Research and Development of On-site Decontamination System for Biological and Chemical Warfare Agents

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Biological and chemical warfare agents (BCWAs) are diverse in nature including synthetic low molecular weight chemical warfare agents (gaseous choking and blood agents), volatile nerve gases and blister agents, non-volatile vomit agents and lachrymators, biological toxins (polar low molecular weight toxins and proteinous toxins), and microorganisms (viruses, rickettsia, chlamydia and bacteria). In the consequence management against chemical and biological warfare terrorism, speedy decontamination of casualties, goods, items and equipments, buildings and field is required for the minimization of the terrorism damage. At present, washing casualties and contaminated materials with large volumes of water is the basic way, and hypochlorite solution is mainly used to decompose BCWAs. However, it still remains unsolved how to dispose the waste water contaminated with BCWAs, and decontaminating reagents have serious problems of high toxicity to human, despoiling nature to environment, long finishing time and non-durability. In addition, the present decontamination technologies are not effective, nonspecifically affecting the surrounding non-target materials. Therefore, it is the urgent matter to build up usable decontamination system surpassing the present system. The author introduces the joint project of research and development of the novel decontamination system against BCWAs, in the purpose of realizing non-dangerous, rapid, specific, effective and economical on-site decontamination. The project consists of establishment of the evaluation methods for decontamination, and verification of the present technologies and utilization of bacterial organophosphorus hydrolase, development of specific adsorptive elimination technologies using molecular recognition devices, and development of deactivation technologies using photocatalysis.

Key words —— decontamination, chemical warfare agent, biological warfare agent, adsorption, molecular-recognition, photocatalysis

INTRODUCTION

In the terrorism cases of 1995 Japan sarin (GB) gas attack1) and 2001 United States postal anthrax attack,2) chemical warfare agents (CWAs)3−5) and biological warfare agents (BWAs)6) were used as mass destruction weapons, and defenseless citizen were suffered (former case: 12 died and more than 5000 injured; later case: 5 died and ten and several injured), causing great shock against the terrorism threat all over the world. Although the volumes of the agents used were not large, these agents were peculiar, and countermeasures performed in those
days were not evaluated adequate. After 11 September Attacks, the governments have strengthening the countermeasure against the mass weapon terrorism. Within the preparedness for adequate counterterrorism, the promotion of the corresponding research and development (R&D) of science and technology should be definitely considered. Especially, detection of biological and chemical warfare agents (BCWAs), composed of hazardous material monitoring, security check and infectious disease surveillance in crisis management stage; on-site detection in consequence management stage; and laboratory analysis and diagnosis in incident management stage, is regarded as the most important, and R&D of the detection technologies has been performed in leading industrialized nations. In review articles and books the author introduces on-site detection of BCWAs7,8) and laboratory analysis of CWAs9,10)
However, concerning decontamination, at present the countermeasure has been only performed based on the experience, and R&D of the related technologies is not done scientifically. Establishment of adequate decontamination system is requested from the frontlines of the counterterrorism, and it is urgent to perform R&D by the large-scale coordination system among company (responding to manufacture), university (responding to seeds creation) and government (responding to need indication). Previously, the author reported the brief review on the decontamination of BCWAs. In this review article, the characteristics of BCWAs, the concept of on-site decontamination, the present situation of decontamination technologies, and R&D of decontamination system performed by the author’s group are stated.

**CHARACTERISTICS OF BCWAs**

CWAs are low molecular weight (MW) compounds, and according to the toxicity to human, they are divided into “nerve gas”, “blister agent”, “chooking agent”, “blood agent”, “vomit agent” and “tear gas (lachrymator).” In physical properties, they are diverse, including gases in normal pressure and temperature (chooking agents and blood agents), volatile liquids (nerve gases and blister agents) and nonvolatile liquids or solids (vomit agents and lachrymators). Figure 1 shows chemical structures of CWAs, and Table 1 shows their toxicities and manifestation times. CWAs are also diverse in the standpoint of usage, from blood and choking agents with high public warfare usage to nerve gases and blister agents with only military usage. The latter groups are designated as “scheduled compounds” in the Chemical Weapon Convention and the related domestic lows “Low on the prohibition of Chemical Weapons and the Regulation of specific Chemicals (enforced 1997/3/19).”

Blood agents manifest toxicity by inhibiting oxygen consumption via blood hemoglobin. Hydrogen cyanide (AC) and cyanogens chloride (CK) inhibit cytochrome oxidase activity in respiratory chains. Arsine destroys red blood cells. Cyanide has high public warfare usage such as gold plating, and CK and arsenic are used in industrial manufacturing. Choking agents manifest toxicity by injuring respiratory system such as nose, throat and lung, and disturb breathing. They possess active chlorine molecule(s), produce irritating hydrochloric acid (HCl) by reaction with water, which leads to inflammation of mucous membranes, and chlorinate biomolecules, which leads to loss of biological function. In the World War 1st, chlorine (CL) and phosgene (CG) were used. Chloropicrin (PS) is used as fumigant for agricultural usage. CG shows toxicity after incorporation into the lungs without particular irritation, and produces HCl several hours latter, leading to pulmonary emphysema. In water they are hydrolyzed to loose volatility and reactivity.

Nerve gases manifest strong toxicity with disturbance of nerve impulse by inhibiting synaptic cholinesterase (ChE) activity and accumulating acetylcholine. They are diverse including very volatile GB, volatile soman (GD) and nonvolatile VX. They resemble structurally with organophosphorus pesticides. G-agents (code names used by North Atlantic Treaty Organization) such as GB, GD and tabun (GA) were newly synthesized in Germany during the World War 2nd. V-agents such as VX were synthesized by the Great Britain and U.S.A. after the World War 2nd. Nowadays, they are still stockpiled as negative legacy of the Cold War, and the disarmament has proceeded internationally. Small quantity of nerve gases incorporated into the body can be detoxified with suicide binding to blood ChE, liver carboxylesterase and with hydrolysis by blood paraoxonase (POX). Nerve gases are easily hydrolyzed by nucleophilic attack of hydroxyl anion to form nontoxic polar hydrolysis products (alkaylmethylphosphonic acid etc.) and loose volatility and toxicity. For example, GB is hydrolyzed to form isopropylmethylphosphonic acid (IMPA). IMPA is further hydrolyzed to form methylphosphonic acid (MPA) under acidic condition (Fig. 2).

