Using GeneSpring (PCA) analysis, 550 genes whose expression was significantly ($p<0.01$) altered by at least 2.5-fold, were selected. The results indicate that the low-level single dose exposure do not always parallel acute toxicity, but can cause a reversible down-regulation of genes and a range of anti-cholinesterase effects.

In contrast, repeated doses produced persistent irreversible down-regulation of genes related to neurodegenerative mechanism at 48h. Real-time PCR and western blot analysis confirmed the reduced expression of presenilin 1 (TMP21), 2 and dopa-decarboxylase (DDC) mRNA and proteins.

Besides providing an in vitro experimental model for studies on the neuropathophysiology and brain cells, this investigation indicate possible mechanisms by which sarin could mediate neuro-degeneration. A comparison will be made with similar study with soman.

51. DEVELOPMENT OF PROCEDURES FOR THE ANALYSIS OF COMPONENTS OF DUMPED CHEMICAL WEAPONS AND THEIR PRINCIPAL TRANSFORMATION PRODUCTS IN SEA WATER *(5)*

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A package of chemical analytical procedures was developed for the detection of products indicative of the presence of damped chemical weapons in the Baltic Sea. The principal requirements imposed upon the procedures were the following: high sensitivity, reliable identification of target compounds, wide range of components covered by survey analysis, and lack of interferences from sea salts.

Thiodiglycol, a product of hydrolysis of sulfur mustard reportedly always detected in the sites of damping chemical weapons in the Baltic Sea, was considered the principal marker. We developed a high-sensitivity procedure for the determination of thiodiglycol in sea water, involving evaporation of samples to dryness in a vacuum concentrator, followed by tert-butyldimethylsilylation of the residue and GCMS analysis in the SIM mode with meta-fluorobenzoic acid as internal reference. The detection limit of thiodiglycol was 0.001 mg/l, and the procedure throughput was up to 30 samples per day. The same procedure, but with BSTFA as derivatizing agent instead of MTBSTFA, was used for preparing samples for survey analysis of nonvolatile components. In this case, full mass spectra were measured in the GCMS analysis. The use of BSTFA was motivated by the fact that trimethylsilyl derivatives are much wider represented in electronic mass spectral databases.

The identification of sulfur mustard, volatile transformation products of sulfur mustard and lewisite, as well as chloroacetophenone in sea water was performed by means of GCMS in combination with SPME. The survey GC-MS analysis was focused on the identification of volatile and nonvolatile toxic chemicals whose mass spectra are included in the OPCW database (3219 toxic chemicals, precursors, and transformation products) with the use of AMDIS software (version 2.62). Using 2 GC-MS instruments, we could perform the survey analysis for volatile and nonvolatile components of up to 20 samples per day.

Thus, the package of three procedures, including target GCMS analysis for thiodiglycol and survey GCMS analysis for listed volatile and nonvolatile toxic chemicals, in combination with atomic absorption analysis for total arsenic allowed express and reliable control of sea water for the suspected presence of chemical weapons.

52. ESTABLISHMENT OF EXPOSURE TO ORGANOPHOSPHORUS WARFARE AGENTS BY MEANS OF SPME-GSMS ANALYSIS OF BODILY FLUIDS *(13)*

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Reliable chemical analytical procedures for revealing an exposure to toxic chemicals, identifying the active substance, and assessing the degree of exposure are necessary as a component of medical and forensic activities in cases of the possible use of highly toxic chemicals in war conflicts and terrorism acts, as well as emergency situations in chemical industry, specifically at chemical weapons storage and destruction facilities. According to Chemical Weapons Convention, Part XI, Appendix 4, e-17, "samples of importance in the investigation of alleged use include ... biomedical samples from human or animal sources (blood, urine, excreta, tissue etc.)".

Urinary metabolites, O-alkyl esters of methylphosphic acid, offer one of the simplest means of confirming an exposure to organophosphorus warfare agents (OPWA). Urine, unlike blood or tissues, does not require invasive collection demanding in terms of sterility. Excretion with urine is the major route of elimination of OPWA from an organism. According to published data, 90% of OPWA metabolites are excreted within 48-72 h after intoxication.

We developed an SPME-GCMS procedure for the determination of O-alkyl esters methylphosphonic acid in urine, with the following detection limits,: isopropyl and isobutyl esters 5 ng/ml and pinacolyl ester 1 ng/ml. The procedure involves derivatization of the target compounds directly on the microfiber. The total analysis time is 1-1.5 h.

In animal experiments in vivo we could establish the exposure to OPWA at a half-LD_{50} level within no less than 48 h after intoxication. In principle, OPWA metabolites could be detected in urine within two weeks after intoxication but at higher doses. Retrospective analysis of urinary metabolites in cases