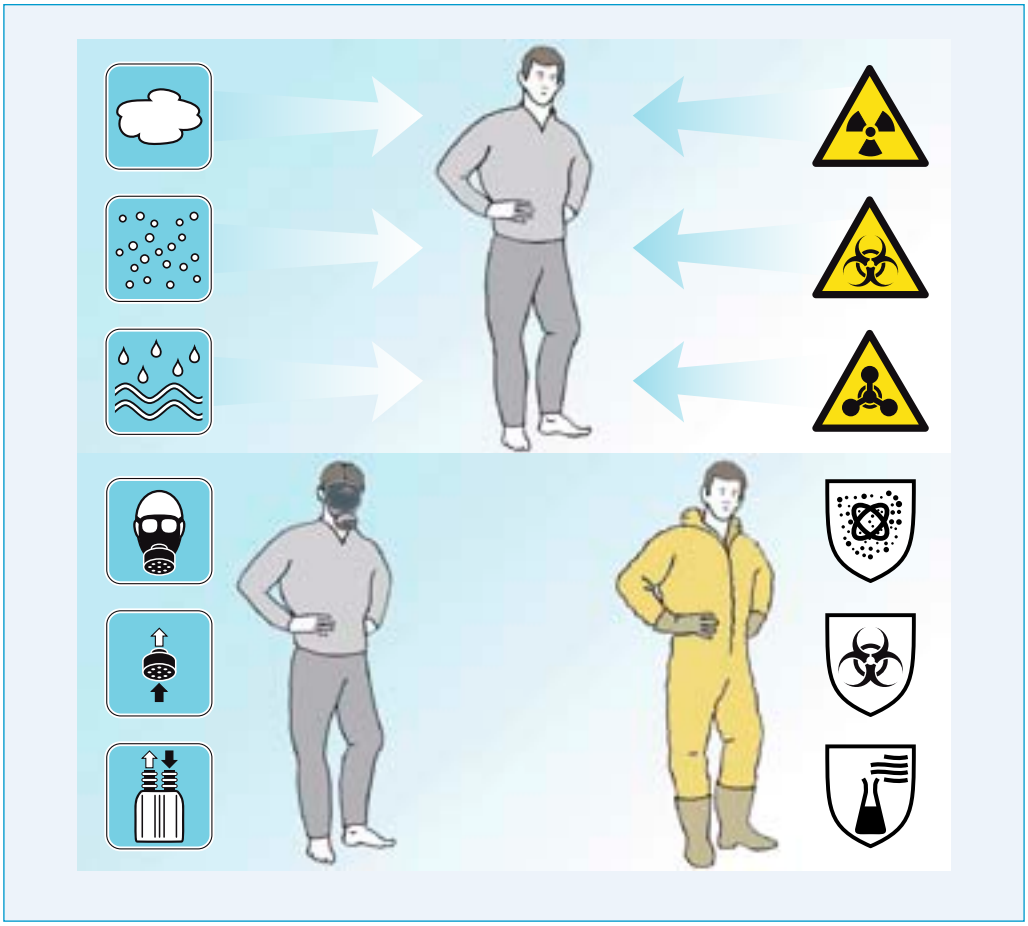
The safe and best use of PPE demands both theoretical understanding and practical skills that enable the correct choice of PPE for a certain hazard.

The first chapter of the handbook provides insight into the hazard from NBC agents: in what form do they arise, how do they affect humans, what classifications exist for chemicals and what are their known threshold values?

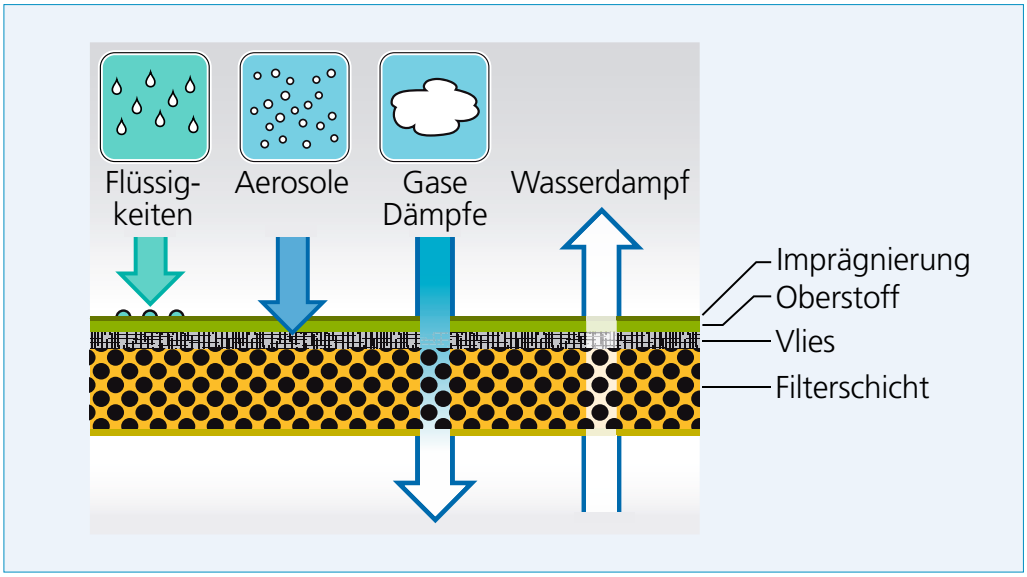
The second chapter describes the NBC protection materials and is subdivided into respiratory and skin protection. Fundamental basic physical and chemical principles are explained that help us to better understand the way the protective equipment functions. Such basic knowledge enables us to derive what equipment is required for what hazard.

Every type of mask, filter or protective clothing is briefly presented in the handbook. The most important features are listed complete with their strengths and weaknesses. Symbols facilitate distinguishing between the various objects and graphs facilitate their overview.

Members of the DDPS task-force check their personal protective equipment during an exercise in Sonthofen, Germany