Blister agents manifest toxicity by causing inflammation of contact surfaces such as skins, eyes and respiratory tracts. They possess heteroatom(s) and active chlorines. Mustard compounds (sulfur mustard and nitrogen mustard) are converted to cyclic ethylene sulfonium (ammonium) as intermediate by leaving one chlorine molecule, showing high reactivity of alkylating biomolecules such as proteins and nucleic acids. By reacting with water, they are converted to nontoxic hydrolysis products such as thioglycol and ethanolamines. Lewisite L1 possesses two arsenic chlorine bonding, which leads to rapid hydrolysis in water to produce 2-chlorovinylarsonic acid (CVAA) and HCl, manifesting inflammation.
bind with thiols such as protein cysteine residues, resulting in loss of biological function. Mustard gas (HD) was used in military from the late of the World War I, and even now large quantity is stockpiled. The Former Japanese Military Forms manufactured HD, used it in the China-Japanese War, and abandoned the chemical shells in the North-East area of China and Japan. Now, Japanese government is due to dispose the abandoned chemical weapons.25)

Vomit agents such as diphenylchloroarsine (DA) and diphenylcyanarsine (DC), manifest toxicity by irritating respiratory mucous membranes and eyes, which cause sneeze, cough, vomit and head ache. They possess one bonding between trivalent arsenic and chlorine (or cyanide). They are not highly toxic, compared to blister agents. They are hygroscopic, and in water converted to nonirritating hydrolysis products such as diphenylarsinic acid (DPAA). DPAA was the cause of poisoning, which showed neurotoxic syndromes to habitats in Kamisu area, Ibaragi, who had been drinking the contaminated well water.26) Lachrymators27) manifest toxicity by irritating mucous membranes of eyes, nose and pharynx, which cause tear, sneeze and
Table 1. Toxicity and Infectivity of BCWAs

<table>
<thead>
<tr>
<th>Agent</th>
<th>Toxic concentration$^{a)}$/ infectious dose</th>
<th>Manifestation time/ incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>4500 mg·min/m$^3$ 4500 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Cyanogen chloride</td>
<td>11000 mg·min/m$^3$ 11000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Arsine</td>
<td>5000 mg·min/m$^3$ 5000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Choking agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosgene</td>
<td>3200 mg·min/m$^3$ 3200 mg·min/m$^3$ hour</td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>19000 mg·min/m$^3$ 19000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Chloropicrin</td>
<td>2000 mg·min/m$^3$ 2000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Nerve gas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarin</td>
<td>150 mg·min/m$^3$ 150 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Soman</td>
<td>60 mg·min/m$^3$ 60 mg·min/m$^3$ minute</td>
<td></td>
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<tr>
<td>Tabun</td>
<td>300 mg·min/m$^3$ 300 mg·min/m$^3$ minute</td>
<td></td>
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<tr>
<td>VX</td>
<td>40 mg·min/m$^3$ 40 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Blister agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mustard gas</td>
<td>1500 mg·min/m$^3$ 1500 mg·min/m$^3$ hour</td>
<td></td>
</tr>
<tr>
<td>Nitrogen mustard 1</td>
<td>1500 mg·min/m$^3$ 1500 mg·min/m$^3$ hour</td>
<td></td>
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<tr>
<td>Nitrogen mustard 2</td>
<td>3000 mg·min/m$^3$ 3000 mg·min/m$^3$ hour</td>
<td></td>
</tr>
<tr>
<td>Nitrogen mustard 3</td>
<td>1500 mg·min/m$^3$ 1500 mg·min/m$^3$ hour</td>
<td></td>
</tr>
<tr>
<td>Lewisite 1</td>
<td>1500 mg·min/m$^3$ 1500 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Vomit agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenylchloroarsine</td>
<td>15000 mg·min/m$^3$ 15000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>Diphenylecyanoarsine</td>
<td>10000 mg·min/m$^3$ 10000 mg·min/m$^3$ minute</td>
<td></td>
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<tr>
<td>Lachrymator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Chloroacetophenone</td>
<td>7000 mg·min/m$^3$ 7000 mg·min/m$^3$ minute</td>
<td></td>
</tr>
<tr>
<td>o-Chlorobenzylidenemalonitrile</td>
<td>61000 mg·min/m$^3$ 61000 mg·min/m$^3$ minute</td>
<td></td>
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<tr>
<td>Capsaicin</td>
<td>13000 mg·min/m$^3$ 13000 mg·min/m$^3$ minute</td>
<td></td>
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<tr>
<td>Biological toxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>10 µg/kg (mouse, iv$^{b)}$ 10 µg/kg (mouse, iv$^{b)}$ hour</td>
<td></td>
</tr>
<tr>
<td>Ricin</td>
<td>3 µg/kg (mouse, ip$^{c)}$ 3 µg/kg (mouse, ip$^{c)}$ hour</td>
<td></td>
</tr>
<tr>
<td>Botulinum toxin A</td>
<td>1 ng/kg (mouse, ip) 1 ng/kg (mouse, ip) day</td>
<td></td>
</tr>
<tr>
<td>Staphylococcal enterotoxin B</td>
<td>30 mg (human, o$^{d)}$ 30 mg (human, o$^{d)}$ hour</td>
<td></td>
</tr>
<tr>
<td>Virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small pox (Variola minor)</td>
<td>10 particles 7 days</td>
<td></td>
</tr>
<tr>
<td>Ebola hemorrhagic fever</td>
<td>1–10 particles 4–21 days</td>
<td></td>
</tr>
<tr>
<td>Venezuelan equine encephalitis</td>
<td>10–100 particles 1–5 days</td>
<td></td>
</tr>
<tr>
<td>Rickettsia and Chlamydia</td>
<td>Q fever (Coxiella burnetti) 1–10 particles 2–14 days</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthrax (Bacillus anthracis)</td>
<td>8000–50000 spores 1–6 days</td>
<td></td>
</tr>
<tr>
<td>Plaque (Yersinia pestis)</td>
<td>100 cells 2–3 days</td>
<td></td>
</tr>
<tr>
<td>Tularemia (Francisella tularensis)</td>
<td>10–50 cells 1–21 days</td>
<td></td>
</tr>
<tr>
<td>Brucellosis (Brucella melitensis)</td>
<td>10–100 cells 5–60 days</td>
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</table>

$^{a)}$ 50% lethal concentration with 1 min respiration (LC50); $^{b)}$ intravenous route; $^{c)}$ intraperitoneal route; $^{d)}$ oral route.

cough. Synthetic 2-chloroacetophenone (CN) and o-chlorobenzylidenemalononitrile (CS) are used for the means of military and riot control. Active ingredients of pepper extracts including capsaicin (OC) are used as pepper sprays for personal protection, and many incidents such as burglaries have been occurred using pepper spray.28) The toxic threshold concentration is considerably higher than the irritating threshold concentration. Synthetic lachrymators manifest toxicity by alkylating biomolecules, and OC by binding to specific receptors.29) Biological toxins produced by living organ-
isms\textsuperscript{30}) are polar high MW compounds. There are quite many toxins, but the agents that can be used as BWAs are limited. These include saxitoxin (STX) and ricin which are designated as the scheduled compounds in Chemical Weapon Convention, and botulinum toxins (BTXs) and Staphylococcal enterotoxin B (SEB) which are designated as those possibly usable for bioterrorism by World Health Organization (WHO)\textsuperscript{31}) and U.S. Center of Disease Control and Prevention (CDC).\textsuperscript{32)} By considering not only the military situations, physical property, toxicity, environmental stability and supply possibility, but also political situations, these were nominated. STX\textsuperscript{33}) is a guanidium compound of MW 299, which is produced by marine dinoflagellates and obtained from paralytic shellfish. STX possesses many structural isomers, and is the most toxic within the paralytic shellfish toxins. They manifest toxicity by inhibiting sodium channel function of cell membranes with disturbance of nervous impulse. Ricin is a proteinous plant toxin,\textsuperscript{34}) included in seeds of \textit{Ricinus communis} as a major constituent. The toxin is a heterodimer composed of A-chain of about 30000 MW with ribonuclease activity and B-chain of about 30000 MW with galactose-binding lectin activity, which are inter-connected by disulfide bonding. Ricin adsorbs to the target cells by binding to the cell membrane carbohydrate chains having terminal galactose via the B-chain, and then is incorporated by endocytosis. The endosomes are merged with the Golgi apparatus, and then ricin traverses the Golgi membrane to the cytosol region. The A-chain cleaves specific site of ribosomal RNA, which results in inhibition of protein synthesis.

BTXs\textsuperscript{35}) are proteinous toxins produced by obligate anaerobic Gram positive store-forming bacteria, \textit{Clostridium botulinum}. The toxins have subtypes of A–G, differing in antigenicity, molecular structure and toxicity (toxic power, host cell sensitivity). The BTXs are heterodimers composed of N-terminal light chain of about 50000 MW and C-terminal heavy chain of about 100000 MW which are inter-connected by disulfide bonding. The toxin adsorbs to the target nervous cells by binding to the specific receptors via the heavy chain, and then is incorporated by endocytosis, and traverses the endosomal membrane into the cytosol region. The light chain has proteolytic activity, cleaving the cytosol proteins responsible for the acetylcholine exocytosolic release. Staphylococcal enterotoxins\textsuperscript{36}) are nonlethal proteinous toxins of about 30000 MW, produced by facultative anaerobic Gram positive rod bacteria, \textit{Staphylococcus aureus}, and have subtypes of A–E, differing in antigenicity. They are superantigen activity, stimulating lymphocytes. Poisoning syndrome is vomit, stomachache and diarrhea. Type B toxin (SEB) shows high toxicity and environmental stability, and so is used as BWA.

Within living microorganisms including virus, rickettsia, chlamydia, bacteria, fungi, protozoa and parasites, BWAs are designated as those possibly usable for bioterrorism by WHO\textsuperscript{31}) and CDC.\textsuperscript{32)}
BWAs are characterized by high infectivity to human, high toxicity and easy dissemination. In this article, fungi, protozoa and parasites are excluded. Viruses are composed of multiple nucleic acids surrounded by envelopes of proteins and or liquids. The size differs from 20 nm long to 300 nm. Small pox, hemorrhagic fever (Ebola, Marburg) and Venezuelan equine encephalitis are typical BWAs. Small pox (Variola major, Variola minor) is an ellipse particle of 230 × 400 nm. They show strong infectivity by air transmission, splash and contact. The fatality is 50–90% with hemorrhage on whole body.

Rickettsia and Chlamydia are obligate parasite bacteria, and transmitted to human by arthropods. Q fever (Coxiella burnetti) is a sub μm long rod, and infected via animal feces and urine. Fever, chill and fatigue occur, and sometimes shows pneumonia and hepatitis, but the fatality is low.

Bacteria possess rigid cell walls beside cell membranes, and are self-proliferating with DNA as genetic information. They are divided into positive and negative types in Gram staining. BWAs include Anthrax, Plague, Tularemia and Brucellosis. Anthrax (Bacillus anthracis) is 1–1.5 × 3–10 μm size, aerobic gram positive spore-forming rod shape bacteria, and divided into inhalational anthrax, cutaneous anthrax and gastrointestinal anthrax in the infectious route, differ in incubation period, symptom and fatality. Inhalational anthrax manifests difficulty in breathing, meningitis and pulmonary edema, leading to more than 86% death. The spores of 1 × 1.5 μm size are stable in environment, and resistant to various kinds of sterilization treatment. In a body, even though they are phagocytized by macrophages a part of them still retain their lives. Besides the toughness of the spores, anthrax is characterized by the formation of capsules resistant to the phagocytosis and three kinds of toxins (protective antigen, lethal factor and edema factor), all which genes are coded in plasmid DNA. Plague (Yersinia pestis) is 0.5–0.8 × 1.5–2 μm size, facultative anaerobic gram negative rod-type bacteria, and infectious by the transmission with flea. Clinically, plague is divided into bubonic, septicemic and pneumonic types, and differs in incubation period, symptom and fatality. Pneumonic plague induces illness with difficulty of breathing, septicemia and malfunction of multi-organs, resulting in death with 100% fatality.