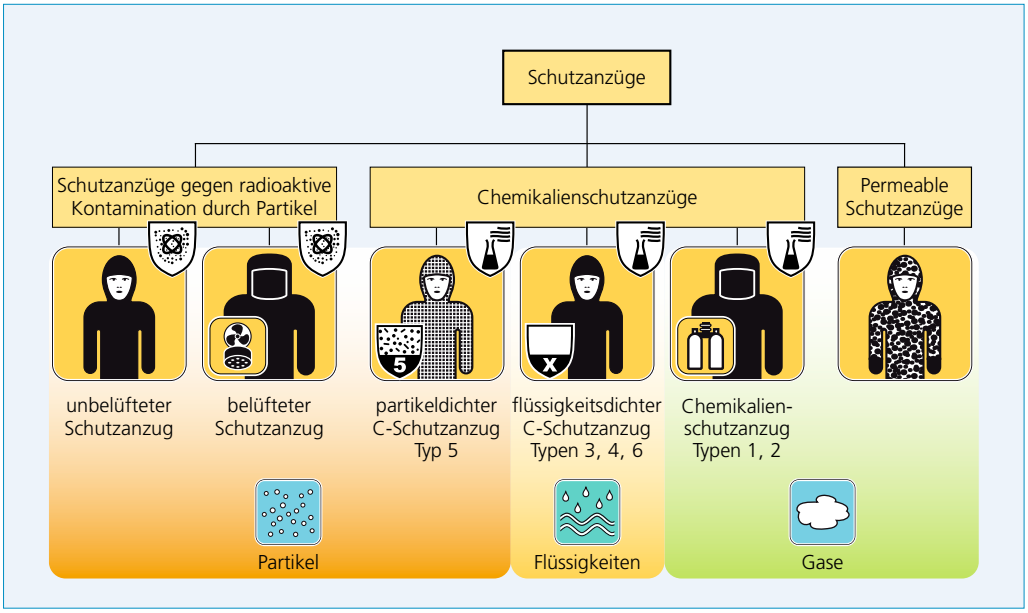


Hazard through NBC agents demand a suitable respiratory and skin protection



Function of a permeable protective suit

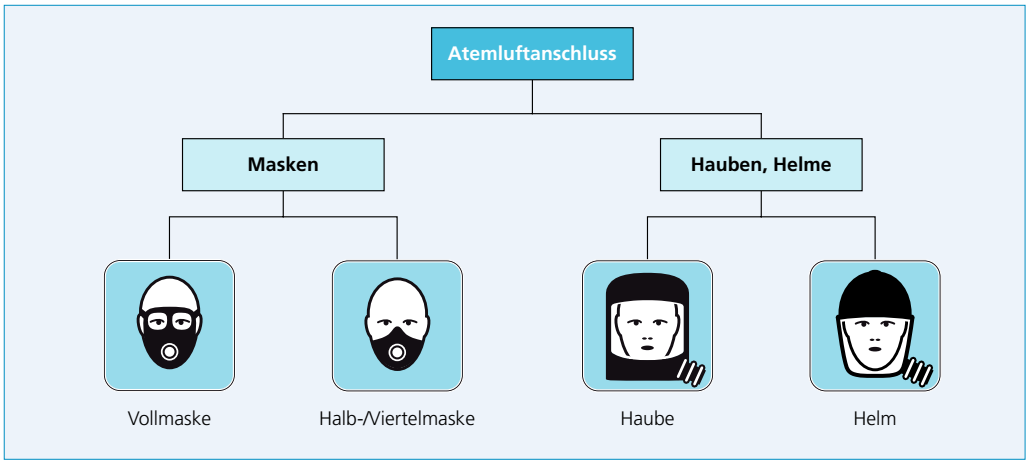
Overview of protective suits



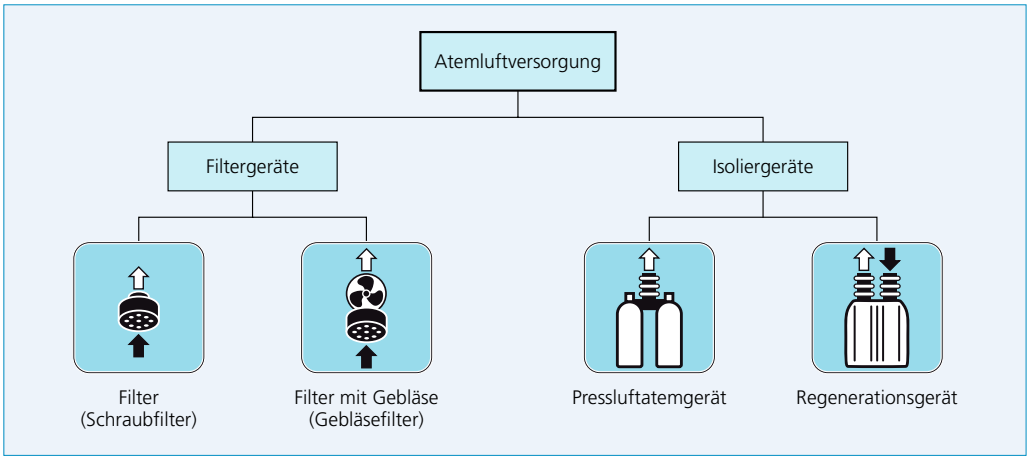
The final third chapter provides notes for evaluation, training, handling and use of personal NBC protective equipment. With these a partner organisation is able to derive what PPE satisfies its needs best on the basis of its operational concepts for defined scenarios. Here the tension between safety and practical comfort should always be taken into account. The following principle always applies: only material that is technically in perfect condition is suitable for operations. Therefore, the life cycle from procurement, storage, use, and decontamination to disposal must be planned in advance.

SPIEZ LABORATORY offers its expertise and supportive advice in procurement issues to the partner organisations of the Federal Office for Civil Protection.

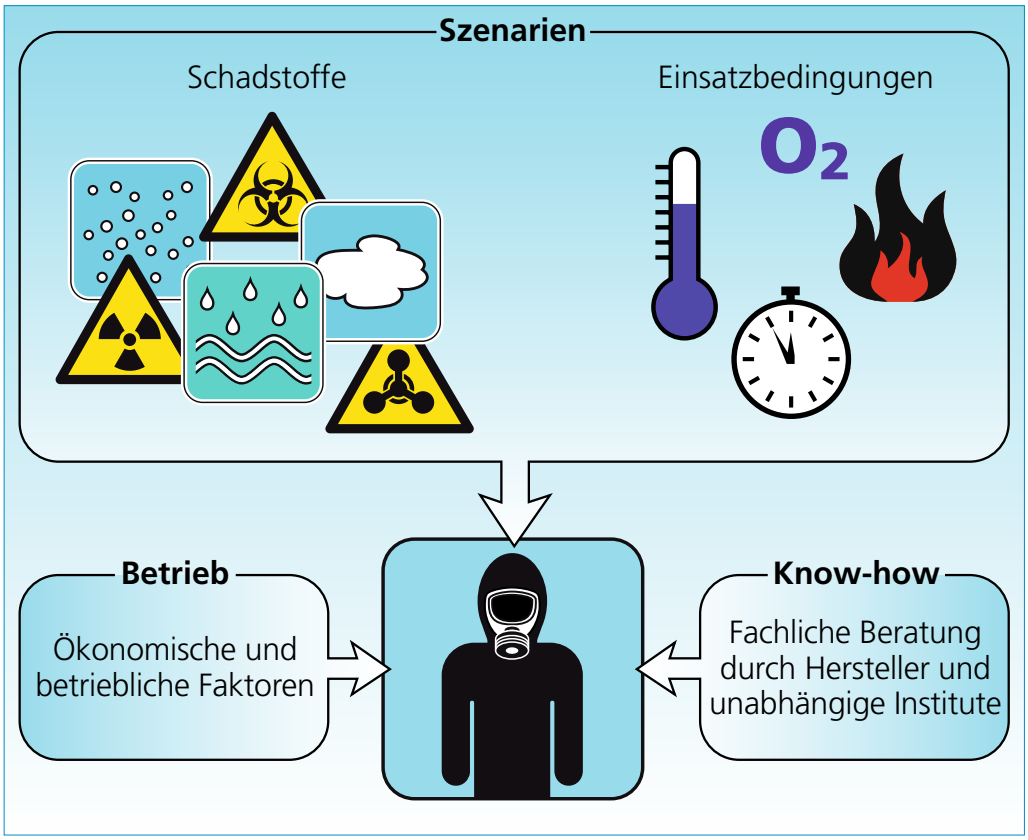
**A full copy of the handbook (in German) can be ordered as of August 2012 at: laborspiez@babs.admin.ch*



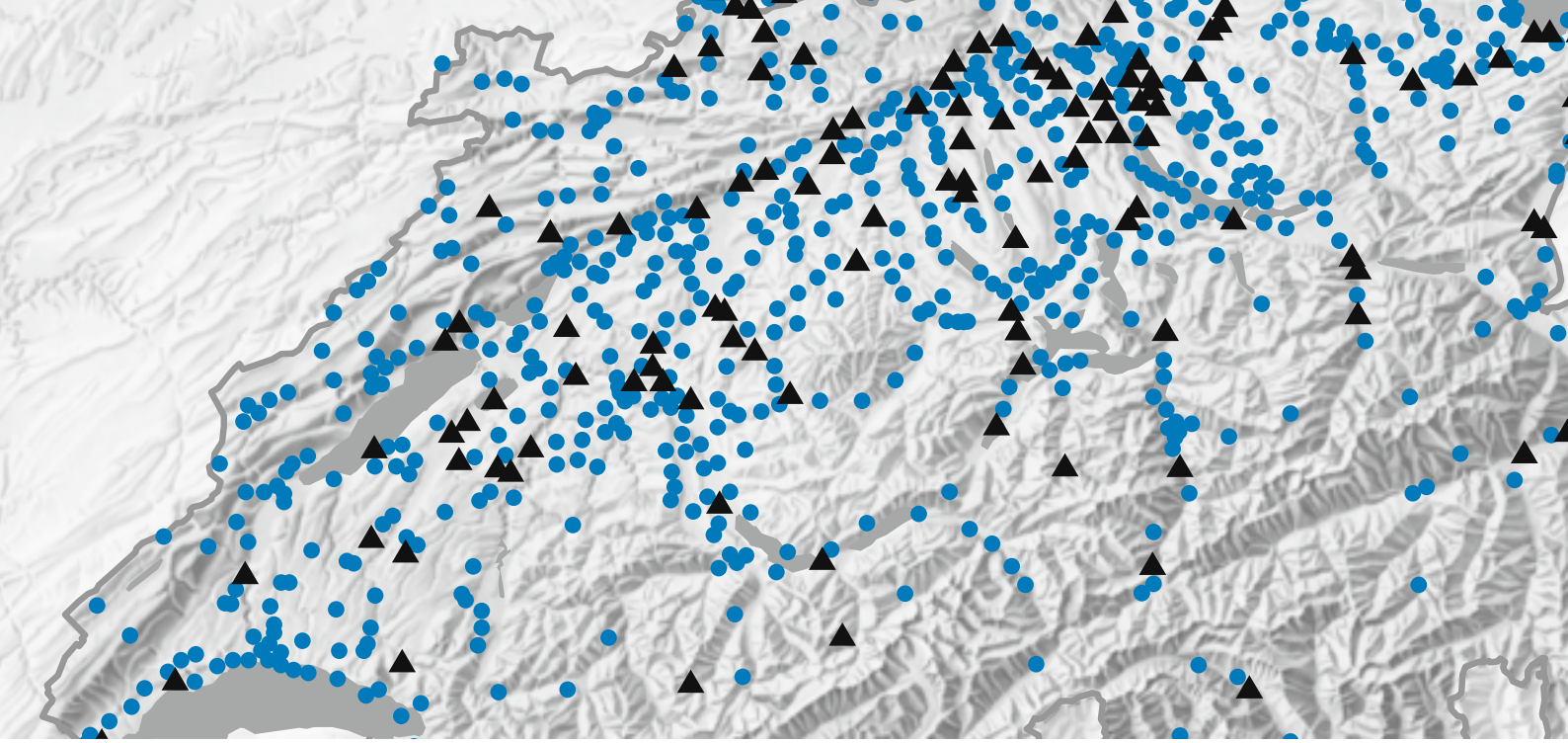
Overview of facepieces



Overview breathable air supply



Choosing the optimal personal protection equipment



NBC Contaminants

Dr. Daniel Storch

Within the context of the «National NBC» project the Federal Council appointed the NBC Protection Commission in 2007 to evaluate the legal foundations for NBC security. It was to clarify to what extent these suffice to prevent the theft or misuse of dangerous NBC agents. The investigations of the National NBC Protection and Coordination Office have shown that the acts and ordinances relating to radiation protection, nuclear energy, incident prevention and transports primarily regulate safety aspects (civil protection) and that the security problem (theft protection) of NBC agents is not always sufficiently taken into account.