**CONCEPT OF DECONTAMINATION**

In biological and chemical warfare terrorisms, BCWAs are disseminated mainly as vapor or aerosol, or contaminated with food and beverages. As counterterrorism, detection of hazardous materials is performed, and if the existence of agents and the expose to the casualties are ascertained, “decontamination” (in the wide sense) is performed for the minimization of the terrorism damage. The concept of decontamination is “detection,” “containment,” “decontamination” (in the narrow sense) and “disposal” (Fig. 2). In this review article, the process of “decontamination” in the narrow sense is focused in research and technical aspects. The principal objective of the decontamination is the suppression of the enlargement of the disaster derived from the disseminated agents. Restoration of the field is the other mission. However, the first responders do not have enough knowledge about BCWAs, and can not adequately determine how to take decontamination procedures. Namely, whether or not the present decontamination system is proper is not scientifically decided. The decontamination methods should be selected, depending on the kinds, volumes/concentrations of agents, dissemination pattern (gas, liquid, solid, mediation), dissemination site (closed space or outside the building), and environmental conditions (season, climate). The goal of the decontamination work also differs, depending on the achieved remaining agent concentrations [perfect or only lowering of the contaminated level to be safe lower than the dangerous level (Table 1)]. The conceptual method is divided into “physical” and “chemical.” The former is the isolation of agents from the contaminated sites into the closed containers (containment), and elimination using the specific absorbing reagents. Even though the agents are not deactivated and sterilized in the exact contaminated field, the agents which are removed from the casualties and field and isolated in the containers, can be later deactivated at the other sites such as the first responder bases. The later is the chemical transformation of the agents to the different materials without toxicity and infectivity, includes the change of chemical structure, change of three-dimensional structure of the macromolecules, and disruption of the hyper-molecular structure (Fig. 2).

Gaseous CWAs such as blood agents and choking agents can be physically decontaminated by dispersing into the atmosphere or by elimination with passing through the absorbing liquid. They can be
also decontaminated chemically by wet decomposi-
tion. PS is decomposed by reaction with sodium
methylthiocarbamate. AC is decomposed by
oxidation reaction and microorganisms.

Volatile nerve gases and blister agents, non-
volatile vomit agents and lachrymators can be de-
contaminated physically by elimination using the
adsorbents such as active carbon. CWAs, which
have leaving functions in nerve gases and active
chlorines in blister, choking and vomit agents, can
be chemically detoxified by degradation of the ac-
tive functions with nucleophilic reagents. In alka-
line conditions, hydrolysis of nerve gases is signifi-
cant, and so sodium hydroxide (NaOH) and sodium
carbonate solution are used. VX is hydrolyzed
weakly, compared to the G-agents, and also their
nonvolatile nature gives high environmental persis-
tency. Nerve gases can be also decomposed by
oxidation. Amines and metals show decomposing
activity toward nerve gases by forming coordina-
tion bonding. In plant nerve gases are decom-
posed. Except for some agents such as VX, nitro-
gen mustard and CN, CWAs are generally un-
stable in water, and easily hydrolyzed. Because
of water solubility, it is possible to remove CWAs
from the casualties, goods, items, equipments and
building surfaces by washing with water. In the
concentrated conditions, blister agents react inter-
molecularly to form the condensed complexes.
Phosphorus carbon bond typical in nerve gases can
be cleaved by chemical and bacteriological ac-
tion. Arsenic CWA degradation intermediates can
be destroyed by microorganisms to be converted
to further detoxified compounds. Even though, re-
main ing arsine compounds pose disposal problem.
OC and STX can not be easily hydrolyzed, and so
should be detoxified by structural destruction
using oxidants. As for decontamination of non-
toxic lachrymators, treatment with toxic hypochlo-
rite leads to formation of rather toxic products.

Proteinous biological toxins can be decontami-
nated physically by elimination using the trapping
filter or using aggregating reagents to form precipi-
tates in solution. They can be also detoxified chem-
ically by destruction of the three-dimensional struc-
tures leading to protein denaturation where their bi-
ological activities (enzymatic and receptor binding
activities) are lost. Heating treatment, detergent ad-
dition and strong acid or alkaline solutions cause
the denaturing effect, and also oxidation or derivat-
tizing reagents modify the side chain functions.

Virus can be decontaminated physically by
elimination using the trapping filter. They can
be also detoxified biochemically by mutation of
the nucleic acids, by denaturation of nucleocapside
components, by inactivation of host-recognizing
molecules, or by structural destruction of viral en-
velopes, which lead to suppression of infectivity and
proliferation in the host cells. Viruses are gener-
ally unstable in environments, and easily inactivated
within several days by natural ventilation and sun
light after falling to the grounds. Even environment-
ally stable viruses such as small pox can be detox-
ified by physical effect of heat, short-wavelength
light irradiation, high pressure, ultrasonication and
addition of chemical reagents such as detergents
-envelope destruction), aldehydes (modification of
amine function) and oxidants.

Bacteria, rickettsia and chlamydia can be de-
contaminated physically by elimination using the
trapping filter. They can be sterilized biochemi-
cally by mutation of genomic and plasmid nucleic
acids using high energy light irrigation, by struc-
tural destruction through rapture of cell membranes
or cell walls, by denaturation of host-recognizing
molecules, or by inhibition of cell metabolism and
proliferation, which lead to suppression of living,
proliferating or infecting activity. Q fever is re-
sistant to heat, drying and disinfectant solution,
and stable in environment (−52–40°C, dryness, 5–
60 days). Bacterial resistance to decontamination
treatment differs in species and cell stages, and bac-
teria with growing stage are easily decontaminated.
In environment, vegetative cells are rather unstable,
and inactivated within several days after falling to
the grounds. Spores are resistant to various kinds
of decontamination treatments because of the spe-
cial spore coat structure and metabolic suppression.
Various kinds of technologies were compared for ef-
fective decontamination of Bacillus spores.

By BCWAs dissemination, the agents are incor-
porated into the surface materials with adsorption
and deposition. The material contaminated by the
agents is called “coupon,” and exert the influence
on the efficiency of the decontamination method.
The typical coupons are wood, carpet, ceiling tiles,
wall board, brick, ceramics, stainless steel and plas-
tics. The decontamination is rather easy for solid
stainless steel surface, but the plastics adsorbed with
CWAs and the carpets incorporated with BWAs are
difficult to be decontaminated due to physical iso-
lation. It is probable that the toxicity and infectiv-
ity are sequestered temporarily. For example, GB is
adsorbed on plastics, and anthrax spore is invaded
into the carpets, and in such situations direct and immediate threat of BCWAs exposure does not matter, but while time elapses GB is vaporized and anthrax particles are brown up. The sequestered BCWAs in coupons retain the hazardous potency, and can be protected against ultraviolet (UV) light irradiation and chemical reagent treatment, and so decontamination treatment can not give enough effect. Also, if spores are included in the food stuff, efficiency of wet decontamination is diminished.