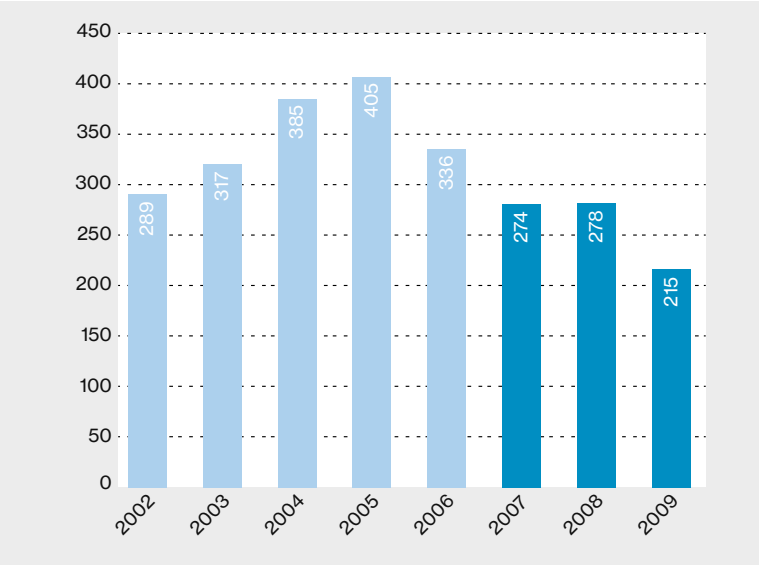
In 2010, the NBC Protection Commission passed the «NBC Security» report with nine recommendations. In 2011, that report was subjected to interdepartmental consultation by the Federal Office for Civil Protection FOCP and taken note of by the departments and federal offices concerned. Amounts of substances were considered dangerous whose misuse could have effects corresponding to the reference scenarios of the Swiss NBC protection strategy and could thus necessitate civil protection measures. For major sources that are suitable for misuse in the sense of the reference scenarios, the NBC Protection Commission considers the legal security requirements to be sufficient at the present time. Currently however, there are international efforts by the «NNSA's Global Threat Reduction Initiative (GTRI)», among other things to secure Caesium-137 sources in order to prevent their deliberate misuse (nuclear terrorism).

Recommendation 1: check whether the directives of the «National Nuclear Security Administrations Global Threat Reduction Initiative (GTRI) can be implemented in Switzerland.

The NBC Protection Commission sees a deficit in the fact that radiation protection experts are not trained in securing and heightening awareness with regard to the misuse of radioactive sources.

Recommendation 2: augmentation of radiation protection legislation so that radiation protection expert training attributes greater weight and commitment to the aspect of security. Furthermore, radiation protection experts should attend regular progressive training and refresher courses.

Recommendations 1 and 2 have been acknowledged and addressed within the context of the revision of radiation protection legislation and in connection with the issue of radiation hazards and training requirements. Here the direc-



Numbers of incidents/ thefts of radioactive materials per year recorded in the INTERPOL Geiger database in 2002-2009, including Project Rutherford data (2007-2009)

tives of the ICRP 103' and the European Union (EU) regarding the security of radiation sources have also been taken into account.

Biologic contaminants

There is currently no official arrangement for the import of human pathogens analogous to the Dual Use Control Act, which regulates export. The Dual Use Control Ordinance merely stipulates that human pathogens defined as dual use goods by the State Secretariat for Economic Affairs SECO require an import certificate. In the revised Epidemics Act this gap is expected to be closed and a permit obligation imposed for the import of certain germs.

Recommendation 5: introduction of a permit obligation for the import of Hazard Group 3 and 4 organisms.

Within the context of the on-going revision of the Epidemics Act (expected to enter into force in 2014) it is planned to augment the containment ordinance in such a way that a permit from the FOPH will be required for the import of organisms that according to Category A of the European Agreement on the International Carriage of Dangerous Goods by Road (ADR, SR 0.741.621) are classified as potentially contagious material.

The following recommendations have not yet been addressed; it is up to the relevant departments to implement them accordingly:

At certain security level 3 (BSL3) and 4 (BSL4) bio-containment laboratories B agents or germs are handled that would be suitable for misuse in the sense of the reference scenarios. As already small amounts of germs (or pathogens) constitute a potential danger, other laboratories – namely those at universities – are also capable of creating B agents that would be suitable for misuse. In the view of the NBC Protection Commission too little importance is in particular attributed to the aspect of security in the containment ordinance.

Recommendation 3: evaluation of legislation, namely the Environmental Protection Act (EPA) and the Gen Technology Act (GTA), as regards adaptation concerning biological security aspects («bio-security»)

The NBC Protection Commission also perceives a deficit in security training of bio-security officers (BSO).

Recommendation 4: evaluation of legislation, namely EPA and GTA, regards schooling (training and regular training or refresher courses) of those in charge of bio-security during activities in contained systems and in particular as regards obligatory schooling

Dangerous chemical substances

The handling of dangerous chemical substances and their security in enterprises and universities (e.g. chemical and pharmaceutical industry, SPIEZ LABORATORY and others) is sufficiently regulated according to the view of the NBC Protection Commission. The FOPH sees no essential deterioration of the situation of free trade with dangerous chemicals and no increase in the number of illegal usage cases since the new Chemicals Act was introduced. In the view of the NBC Protection Commission, however, it would be helpful if the issue of chemicals listed in the Chemical Control Ordinance would be reported to a central authority.

Recommendation 6: evaluate whether the issue of chemicals listed in the Chemical Control Ordinance and whether enterprises dealing with such substances are to be reported to a central federal or cantonal authority, whether this obligation to report can be extended to include other chemicals as well that would be eligible for terrorist purposes and whether the security gain would justify the additional administrative expenditure

The NBC Protection Commission perceives a deficit in the unequal treatment of experts in the N, B and C fields. All enterprises dealing with dangerous chemicals (hazardous incident plants and enterprises not subject to the MAO [Major Accidents Ordinance]), should not only designate a contact person for chemicals, but a respectively trained chemicals expert.

Recommendation 7: adaptation of the Chemicals Act and the Ordinance of the DHA of 28 June 2005 on the Chemicals Contact Person (SR 813.1 13.1 l), so that every hazardous incident plant has to employ a respectively trained chemicals expert for handling dangerous chemicals. Furthermore, the aspect of security is to be regulated more comprehensively and more bindingly. Furthermore, it should be evaluated whether all enterprises dealing with dangerous chemicals should employ a correspondingly trained chemicals expert.

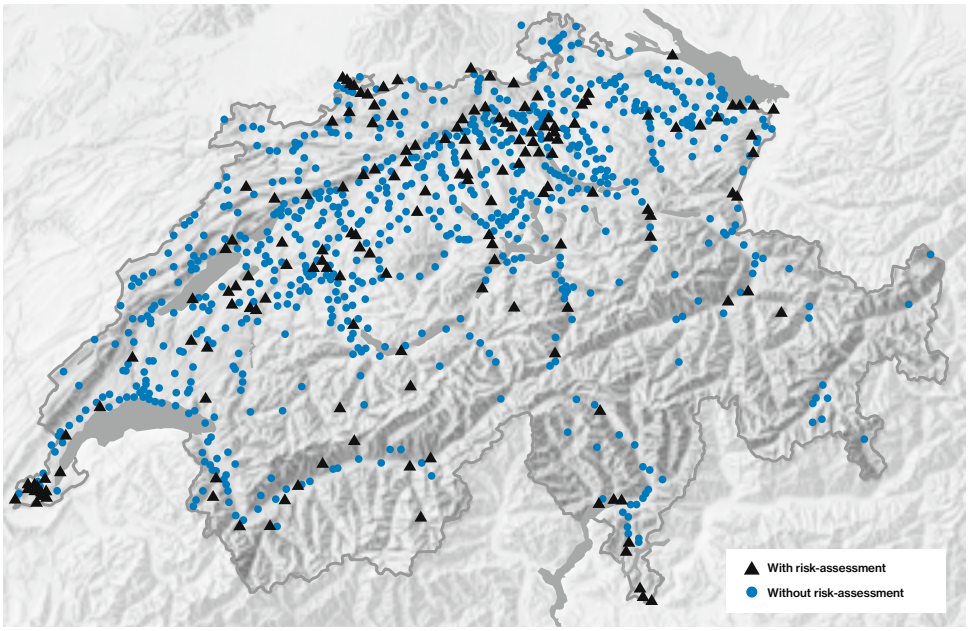
Transports of dangerous goods
In the opinion of the NBC Protection Commission the security measures for transports of dangerous goods are sufficiently regulated in various ordinances or in the international legal frameworks (ADR / RID / ADN) they refer to. Vehicles and trucks must be protected against theft or their cargo being stolen. In the case of transports of high-risk dangerous goods a security plan should be compiled. It must correspondingly take all security aspects and legal requirements into account. Suitable implementation aids are, however, inexistent or of a very general nature only.