Treatment using chemical modifier, high pressure and high temperature is enough to decontaminate BCWAs through destruction of toxic and infectious machinery, and it is not necessary to destroy the targets completely to convert to the broken pieces. If the perfect destruction of BCWAs is desired, perfect oxidation can be performed to convert to carbon dioxide, water, nitric acid, sulfuric acid etc. Chemical decontamination is a process of detoxification of the hazardous BCWAs, and from the phrase “poison fights one evil with another,” decontamination means destroying the dangerous targets by using dangerous reagents. Such decontaminating reagents are so hazardous to not only human but also environment, and the casualties may be poisoned and the environment be destroyed.

THE PRESENT SITUATION OF ON-SITE DECONTAMINATION AGAINST BCWAs

The decontamination in military, industry and laboratory is performed by the determined protocols, because the target hazardous materials are assumed. On the other hand, in the biological and chemical warfare terrorisms, kinds and volumes of the BCWAs disseminated can not be assumed, and excess decontamination may be performed in order to ensure safety level. In addition, the completion of decontamination work is difficult to be decided. In 1994 Matsumoto sarin attack, decontamination was not particularly performed, and GB was left to scatter and decompose spontaneously. In 1995 Tokyo subway sarin attack, victims rescued from the scenes were transported to the emergency hospitals, and medical stuffs were suffered from secondary exposure to GB. The subway trains where GB liquids were dispersed were treated to neutralization with sodium carbonate solution by the staffs of Japanese Ground Self-Defense Forces. In 2001 U.S. postal anthrax attack, the buildings where anthrax spores were disseminated were fumigated with chlorine dioxide.

In the present situation, the first responders get information about the kinds and volumes of the contaminated BCWAs using on-site detection equipments. Against gaseous and volatile CWAs, detection devices such as ion mobility spectrometer provide identification of some volatile CWAs. Against nonvolatile CWAs, it is impossible to detect in the field except for the special instrument (counter-flow atmospheric pressure chemical ionization mass spectrometry). Some biological toxins are detected by commercial lateral flow immunoassay. This method has the merit of the detection specificity but the drawback of limited detectable toxins (ricin, BTX A and B, SEB, STX). Against viruses, rickettsia, chlamydia and bacteria, protein assay kit, nucleic acid assay kit, flow cytometer and biological luminescence kit are used as screening test, and then the specific methods such as lateral flow immunoassay and real-time polymerase chain reaction (PCR) are adopted. However, the number of the threat BWAs is too numerous, and repertoire detectable by the specific methods is limited. Anyway, it is necessary to identify the agents by laboratory analysis after transporting the on-site samples and victim’s samples to the specific facilities.

According to the contamination situation monitored by on-site detection, decontamination is performed. As shown in Fig. 3, gaseous CWAs are eliminated by passive vaporization and active ventilation. Against the liquid and solid materials observable by naked eyes, they are scrapped physically, by isolation into the containers, or by covering with plastic seats. Against the liquid CWAs, adsorbents such as active soils and white flours are sprinkled over to adsorb and isolate agents. Usually, BCWAs adsorbed on the surfaces of the facilities and casualties are washed with water. More than 5% active chlorine solution such as sodium hypochlorite (NaClO), breech or solution which is prepared by dissolving bleaching powder with water, is particularly used as universal BCWAs decontamination method. Against BWAs, aldehyde solution such as formaldehyde and glutaraldehyde is also used.

Casualties are rescued from the hot zone (area with high BCWAs level) to the warm zone (area with low BCWAs level), and first-aid is performed after triage check. Then, they are transported to the emergency hospitals. At the entrance site
the clothes of casualties are stripped off to isolate BCWAs into the closed plastic bags and whole bodies were washed with warm water and soup water. Hypochlorite solution of 0.5% active chlorine level is also used for casualty decontamination.

Against CWAs and biological toxins which are dispersed in the closed space and partly adsorbed to the surface materials, water and diluted hypochlorite solution are showered or sprayed over. How the disseminated agents are removed efficiently by the decontaminant liquid depends on the contact efficiency. The wet decontamination may spoil the building facilities and equipments, and it is necessary to wash out or disperse the decontaminants after the termination of the decontamination work to mitigate the corrosive influence of the remaining decontaminates.

Against bacteria and viruses, heating treatment and spraying disinfection reagents are generally performed. Particular viruses are inactivated by heating at 80°C for 30 min, incubation in 0.1% tertiary ammonium salt solution for 30 min, incubation in 0.5% hypochlorite solution for 10–15 min. Particular bacteria were sterilized by heating at 117°C for several sec, at 80°C for 10 min, incubation in 0.1% tertiary ammonium salt solution for 30 min, incubation in 0.5% hypochlorite solution for 10–15 min, incubation in 1% phenol for several min. Spore forms of bacteria are killed by heating at 140°C for 60 min and incubation in iodine and chlorine solution. Biological toxins are weak to heating and disinfectant treatment, and BTXs and ricin are inactivated by heating at 100°C and 80°C for 10 min. SEB and STX can not be inactivated by heating at 100°C for 30 min. Detectability of BTXs, ricin, SEB and STX in lateral flow immunoassay was lost by incubation in 0.05% hypochlorite solution for 10 min. 

Against BWAs dispersed in the buildings, fumigation using formaldehyde, hydrogen peroxide, chlorine dioxide, ethylene oxide and methyl bromide is the general countermeasure. The concentrations of the remaining BWAs after fumigation are lowered depending on the product of the fumigant gas concentration (ppm) and treatment period (hr). The recommended values of the products for effective fumigation are reported to be 8000 ppm·hr for chlorine dioxide and 1000 ppm·hr for hydrogen peroxide. After fumigation, ventilation and monitoring of the fumigant gas should be performed.

Decontamination of the equipments of the first responders is performed by wet method using hypochlorite etc. Inner construction of precision machines is weak to corrosive action by disinfectant or fumigant gas, and so BCWAs are eliminated by air blowing or drying.

Recently, washing with large volume of water is chosen to eliminate the adsorbed and attached BCWAs from the casualties, goods, items, equipments and buildings. However, the disposal of the
waste water containing BCWAs is a big problem. BCWAs remain in the waste water, and it is not permitted to dispose the dangerous waste water at the field. The first responders must store the several m³ of waste water during on-site work and bring this water back to the base to dispose it later. It is obscure whether or not the waste water can be disposed as industrial waste.

**RESEARCH AND DEVELOPMENT OF ON-SITE DECONTAMINATION TECHNOLOGY**

To sum up, the present BCWAs decontamination system is not satisfactory. The problems which should be pointed out are followings. The evaluation methodology and the efficiency are not verified, and there is no clear scientific evidence concerning decontamination. It is not safe toward human and environment. Universal decontaminant, hypochlorite is very toxic and burdens the environment heavily. It is not valid against all the BCWAs. There are some BCWAs (VX, anthrax spore etc.) resistant to usual decontamination treatment. It is not effective, and it takes long time to complete the decontamination process perfectly. It is not long-standing, and the decontamination effect is only one use. It is serious to dispose large volume of contaminated waste water. Concerning R&D of decontamination system against BCWAs, summed-up national project was not planned in Japan. The present situation was only to import and imitate the system done in the advanced nations. However, the research levels even in the advanced nations can not be satisfactory. So, the research on decontamination should be spotlighted, and evidence based decontamination should be promoted. The author’s group applied the project in 2006 for “Research and Development Program for Resolving Critical Issue” sponsored by the Special Coordination Funds for Promoting Science and Technology, which is supported by the Ministry of Education, Culture, Sports, Science and Technology, and the theme “Development of effective decontamination technologies in biological and chemical warfare terrorism” was accepted. This three-years project is the coordination research among companies, academia and government, and the representative organization is National Research Institute of Police Science (NRIPS) realizing proper on-site needs, possessing authentic agents, specialized facility and detection technologies. As participating organizations, National Institute of Advanced Industrial Science and Technology, Chiba University, Kantogakuin University and Saga Prefectural Ceramic Research Laboratory possessing at-front technologies for biotechnology, molecular-recognition and photocatalysis, and G-L Science Ltd. and Axtis Company possessing achievement for manufacturing useful materials entered into the team. The respective organizations are leagued together systematically, and have promoted research, extensively utilizing at-front technologies. Figure 4 shows the concept of the project.

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**Fig. 4.** Project of Research and Development for Effective Decontamination System Devoted for Counterterrorism
Method for Estimating Decontamination Technology

In the verification of the present decontamination methods and R&D of new decontamination technologies, it is necessary to establish the method for scientifically evaluating the methods. Instead of using dangerous and law-regulated BCWAs, stimulants possessing similar characteristics of real BCWAs may be used for construction of the essential parts of the technologies, and in final steps for the development the authentic agents should be used. For gaseous CWAs (blood agent), volatile CWAs (nerve gas, blister agent) and nonvolatile CWAs (lachrymators), the decontamination level can be estimated by qualitative and quantitative determination of the remaining CWAs and degradation products. The CWAs and volatile degradation products are analyzed by gas chromatography-mass spectrometry (GC-MS)\(^\text{10,70}\) and polar decomposition products are structurally analyzed by liquid chromatography (LC-MS).\(^{71}\) The low MW biological toxins and the degradation products are analyzed by LC-MS,\(^{72}\) and the toxin activity is measured by bioassay\(^{73}\) or lateral flow immunoassay.\(^{61}\) The proteinous toxins are analyzed quantitatively by spectroscopy (protein assay, organic materials index) and enzyme-linked immunoabsorbent assay,\(^{74}\) and qualitatively by capillary electrophoresis,\(^{75}\) gel electrophoresis,\(^{76}\) surface plasmon resonance (SPR) spectrometry,\(^{77}\) LC-MS\(^{78}\) and matrix-assisted laser ionization MS.\(^{75,76}\) The cytotoxic activity is measured by cell culture assay\(^{79}\) and bioassay,\(^{80}\) and the enzymatic activity is measured by colorimetric assay.\(^{81,82}\) The viruses are measured by plaque forming test, and analyzed structurally by electron microscopy.\(^{83}\) The host-binding activity is measured by SPR. The rickettsia, chlamydia and bacteria are quantitatively analyzed by ATP bioluminescence assay,\(^{63}\) and by colony-forming assay, and structurally analyzed by light microscopy after staining\(^{84}\) and electron microscopy. The biological activity relating to toxicity is measured by enzyme assay. Nucleic acids of viruses, rickettsia, chlamydia and bacteria are analyzed by gel electrophoresis and real-time PCR methods.\(^{85}\)

It is also necessary to ascertain the completion of the decontamination in the field. Surface materials or air during and after the decontamination work are sampled for measuring the remaining BCWAs concentrations. However, existence of the decontaminating reagents in the samples may interfere with the accurate quantification.\(^{56,86}\)

Verification of the Existing Decontamination Technologies

The performance of the present decontamination methods should be verified, and the suitable procedures should be built up as recommended system for first responders. The author’s group examined the efficiency of the existing technologies experimentally. As for gaseous CWAs, wet absorption method was verified. Five hundred ml of AC gas (200 mg/m\(^3\)) produced by combining potassium cyanide and sulfuric acid in closed 10 l glass container was passed through the absorbent solution (0.1 M NaOH, 320 ml, 12 cm travelling path) with the flow rate of 500 and 1000 ml/min, and the effluent gas was analyzed by gas chromatography\(^{10}\) [column: HP-PLOT Q (Agilent Technologies, Palo Alto, CA, U.S.A., 0.53 mm \(\times\) 30 m, 40 \(\mu\)m film thickness), 120°C; carrier: helium 5 ml/min; injection: 200°C, split (ratio 5); detection: nitrogen phosphorus detection (NPD), 250°C]. As a result, AC was completely eliminated to be 0.015 and 0.12 mg/m\(^3\) in the effluent gas which was lower than the background air levels.

As for volatile CWAs, wet decomposition method using acid, base and hypochlorite was verified. As shown in Fig. 5, G-agent nerve gases were hydrolyzed in weak alkaline solution (below 10 mM NaOH) during 2 min incubation. In contrast, VX was not hydrolyzed even under the high alkaline solution. Six % VX was remained after 10 min incubation with 3 M NaOH treatment. Mustard type CWAs were hydrolyzed in water, with pH independent manner. Under the neutral pH condition, HD and nitrogen mustard 1 (HN-1) were considerably hydrolyzed within 1 hr, but nitrogen mustard 2 (HN-2) and 3 (HN-3) were not hydrolyzed. Under the weak alkaline conditions, HN-2 and HN-3 were considerably hydrolyzed within 1 hr. In the aqueous conditions, mustard compounds did not show complete hydrolysis, and this may be due to the reverse condensation reaction of mustard hydrolysis products and chloride ions.