Recommendation 8: evaluate whether it is necessary to compile execution or implementation aids for security plans in accordance with the international legal frameworks ADR, RID, ADN for transports of dangerous goods involving other than bio-hazard material

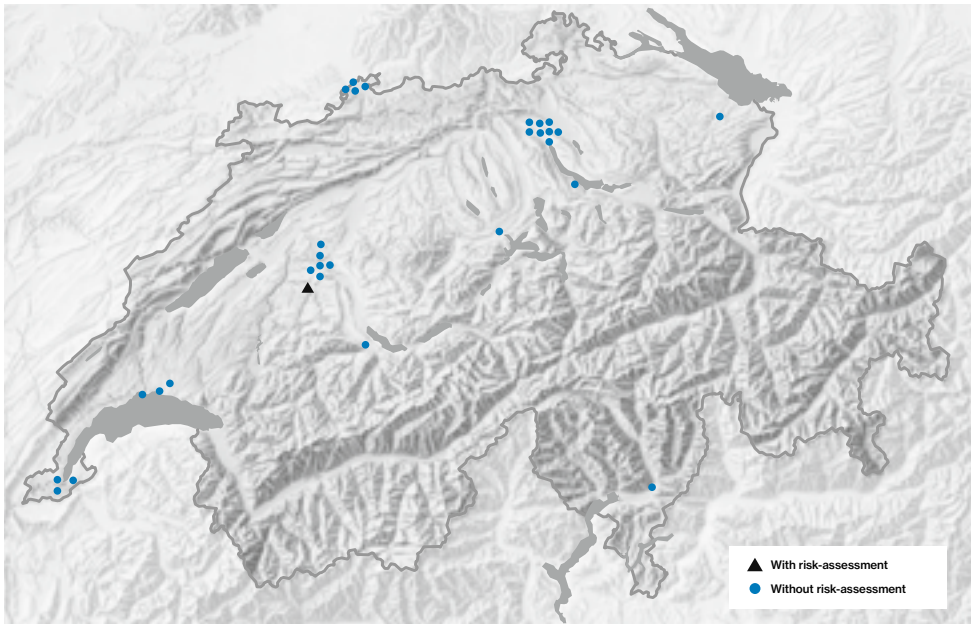
The Federal Office of Transport [FOT] is the authority for rail traffic and supervises though random-type security audits the security plans for transports of dangerous goods. For road traffic there is neither an obligation to register nor a central authority for evaluating security reports or eventual hazard studies.

Recommendation 9: evaluate whether a central authority would be useful for the appraisal of security plans for road transports of dangerous goods and whether a federal or a cantonal authority should be responsible

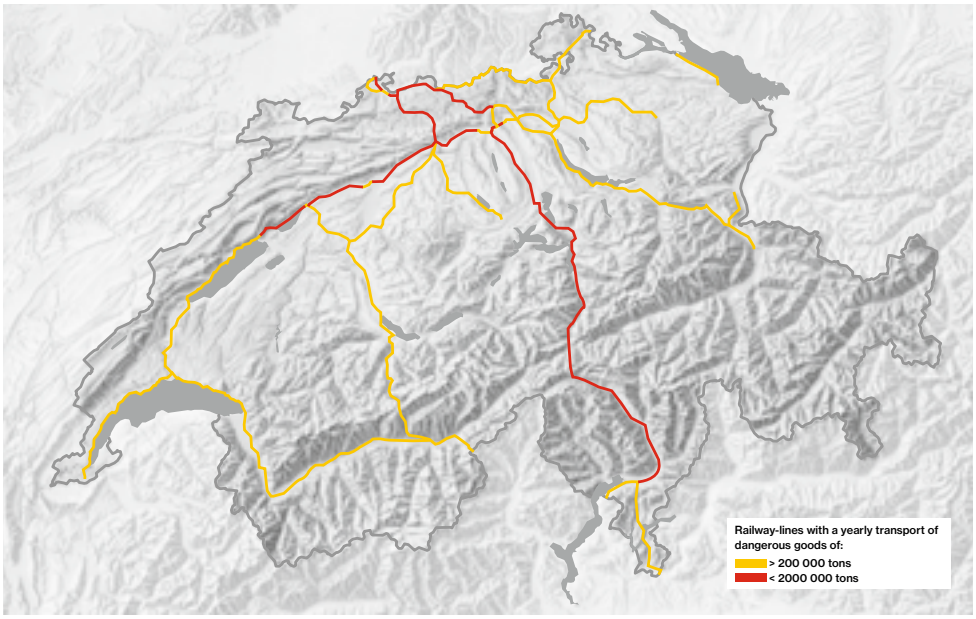
The NBC Protection Commission will periodically require information on to what extent its recommendations have been implemented. The legal adaptations should eliminate or at least reduce the potential misuse of dangerous NBC materials.



Chemical installations with high hazard potential subject to the Major Accidents Ordinance in 2005 (Source: ERKAS)



Biological installations with high hazard potential subject to the Major Accidents Ordinance in 2005



Transport of dangerous goods (rail) in 2005

New Energy Supply in Spiez

Mauro Zanni

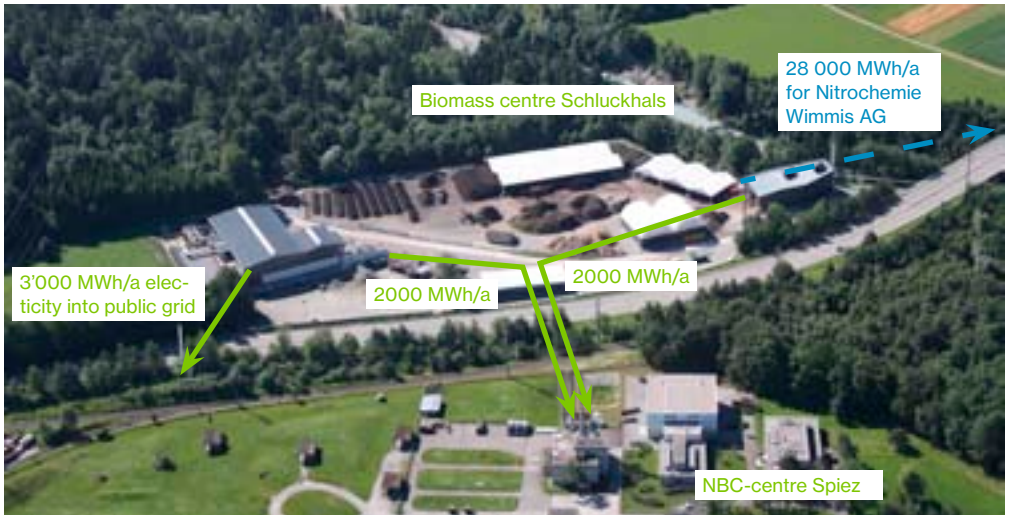
Today, the NBC Centre Spiez draws a large portion of its energy from the new biomass centre Schluckhals in Spiez which converts old and waste wood as well as organic garbage in a fermentation plant into electricity, steam heat and compost. Thanks to this recycling system, the NBC Centre Spiez can save hundreds of thousands of litres of heating oil. The facility won the «Watt d'Or» award in 2011.

The biomass centre consisting of a fermentation plant, a composting facility as well as a furnace for old and waste wood was planned by the companies Oberland Energie Ltd. and Dr. Eicher+Pauli Ltd. and built for CHF 30 million. The plants provide electricity, heat and steam from biological waste products for the NBC Centre Spiez, Nitrochemie Ltd and other recipients.

The fermentation plant produces more than 1.5 million cubic metres of gas annually that power two combined heat and power stations. These generate more than 3500 megawatt-hours worth of tele-heating and more than 3000 megawatt-hours of electric power per annum. The NBC Centre Spiez makes use of some of the tele-heat, thus substituting some 400 000 litres of heating oil annually.

In conventional composting plants a large amount of the energy contained in the material escapes unused as heat and climate-active methane is released. In addition, plants or their components that have been introduced to our latitudes due to globalisation and warmer climate are not destroyed but are spread further with the compost. Then, the more widespread separation of biogenic refuse and the ensuing increase of foreign materials (plastic, metals, paper etc.) also make composting more demanding. The bio gas plant in Spiez circumvents these problematic composting aspects as the biomass – contrary to the past – is not only materially exploited but energetically too. In this process biogenic substances are sanitised over a period of 30 days at a temperature exceeding 55 degrees Celsius. The fermentation plant works with six box-shaped fermenters; biogenic substances are pumped into the fermenter and subjected to the fermentation process. The easily degradable biomass is subsequently digested by microbes under anaerobic conditions. Each fermenter can be controlled autonomously, a fact that enables to create optimum fermentation conditions as required by the material being treated. The compact construction of the fermentation containers also ensures that the plant consumes no more than a minimum amount of heat.