Hypochlorite showed extensive degradation against CWAs (Fig. 5). G-agent nerve gases and HD were perfectly decomposed in diluted hypochlorite solution (below 0.05% effective chlorine) during 2 min incubation. In contrast, VX, HN-2 and HN-3 were not decomposed even in 0.5% hypochlorite solution. This 0.5% level is the recommended level used for BCWAs decontamination.\(^{14,59}\) By the incubation for 5 min in 1% hypochlorite solution, all the CWAs tested were completely decomposed.
Fig. 5. Decomposition of Nerve Gases and Blister Agents by Wet Methods

(A) Degradation plot of nerve gases in the presence of sodium hydroxide (NaOH). (B) Degradation plot of blister agents under various pH conditions [pH value was theoretically calculated from the added concentration of hydrochloric acid (HCl) and NaOH]. (C) Degradation plot of nerve gases and blister agents in the presence of sodium hypochlorite (NaClO). GB, GD, GA, cyclohexylsarin (GF), VX, HD, HN-1, HN-2 and HN-3 (5 ml, final 100 µg/ml) were incubated in water containing various concentrations of HCl, NaOH and NaClO at 25°C for 2 min, and then the CWAs were extracted with 1 ml of dichloromethane containing internal standard (nonadecane: final 100 µg/ml). VX and nitrogen mustards were extracted under basic condition supplemented with 0.04 M tris(hydroxylmethyl)aminomethane. The extracts were analyzed by gas-chromatograph mass spectrometer [S10] using DB-5MS column (0.25 mm × 30 m, film thickness 0.25 µm), temperature program [40°C (1 min) to 290°C by 40°C/min] with 0.7 ml helium/min flow and electron ionization (m/z 40–550).
Table 2. Decomposition of CWAs by Commercial Decontamination Products

<table>
<thead>
<tr>
<th>Decontaminant</th>
<th>Concentration</th>
<th>GB</th>
<th>GD</th>
<th>GF</th>
<th>GA</th>
<th>VX</th>
<th>HD</th>
<th>HN-1</th>
<th>HN-2</th>
<th>HN-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasyDECON</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>MODEC DECON</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
<td>0</td>
<td>1.7</td>
<td>2.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Acecide</td>
<td>10%</td>
<td>85</td>
<td>110</td>
<td>108</td>
<td>82</td>
<td>59</td>
<td>0</td>
<td>1.6</td>
<td>21</td>
<td>62</td>
</tr>
<tr>
<td>FAST ACT</td>
<td>0.5 g</td>
<td>0.3</td>
<td>1.8</td>
<td>1.6</td>
<td>0</td>
<td>21</td>
<td>62</td>
<td>55</td>
<td>57</td>
<td>98</td>
</tr>
<tr>
<td>Eliminator</td>
<td>100%</td>
<td>68</td>
<td>98</td>
<td>93</td>
<td>53</td>
<td>125</td>
<td>75</td>
<td>25</td>
<td>53</td>
<td>85</td>
</tr>
<tr>
<td>Hyper Ion Water Acid</td>
<td>20%</td>
<td>64</td>
<td>96</td>
<td>94</td>
<td>2.9</td>
<td>96</td>
<td>89</td>
<td>53</td>
<td>59</td>
<td>86</td>
</tr>
<tr>
<td>Hyper Ion water Alkaline</td>
<td>20%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>95</td>
<td>111</td>
<td>23</td>
<td>15</td>
<td>54</td>
</tr>
</tbody>
</table>

GB, GD, GA, GF, VX, HD, HN-1, HN-2 and HN-3 (5 ml, final 100 µg/ml) were incubated in the decontamination products at 25°C for 2 min, and then the CWAs were extracted with 1 ml of dichloromethane containing internal standard (nonadecane: final 100 µg/ml). VX and nitrogen mustards were extracted under basic condition supplemented with tris(hydroxylmethyl)aminomethane. For FAST ACT, 10 µl of 3% CWAs (w/v) in n-hexane was mixed with 0.5 g of the powder, stood at room temperature for 2 min, and then extracted with 1 ml of dichloromethane including internal standard. The extracts were analyzed by gas-chromatograph mass spectrometer. Values show the percentage of the remaining CWAs.

Foam-type decontamination products have been developed, and are reported to be effective for long-term decontamination. Various types of mixture solution of active ingredients has been already developed and put to practical usage. Although the detailed information about the inclusions has not been opened, detergents, amines and oxidative reagents may be included, and these decontaminants are reported to detoxify not only CWAs but also BWAs. Commercial products [EasyDECON (EnviroFoam Technologies, Inc., Huntsville, AL, U.S.A.), MODEC DECON (Modec, Inc., Denver, CO, U.S.A.) etc.] which work as foam with several minute-lasting by mixing two packaged reagent bottles, have brought to the markets for not only military usage but also civil defense. Liquid disinfectants such as Eliminator (Sea To Sky Innovations, Inc., Auckland, New Zealand) have been commercially available for decontamination of viruses, bacteria and spores. Commercial peracetic acid (Acecide 6% disinfectant, Saraya Co. Ltd., Osaka, Japan) are used in medical region, which replaces with hydrogen peroxide as safer decontaminant. Peracetic acid shows higher hydrobobicity compared to hydrogen peroxide, and works effectively by the elevated penetration potency into the microorganism membranes. Fine particle materials possessing higher adsorption capacity have been developed such as vanadium oxide nanotubes and impregnated silica nanoparticles. Commercial titanium oxide particles (FAST ACT, NanoScale Materials, Inc., Manhattan, KS, U.S.A.) adsorb and immobilize CWAs quickly when they are sprinkled on the liquid form of CWAs. Deep sea water is used for healthcare etc., and processed to produce decontaminating reagents through physical separation and concentration steps. Acid and alkaline forms of Hyper Ion Water (Fine Crest Co., Tokyo, Japan) appear for environmental hygienic means. We examined the efficiency of the new commercial decontamination products for CWAs decomposition. As shown in Table 2, oxidant-containing foam-forming decontaminating liquids, EasyDECON and MODEC DECON decomposed CWAs significantly even for VX. Peracetic acid-containing disinfectant liquid, Acecide was not effective for decompose CWAs except for HD. Fine titanium oxide powder, FAST ACT showed extensive degradation against nerve gases, but did not decompose blister agents. Disinfectant liquid, Eliminator was not effective in decomposing CWAs. Although Hyper Ion Water Acid did not decompose CWAs except for GA, Hyper Ion Water Alkaline was effective for CWAs decomposition except for VX, HD and HN-3.