Biomass centre Schluckhals



Overview and distribution

Thus biogas is generated under controlled conditions out of organic waste. 50 to 70 percent of this gas consists of methane (CH4), correspondingly it contains 30 to 50 percent of carbon dioxide (CO2) and small amounts of hydrogen and hydrogen sulphide. This gas is transferred by pipes to two combined heat and power stations for generating electricity, surplus heat is fed into a local tele-heating grid.

Apart from wood from rootstocks and tree trunks, untreated old wood is also burned in the furnace for old and waste wood. Steam is generated in two boilers (6 + 6 tons of steam/h) with two lines for flue gas cleaning. Process steam is delivered to the Nitrochemie Wimmis Ltd company while a separate pipe to the NBC Centre allows the biogas plant to compensate for seasonal fluctuations in tele-heating requirements. The 28 000 megawatt-hours worth of steam provide for the equivalent of approximately 2.8 million litres of heating oil the Nitrochemie company would otherwise require which corresponds to about 140 tanker truckloads.

The purified products generated by fermentation and wood preparation are processed into various types of compost and substrates. The designation of the compost with the Bio Suisse label «Knospe» certifies its high quality. The facility won the «Watt d'Or» award in 2011. This prize is awarded by the Swiss Federal Office of Energy SFOE every year. With the «Watt d'Or» the SFOE honours persons and organisations whose projects and initiatives indicate the way to a future with sustainable energy.

Furnace for old and waste wood

Organisation and Employees

SPIEZ LABORATORY
Director: Dr. Marc Cadisch[2)]

Secretariat: Irma Lehnher
Staff: 2/FTE: 2

PHYSICS SECTION
Staff: 14/FTE: 13.8
Head: Dr. Mario Burger[2)]
Markus Astner
Dr. Béatrice Balsiger
François Byrde
Dr. Emmanuel Egger
Ruth Holzer
Alfred Jakob
André Pignolet
Dr. Stefan Röllin
Hans Sahli
Thomas Sarbach
Marc Stauffer
Dr. Christoph Wirz
Dieter Zehr

BIOLOGY SECTION
Staff: 16/FTE: 15.1
Head: Dr. Marc Strasser a.i.[2)]
Werner Arnold
Marc-André Avondet
Cindy Ayer
Dr. Christian Beuret
Dr. Olivier Engler
Marcelle Holzer
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Dr. Matthias Wittwer
Fritz Wüthrich

CHEMISTRY SECTION
Staff: 15/FTE: 14.8
Head: Stefan Mogl[2)]
Dr. Beat Aebi
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Thomas Clare
Dr. Christophe Curty
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Nicolas Fragnière
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Roland Kurzo
Dr. Urs Meier
Benjamin Menzi
Dr. Martin Schär
Dr. Beat Schmidt
Dr. Peter Siegenthaler
Andreas Zaugg

NBC PROTECTION TECHNOLOGY SECTION
Staff: 11/FTE: 11
Head: Peter Hunziker [1)] [2)]
Kurt Bachmann
Thomas Friedrich
Markus Gurtner
Lukas Gyseler
Marco Hofer
Christian Krebs
Roland Liebi
Dr. Patrick Wick
Andres Wittwer
André Zahnd

LOGISTICS, QUALITY, SAFETY AND SECURITY SECTION
Staff: 30/FTE: 25.2
Head: Mauro Zanni[2)]
Aly Beer
Werner Berger
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Stefan Breitenbaumer
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Barbara Fischer
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Nicole Rothenbühler
René Scherz
Hans Schmid
Isabelle Strasser
Marianne Tadmoute
Roger Tschirky
Alexander Werlen[2)]
Marianne Wittwer

STRATEGY AND COMMUNICATION
Staff: 1/FTE: 1
Dr. Andreas Bucher[2)]

DDPS RADIATION PROTECTION TECHNOLOGY
Staff: 1/FTE: 1
Markus Zürcher

NATIONAL NBC PROTECTION AND COORDINATION OFFICE
Staff: 2/FTE: 2
Dr. Marc Kenzelmann[2)]
Dr. Daniel Storch

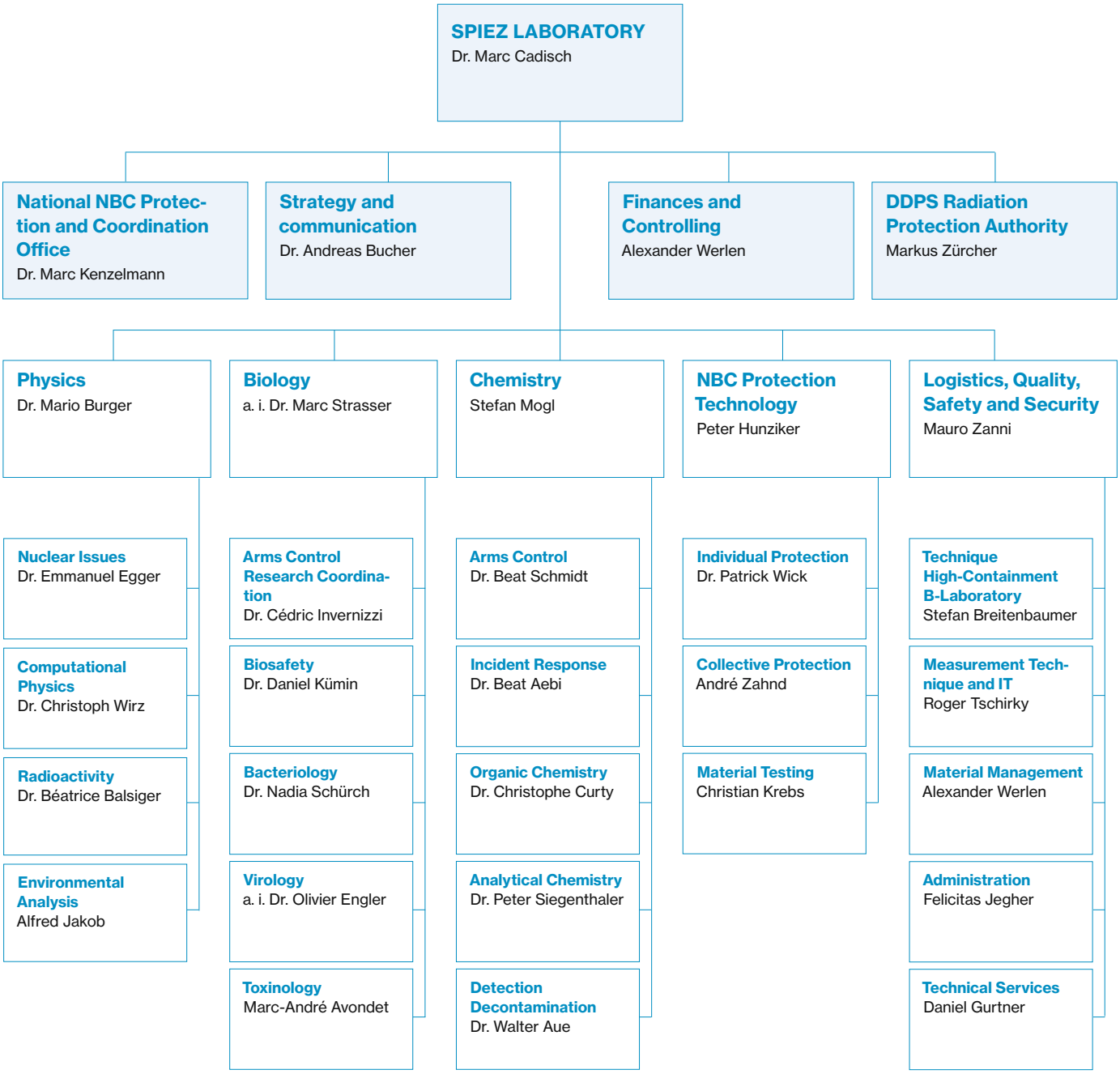
TRAINEES
Staff: 6/FTE: 6
Fabian Hauenstein
Joel Trummer
Simon Burn
Yannick von Känel
Miriam Champion
Roger Noti

NOTES
Staff: Total number of employees
FTE: No. of paid full-time-equivalent posts (FTE)

[1)] Deputy Director SPIEZ LABORATORY
[2)] Member of the SPIEZ LABORATORY executive management team

01.01.2012

Organigram


SPIEZ LABORATORY
Dr. Marc Cadisch
National NBC Protection and Coordination Office
Dr. Marc Kenzelmann
Strategy and communication
Dr. Andreas Bucher
Finances and Controlling
Alexander Werlen
DDPS Radiation Protection Authority
Markus Zürcher
Physics
Dr. Mario Burger
Biology
a. i. Dr. Marc Strasser
Chemistry
Stefan Mogl
NBC Protection Technology
Peter Hunziker
Logistics, Quality, Safety and Security
Mauro Zanni
Nuclear Issues
Dr. Emmanuel Egger
Computational Physics
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Radioactivity
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Environmental Analysis
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Arms Control Research Coordination
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Biosafety
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Bacteriology
Dr. Nadia Schürch
Virology
a. i. Dr. Olivier Engler
Toxinology
Marc-André Avondet
Arms Control
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Incident Response
Dr. Beat Aebi
Organic Chemistry
Dr. Christophe Curty
Analytical Chemistry
Dr. Peter Siegenthaler
Detection Decontamination
Dr. Walter Aue
Individual Protection
Dr. Patrick Wick
Collective Protection
André Zahnd
Material Testing
Christian Krebs
Technique High-Containment B-Laboratory
Stefan Breitenbaumer
Measurement Technique and IT
Roger Tschirky
Material Management
Alexander Werlen
Administration
Felicitas Jegher
Technical Services
Daniel Gurtner

Accredited activities

ISO/IEC 17025 accredited laboratories		No. of reports in 2011
STS 019	Testing laboratory for the analysis of samples for chemical warfare agents and related compounds	1
STS 022	Testing laboratory for adsorbents and respiratory protective filters	37
STS 028	Testing laboratory ort he determination of radionuclide concentrations	18
STS 036	Testing laboratory for polymers and rubber	196
STS 054	Testing laboratory for the detection of biological agents	244
STS 055	Testing laboratory for NBC protection material	66
STS 101	Testing laboratory for the determination of main and trace elements, their compounds and selected air pollutants	47
Total reports		**609**

Visits

Besides visits from our partner organizations, we also welcomed experts from abroad. These visits provide a valuable opportunity to share knowledge and expertise. Below are some of the visits we received in 2011. The list is not exhaustive.

Date	
28.11.2011	Korea Gas Safety Corp.
25.11.2011	Swiss Young Chemists' Association
23.11.2011	Swiss Federal Railways SBB - Real Estate, Safety & Security
18.11.2011	Berne Cantonal Police
16.11.2011	Chief Armed Forces Joint staff, Major General Jean Marc Halter
14.11.2011	Bundeswehr Research Institute for Protective Technologies and NBC-Protection (Munster)
31.10.2011	Swiss Accreditation Service SAS
26.10.2011	GCSP Middle East Course
25.10.2011	Agroscope Liebefeld-Posieux ALP
14.10.2011	Deputy Army Commander, Major General Fritz Lier
29.09.2011	Federal Office of Police, Police Attachés
28.09.2011	Major General Bert Mizusawa, Dep. Dir. for Strat. Initiatives J-5, Joint Chiefs of Staff, USA
01.09.2011	Swiss Federal Institute of Technology, Zürich, Life Sciences
26.08.2011	RUAG Network Enabled Operations
17.06.2011	Delegation Ministry of Environmental Protection, Republic of China
15.06.2011	Swiss Federal Customs Administration
10.06.2011	Green Cross International
26.05.2011	NBC Defence School, Sonthofen, Germany
23.05.2011	Delegates of the 14th Chemical Weapons Demilitarisation Conference
15.04.2011	The National Academies Washington DC
09.03.2011	RLS Rheinmetall Defence
07.02.2011	General Gérard Nédellec, Directeur du service de santé des armées (F)
04.02.2011	Federal Criminal Police Office, BKA, Germany
01.02.2011	HE Ms. Sarah Gillett, HM Ambassador to Switzerland and Liechtenstein
20.01.2011	Oeschger Centre for Climate Change Research
20.01.2011	Swiss Tropical and Public Health Institute
11.01.2011	International Committee of the Red Cross , ICRC

Presentations

Our scientists attend and actively contribute to conferences and offer their input to training courses dealing with NBC protection issues. Below are some of the English presentations given by our specialists during 2011.

Date	Subject
18.11.2011	Dr. Cédric Invernizzi: Awareness-Raising and Education – Report from Switzerland, Biosecurity Workshop, Landau Network CV, Como
27.10.2011	Dr. Matthias Wittwer: MALDI-TOF, Medical Biodefence Conference, Munich
17.10.2011	Dr. Marc Cadisch: Switzerland's CBRN Protection System and its Response to CBRN Threat Samples, Academy of Military Medical Sciences, Beijing
27.09.2011	Dr. Daniel Kümin: How to Facilitate the URS Process – Lessons Learned at SPIEZ LABORATORY, ERINHA-Workshop, Lyon
02.09.2011	Dr. Daniel Kümin: Best Practices in Training – Smoke Generators, SB-Net Meeting, Neuchâtel
13.04.2011	Peter Hunziker: Testing the Resistance of Polymer Materials and Textiles against Warfare Agents, CBMTS-Industry VII, Cavtat
11.04.2011	Stefan Mogl: OPCW WS Security and Non-Proliferation, S+A in Industry, OPCW, Den Haag
02.02.2011	Dr. Marc Cadisch: Responses to Chemical Attacks or Incidents, NATO WMD Forensics Conference, Prague

Publications and Lab-Reports

Please note that the list is not exhaustive. Some of the reports are classified.

Physics Section

Laboratory Note LN-2011-01 (in German)
Validation report for the determination of uranium, thorium and their daughter products with ICP-MS

Dr. Stefan Röllin
The analysis method L 028 075 describes the determination of uranium, thorium and their daughter products with external calibration. In this laboratory note the method is validated for the determination of radium-226 in water. As Ra-226 concentrations in water are often very low the samples cannot be diluted until no matrix suppression occurs. It was shown that due to different matrix suppression for analyte and internal standard, the Ra-226 concentrations were until 20 % overcorrected. Therefore, water samples from a proficiency test were reanalyzed with standard addition. The results were within the uncertainty range, but more likely still too high.

Laboratory Note JK 2011-01 (in German)
Analytics of Soil, Water and Plant samples in the accredited laboratory STS 101 – Overview of Methods

Alfred Jakob
The report describes analytical methods to identify elements and species hazardous to the environment, especially heavy metals originating from ammunition. For each of the different matrices the sample preparation, chemical extraction and measurement technique as well as the detection limit and expected uncertainty is clearly represented.

Laboratory Note 2011-02 (in German)
Recommendations on the usage of Field Portable X-Ray Fluorescence Spectrometers (FP-XRF) in the framework of shooting range assays.

Alfred Jakob, Thomas Sarbach, Marc Stauffer
As a consequence of army downsizing many shooting ranges have been closed. The procedures to assay and to clean up contaminated sites are specified by law. The examination of shooting ranges is expensive (laboratory analytics). In order to expedite the examinations the use of Field Portable X-Ray Fluorescence Spectrometers (FP-XRF) was studied in the framework of measuring the former shooting range Bodenänzi/LU. It was found that although FP-XRF cannot replace the chemical reference methods it can greatly reduce the number of samples which have to be chemically analyzed.

Laboratory Note SAR 2011-02 (in German)
Influence of Chlorine Content on the Measurement of Silver in impregnated activated Charcoal with ED-XRF

Thomas Sarbach
Measurements of zinc containing activated charcoal with ED-XRF (Energy Dispersive X-Ray Fluorescence) showed that high chlorine content may have an effect on the evaluation of the silver content. According to the mass budget of the producer of the charcoal there should be more silver in the activated charcoal than what was found by measurement.

Laboratory Note EGM 2011-02
Comments on the Nuclear Famine Study

Dr. Emmanuel Egger
In 2010 Robock and co-workers published a study on the consequences of a nuclear war between India and Pakistan, assuming the detonation of 100 nuclear weapons. They concluded that fires induced by the explosions would lead to carbonaceous smoke emission reaching the stratosphere in an amount that sunshine on earth would be dramatically reduced, and thus, temperature would fall, leading to longer winters. Time available for growing plants, grain, fruits, vegetables would be shortened. As a consequence of such a nuclear war, the authors expect a famine spreading to the entire earth. [Local nuclear war, Scientific American, Jan. 2010] The Department of Foreign Affairs has asked the ETH Zürich to reproduce the simulation of Robock et al. using a different model. SPIEZ LABORATORY was asked to give an opinion on the study. We concluded that the input parameters used in the study are those of a "worst case" and may not be reproducing the reality: The number of operational number of nuclear weapons in the considered countries is not known. The same applies to their yield, as well as to the vectors to bring the bombs into their target. We also expressed doubts on the choice of the targets assumed in the study, as well as on the fuel load available in these areas. No data are available in the open source literature on the fuel load and we doubt that data on American or European cities can simply be taken over, as has been done by the authors.

United Nations Environment Programme,
Synthesis for Policy Makers, Nairobi 2011
The Democratic Republic of Congo - Post-Conflict Environmental Assessment

United Nations Environment Programme, Technical Report, Nairobi 2011
Water Issues in the Democratic Republic of Congo - Challenges and Opportunities

International Atomic Energy Agency - Radiological Assessment Reports Series, Vienna, 2010
Radiological Conditions in Selected Areas of Southern Iraq with Residues of Depleted Uranium
Report by an international group of experts

Biology Section

J Med Virol. 2011 May;83(5):853-63. doi: 10.1002/jmv.21993.
Phylogenetic and virulence analysis of tick-borne encephalitis virus field isolates from Switzerland.

Gäumann R, Ružek D, Mühlemann K, Strasser M, Beuret CM.
Institute for Infectious Diseases, University of Bern, Bern, Switzerland. SPIEZ LABORATORY, Switzerland
Tick-borne encephalitis (TBE) is an endemic disease in Switzerland, with about 110-120 reported human cases each year. Endemic areas are found throughout the country. However, the viruses circulating in Switzerland have not been characterized so far. In this study, the complete envelope (E) protein sequences and phylogenetic classification of 72 TBE viruses found in Ixodes ricinus ticks sampled at 39 foci throughout Switzerland were analyzed. All isolates belonged to the European subtype and were highly related (mean pairwise sequence identity of 97.8 % at the nucleotide and 99.6 % at the amino acid level of the E protein). Sixty-four isolates were characterized in vitro with respect to their plaque phenotype. More than half (57.8 %) of isolates produced a mixture of plaques of different sizes, reflecting a heterogeneous population of virus variants. Isolates consistently forming plaques of small size were associated with recently detected endemic foci with no or only sporadic reports of clinical cases. All of six virus isolates investigated in an in vivo mouse model were highly neurovirulent (100 % mortality) but exhibited a relatively low level of neuroinvasiveness, with mouse survival rates ranging from 50 % to 100 %. Therefore, TBE viruses circulating in Switzerland belong to the European subtype and are closely related. In vitro and in vivo surrogates suggest a high proportion of isolates with a relatively low level of virulence, which is in agreement with a hypothesized high proportion of subclinical or mild TBE infections

J Appl Microbiol. 2011 Oct;111(4):997-1005.

Characterization of a Yersinia enterocolitica biotype 1A strain harbouring an ail gene.

Kraushaar B, Dieckmann R, Wittwer M, Knabner D, Konietzny A, Mäde D, Strauch E

The chromosomal ail gene (attachment and invasion locus) is commonly used as target gene for the detection of pathogenic Y. enterocolitica strains in food testing. The ail PCR does not detect strains of biotype 1A (BT1A), which are regarded as non-pathogenic because BT1A strains lack the virulence plasmid and chromosomally encoded virulence genes. In some recent reports, however, BT1A strains were discovered that harboured the ail gene. We isolated an ail-positive strain and characterized this strain with phenotypic and genotypic methods to study its possible relation to pathogenic Y. enterocolitica strains. The ail region of the BT1A strain was sequenced and compared with the corresponding region of nonpathogenic BT1A strains and pathogenic strains. Pulsed field gel electrophoresis (PFGE) analysis was applied revealing no similarity of the PFGE pattern of this strain to the patterns of pathogenic strains. Virulence-gene-based PCR analyses showed the strain to be positive for ystB, but negative for virulence genes ystA, virF and yadA. Whole-cell MALDI-TOF MS combined with a shrinkage discriminant analysis approach was applied and clearly classified the ail-positive biotype 1A strain within the cluster of BT1A strains. PCR detection of ail sequences in food matrices should be followed by the isolation of the responsible strain and its characterization using phenotypic or genotypic methods. Significance and Impact of the Study: The ail gene may be present in Y. enterocolitica BT1A strains, which are commonly considered as nonpathogenic. Efficient methods such as PCR typing of other virulence genes or rapid MALDI-TOF MS-based bacterial profiling allow a more comprehensive assessment of the pathogenicity potential of Yersinia strains.

Syst Appl Microbiol. 2011 Feb;34(1):12-9. Epub 2011 Jan 15.

Tapping the potential of intact cell mass spectrometry with a combined data analytical approach applied to Yersinia spp.: detection, differentiation and identification of Y. pestis.

Wittwer M, Heim J, Schär M, Dewarrat G, Schürch N.

In the everyday routine of an analytic lab, one is often confronted with the challenge to identify an unknown microbial sample lacking prior information to set the search limits. In the present work, we propose a workflow, which uses the spectral diversity of a commercial database (SARAMIS) to narrow down the search field at a certain taxonomic level, followed by a refined classification by supervised modelling. As supervised learning algorithm, we have chosen a shrinkage discriminant analysis approach, which takes collinearity of the data into account and provides a scoring system for biomarker ranking. This ranking can be used to tailor specific biomarker subsets, which optimize discrimination between subgroups, allowing a weighting of misclassification. The suitability of the approach was verified based on a dataset containing the mass spectra of three Yersinia species Yersinia enterocolitica, Y. pseudotuberculosis and Yersinia pestis. Thereby, we laid the emphasis on the discrimination between the highly related species Yersinia pseudotuberculosis and Y. pestis. All three species were correctly identified at the genus level by the commercial database. Whereas Y. enterocolitica was correctly identified at the species level, discrimination between the highly related Y. pseudotuberculosis and Y. pestis strains was ambiguous. With the use of the supervised modelling approach, we were able to accurately discriminate all the species even when grown under different culture conditions.

Toxins 2011, 3, 1332-1372; doi:10.3390/toxins3101332

Ricinus communis Intoxications in Human and Veterinary Medicine – A Summary of Real Cases

Sylvia Worbs, Kernt Köhler, Diana Pauly, Marc-André Avondet, Martin Schaer, Martin B. Dorner and Brigitte G. Dorner

Accidental and intended Ricinus communis intoxications in humans and animals have been known for centuries but the causative agent remained elusive until 1888 when Stillmark attributed the toxicity to the lectin ricin. Ricinus communis is grown worldwide on an industrial scale for the production of castor oil. As by-product in castor oil production ricin is mass produced above 1 million tons per year. On the basis of its availability, toxicity, ease of preparation and the current lack of medical countermeasures, ricin has gained attention as potential biological warfare agent. The seeds also contain the less toxic, but highly homologous Ricinus communis agglutinin and the alkaloid ricinine, and especially the latter can be used to track intoxications. After oil extraction and detoxification, the defatted press cake is used as organic fertilizer and as low-value feed. In this context there have been sporadic reports from different countries describing animal intoxications after uptake of obviously insufficiently detoxified fertilizer. Observations in Germany over several years, however, have led us to speculate that the detoxification process is not always performed thoroughly and controlled, calling for international regulations which clearly state a ricin threshold in fertilizer. In this review we summarize knowledge on intended and unintended poisoning with ricin or castor seeds both in humans and animals, with a particular emphasis on intoxications due to improperly detoxified castor bean meal and forensic analysis.

Laboratory Report LS 2011-01 (in German)

Identification of Flavi Viruses using Pan-Flavi RT-PCR and Sequencing techniques

Jasmine Portmann, Dr. Olivier Engler

Laboratory Report LS 2011-02 (in German)

Quantitative determination of N-methyl-L-tryptophan (L-abrin) in Abrus praecatorius and its derived extracts

Werner Arnold, Dr. Martin Schär

For the rapid assessment of abrin-suspect samples, the HPLC-UV detection of abrin-L (= N-methyltryptophan), a specific marker for Abrus praecatorius (= paternoster pea), is an excellent and quick detection method. The HPLC-UV detection of L-abrin was introduced within the working Group Toxinology as a non-accredited method. Clinical samples (urine) were performed with LC-MS/MS-measurements in collaboration with the working Group Analytical Chemistry.

Laboratory Report LS 2011-03 (in German)

Evaluation of different Disinfectants for the application in BSL-3 laboratory dunk tanks

Dr. Nadia Schürch, Jasmine Portmann, Johanna Signer

Laboratory Report LS 2011-05 (in German)

Determination of domoic acid in mussels by HPLC-DAD according to DIN EN 14 176

Werner Arnold

Domoic acid (DA) is the cause of amnesic shellfish poisoning (ASP), which is a des-ignated human disease that can occur after eating contaminated seafood (especially shellfish) or fish. The limit is set in the Swiss Food Manual with 20 mg/kg, the same source also defines the method of detection (HPLC-UV method according to DIN EN 14 176). To establish this method, various optimization tests (laboratory precision, recovery, chromatographic conditions, etc.) were performed.

Laboratory Note 2011-01-AW
Establishment of the AOAC method for determination of PSP toxins in shellfish

Werner Arnold
The AOAC method for determination of PSP toxins is commercially established worldwide and represents a further development of the DIN EN 14 526. The major change to the EN method is the additional purification or separation of the toxins in three fractions over a cation exchange column (SPE-COOH). All commercially available PSP toxins can be determined quantitatively with an HPLC method with pre-column oxidation and fluorescence detection.

Chemistry Section

Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament 2011 Edition

Paula Vanninen (Ed.), Ministry of Foreign Affairs of Finland/Univ. of Helsinki
In collaboration with our Finnish research partner VERIFIN (Finnish Institute for Verification of the Chemical Weapons Convention) various projects on processing and analysing difficult matrices were worked on in 2011 and the results were documented in several reports. In addition, after two years of intensive work by VERIFIN and various institutes from all over the world, the new 2011 edition on «Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament» could be published in December 2011. In the latest edition the staff of the Analytical Chemistry group made contributions from all fields as chapter coordinator, authors or reviewers.

Laboratory Report LS 2011-04 (in German)
Identification of chemicals relevant to the CWC in decontamination solutions with the LC/MS System Agilent 1200-AB/MDS Sciex 3200QTrap

Dr. Martin Schär
This study evaluated the influence of sample dilution and neutralisation on the detectability of relevant chemicals in decontamination solutions using liquid chromatography mass spectrometry (LC/MS).

Laboratory Note 2010-01-MRU (in German)
Analysis of sulfur mustard degradation products using a ZrO_2-graphite column with reversed phase chromatography and UV detection
Dr. Urs Meier

Laboratory Note 2011-01-CLA (in German)
Determination of TDS-GC-MS detection limits for selected chemical warfare agents
Thomas Clare, Dr. Peter Siegenthaler

Laboratory Note 2011-01-MRU (in German)
Analysis of sulfoxides from sulfur mustard degradation products using HILIC chromatography and UV detection
Dr. Urs Meier

Laboratory Note 2011-01-DUT
Degradation of chemical warfare agents
Dr. Jean-Claude Dutoit

Laboratory Note 2011-03-DUT (in German)
Extraction of phosphonic acids and monoesters from aqueous samples using anion exchange and derivatisation with different reagents for sylilation and methylation
Dr. Jean-Claude Dutoit, Thomas Clare, Dr. Peter Siegenthaler

MSc Thesis under the supervision of Prof. Christian Bochet, Dept. of Chemistry, University of Fribourg and Dr. Christophe Curty, SPIEZ LABORATORY

Synthesis of D-labelled biomarkers for sulfur mustard exposure

Julien Ducry

The actual trend in analytical chemistry is the use of deuterated internal standards. They permit a fast and direct quantification. During the analysis of samples exposed to sulfur mustards, the hydrolysis, oxidation products and side-products are observed in environmental samples, and further to them, the adducts formed with macromolecules or amino acids in biomedical samples. Existing synthetic methods of these standards are evaluated and the best methods are used for the synthesis of the deuterated counterparts. Thiodiglycol-d8 and bis-1,2-(2-hydroxyethylsulfanyl)ethane-d12 have been synthesized in good yield. Concerning the adducts between sulfur mustard and the aspartic acid and glutamic acid, the procedure has undergone minor modifications and the optimization permits the obtention of the deuterated adducts in moderate yield. Because of the low yields obtained in the literature for the coupling of histidine with sulfur mustard, regioselective synthesis attempts have been pursued. Based on the obtained results for mustard gas, the synthesis of adducts with sesquimustard has been investigated.

Department of Biomolecular Systems Laboratory, Max-Planck-Institut für Kolloid- und Grenzflächenforschung. Berlin, Juni 2011

Pact of Microreactors on the Chemical Weapons Convention's Chemistry – Screening of some Basic Key-Reactions

Dr. François Lévesque

Over the past ten years, microreactors and continuous flow reactors have begun to find broad applications in organic synthesis. Although these devices have been used by academic and industrial researchers, to date, no study of the preparation of chemical warfare agents (CWAs) has been done, either the chemicals scheduled in the Chemical Weapons Convention (CWC) or their related products. Futhermore, the main key-reactions allowing the preparation of this category of chemicals were also not investigated. The convention prohibits the development, use, production and stockpile of chemical weapons and was signed by 188 countries (status June 2011). In addition, all chemical weapons must be destroyed before April 2012. The collaboration with SPIEZ LABORATORY aims to determine the impact of microreactors on the Chemical Weapons Convention's chemistry. The project consists of the study of the feasibility of ten key-reactions using a microreator or a continuous flow reactor (Scheme 1). These reactions have been chosen because they are highly important for CWC chemistry (chlorination, sulfurisation, ester formation, phosphoramide formation and oxidation) and yields key precursors for the preparation of CWAs.

OPCW – Contributions in the Sphere of Security and Non-Proliferation 2011

Sampling and analysis in chemical industry inspections – not an easy start and important for the future (?)

Stefan Mogl

The laboratory analysis of samples for the presence or absence of chemical warfare agents, their precursors and degradation products – Sampling and Analysis (S+A) – plays an important role in chemical arms control and chemical disarmament.

The utility of S+A in OPCW industry inspections is to validate inspectors findings and to confirm through a measurement that Schedule 1 chemicals are absent at OPCW inspected facilities. There are many examples, where S+A in a similar manner is routinely utilised to ensure that measures taken or systems installed for everyday life are functioning correctly and that unwanted substances are absent or below threshold values (food quality, pharmaceuticals, illicit drugs, doping etc.).

The preparations to conduct S+A started early, several years before entry into force (EIF) of the Chemical Weapons Convention (CWC). Expert groups and laboratories were developing procedures and organised inter laboratory comparison tests. In 2006 the Technical Secretariat (TS) introduced S+A as a routine verification tool in Schedule 2 industry inspections. Today, the OPCW

has at its disposal a mobile on-site analysis capability that can be used worldwide, as well as a network of designated laboratories for off-site analysis.

Developing this capability has brought a significant gain in analytical expertise to many Member States and the international community as a whole. There are however critical lessons to be learnt from this process for the further improvement of the current system and its adaptation to future requirements. An evaluation might be necessary in terms of technical objectives, as well as the involvement of stakeholders: the policy making organs of the OPCW, and most importantly, the chemical industry.

S+A in industry inspections will continue to play a vital role for ensuring the non-production of Schedule 1 chemicals and for supporting the non-proliferation aims of the Convention. It is an important verification tool that demonstrates to Member States and the general public that OPCW inspected chemical industry facilities are only used for activities not prohibited by the Convention.

NBC Protection Technology Section

CBMTS Industry VII
Testing the Resistance of Polymer Materials and Textiles against Warfare Agents

Peter Hunziker
One of the key tasks of SPIEZ LABORATORY is to support the national authorities in the procurement of NBC protective material and systems. In this context the resistance of plastics, rubbers and textiles to chemical warfare agents is of prime importance. In the absence of commercially available testing facilities SPIEZ LABORATORY has developed YPAP 21, a fully automatic operating facility allowing the determination of the time needed by chemical warfare agents to permeate or penetrate permeable and impermeable plastics, rubbers and textiles. YPAP 21 makes it possible to test up to 38 swatches simultaneously using a wide range of static and dynamic test methods. The facility is designed for the application of four different test agents. In a first step only sulfur Mustard (HD) in gaseous or liquid form is used. Number (1-9) and volume (1-50 µl) of liquid agent-droplets applied can be chosen by the customer. The test agent can be laid, reamed, or pressed on the test sample or it can be applied as fallen droplets. It is also possible to expose the test samples to a defined stream of contaminated air. Breakthrough time of the chemical agent penetrating or permeating through the test samples is detected by the color change of indicator paper or by the conductivity measuring method which also allows a continuous recording of the breakthrough concentration.

Applied Biosafety Vol. 16, No. 2, 2011
How to Choose a Suit for a BSL-4 Laboratory - The Approach Taken at SPIEZ LABORATORY

Dr. Daniel Kümin, Christian Krebs, Dr. Patrick Wick
Choosing the appropriate suit for a BSL-4 laboratory is of vital importance to create a safe work environment within such a facility. The suit has to provide pro-tection for the wearer and be compatible with the infrastructure at the facility, while still providing some level of comfort. A number of manufacturers are currently in the market, but with the increased number of new BSL-4 facilities worldwide, new manufacturers are entering this market. Unfortunately, apart from the information provided by the manufacturers, not a lot of data exists in the literature on what to look for in a BSL-4 suit. Thus, the authors decided to develop a test program to compare different suit models and to guarantee that the chosen suit can be used in the specific conditions encountered at their new facility. Tests ranged from studies on material compatibility and determination of protection factors to questionnaires on wearer comfort. Results as well as some conclusions that could be drawn from the tests are presented in this article.

National Protection and Coordination Office

Journal «CBRNE Terrorism Newsletter» (http://www.cbrne-terrorism-newsletter.com/new-issue.php). Autumn 2011 issue

Swiss CBRN Protection Goes National

Dr. Marc Kenzelmann

In 2003 the National NBC Protection project was launched in Switzerland, the aim of which was better coordination of all NBC partners and ultimately more effective CBRN protection in Switzerland in the long term. It led to a flurry of activity in the field of CBRN protection, which continues today. The present article outlines some of the activities that have helped to identify and define how Switzerland can sharpen its response to a CBRN incident. The years to come will show whether Swiss policy makers are really willing to implement these recommendations and accept the consequences they entail. Only then can we say that we have learnt lessons from major emergencies like Fukushima.

Kerntechnik - Independent Journal for Nuclear Engineering, Energy Systems, Radiation and Radiological Protection – June 2011

Switzerland: a culture of preparedness

Dr. Daniel Storch and Dr. Marc Kenzelmann

The National NBC Protection and Coordination Office supports the activities of all members of the National NBC protection network. These include the development and implementation of operational principles, as well as training and protection concepts on behalf of the head of the Steering Committee on Radioactivity (LAR) and the Federal Office for Civil Protection (FOCP), the chair of the Federal Commission for NBC Protection (ComNBC) and the chair of the Cantonal NBC Coordination Platform (KPABC). Besides exercises at the political-strategic level (civilian and military), operational training exercises are held on a regular basis.

SPIEZ LABORATORY
The Swiss Institute for NBC-Protection
CH-3700 Spiez
Tel. +41 (0)33 228 14 00
Fax +41 (0)33 228 14 02
laborspiez@babs.admin.ch
www.labor-spiez.ch

88.003.e 04.12 700 860288113