Special enzymes possess the decomposing activity toward CWAs, especially against nerve gases. It is reported that bacteria isolated from some soils showed decomposition of organophosphorus pesticides, and fundamental and applied research on these enzymes have been extensively performed. Recently, such decomposing enzymes are co-tailed with other chemical decontaminants, and used as composite decontaminants having additional merit. Besides using soluble enzymes, bacteria themselves expressing the enzymes on the cell surfaces are utilized. Organophosphorus hydrolase (OPH) shows high hydrolytic activity toward GB but low toward VX. By site-directed mutagenesis on OPH active site, the resulting transformants showing higher hydrolytic activity toward VX is constructed. The antibodies possessing hydrolytic activity toward nerve gases are reported. Enzymatic methods for decontaminating not only CWAs but also BWAs are summarized by Richardt.
and Blum.\textsuperscript{101} Kantogakuin University (Prof. Kawahara) and NRIPS investigated the decomposing power against nerve gases using \textit{Flavobacterium} OPH. In consideration to the improvement of the hydrolytic activity by genetic mutation, the DNA coding OPH was incorporated into pET32b plasmid, and OPH was expressed in the transformed \textit{Eschericia coli} \textsuperscript{102}. The expressed OPH enzymes which contained extended signal peptide sequences showed different metal ion (cobalt, zinc) requirement profiles for hydrolytic activity compared to the parent strain OPH. The recombinant OPH enzymes showed the similar paraoxane hydrolytic activity, and stronger activity against GB. Although site-directed mutagenesis was introduced to the active site region in DNA coding OPH,\textsuperscript{99} the recombinant enzyme (136Leucine→Tyrosine) did not hydrolyze VX efficiently. Recombinant OPH was modified to contain N-terminal His-tag sequence, and after activation with zinc, immobilized to the nickel-column (1.3 ml). CWAs (0.3 ml, final 50 µg/ml, pH 8.5 buffer) were incubated in the OPH immobilized column at 37°C for 20 min. The column eluate was extracted with dichloromethane, and analyzed for GC-MS. As a result, GB and GA were completely hydrolyzed, and 20–40% of GD and cyclohexylsarin were hydrolyzed. This bioreactor technology seems promising, as clean, safe and continuous decontamination tool against toxic nerve gases.

As for proteinous toxins, the author’s group ascertained that the detectability of ricin in BTA lateral flow immunoassay was lost by heat treatment, addition of low level of hypochlorite and high level of formaldehyde.\textsuperscript{66} Hypochlorite was also examined for inactivating ricin. Ricin (1.3 mg/ml) was incubated at 37°C for 20 min with NaClO, and mixed with the substrate solution [2 µM RNA (5′-CGCGCGAGAGCGCG-3′), 5 mM ammonium acetate, pH 4.0; ricin: 0.5–20 nM], and further incubated for 30 min. The reaction was terminated by the addition of 20 mM ammonia, and subjected to the quantification of the released adenine by LC-MS (column: X-bridge C18 3.5 µm, 2.1 × 100 mm, Waters Co., Ltd., Milford, MA, U.S.A.); elution: 2.5% (1 min)–25% (2 min) acetonitrile in 1 mM ammonium acetate pH 5.3 by 6 min; temperature 40°C; injection 10 µl; electrospray ionization (capillary +3.5 kV, cone 35 V); Q-pole: 100°C, drying gas N\textsubscript{2} 350 l/hr 300°C, selected ion monitoring at m/z 136). Ricin ribonuclease activity (adenine releasing) was disappeared by the incubation in higher than 0.1% hypochlorite solution.

Ricin (≦250 µg/ml) was incubated at 25°C for 10 min in NaClO (≦1%) solution, and 1/250 part was mixed with the human pre-monocyte U-937 cell culture (1–2×10\textsuperscript{6} cell/ml, RPMI-1640 medium) and further incubated at 37°C for 39–44 hr, and then the number of the living cells were counted by trypan blue exclusion method. More than 12.5 µg/ml of ricin showed the culture cell lethality, and the preincubation of ricin with more than 0.1% hypochlorite suppressed the culture cell lethality.

As for bacteria, hypochlorite was examined for sterilizing anthrax stimulant \textit{Bacillus subtilis}. As shown in Fig.6, although vegetative cells were sterilized by incubation with 0.005% hypochlorite, spores showed proliferation at this level. Spores were sterilized with more than 0.1% hypochlorite. \textit{Bacillus anthrax} was incubated at 25°C for 20 min with various concentrations of NaClO, and subjected to colony-forming test. Vegetative cells were sterilized by the incubation with 0.01% hypochlorite, and spores were sterilized with more than 1% hypochlorite. Spore forms of anthrax were resistant to the hypochlorite (0.5%) treatment which level is the recommended level used for BCWAs decontamination.\textsuperscript{14, 59} Development of Novel Adsorption Technology Using Molecular Recognition Devices Elimination of BCWAs seems effective as decontamination. However, the present elimination technologies such as active carbon adsorption against low MW CWAs and membrane filtration against BWAs are nonspecific, and the trapping ca-
Lactose (40.5 mg) was immobilized to the carboxyl-terminal monolithic silica with 2 µm through-pore and 20 nm mesopore, and this sugar monolithic silica was formed to be a disc (20 mm φ × 5 mm long disc, left inserted figure), and then set to the stainless steel cartridge (right inserted figure). Ricin solution (150 µg/ml in 10 mM phosphate buffer, pH 7.0) was flown into the cartridge with the rate of 5 ml/min, and the effluent was monitored with UV light absorbance (280 nm). The non-adsorption fraction and break-through fraction were collected, and subjected to the protein assay, sodium dodecylsulfate polyacrylamido gel electrophoresis, surface plasmon resonance assay for lactose binding, ribonuclease assay and cytotoxic assay.

Many biological toxins, viruses and bacteria show toxicity and infectivity by invading the host cells by the specific recognition of the complex carbohydrates on the host cell surface. We utilized carbohydrate chains as the molecular recognition devices for wet decontamination. We have already developed the sensitive on-site ricin detection technology, which is composed of galactose ligand and SPR sensor. If carbohydrate chains can not be used, the other types of molecular recognition devices can be searched such as DNA and RNA aptamers and molecular imprinted polymers. As for the adsorbent support materials, we considered “monolithic silica” because of high flow-through capacity and possible control of pore size. This technology has been applied to sample pretreatment methods such as protein digestion using trypsin-immobilized monolithic silica. Lactose was densely immobilized on the pipette scale of silica monolith disc via triethylene glycol spacer using microwave. The resulting adsorbent provided specific elimination of ricin and not the other proteins even under the high salt conditions. The established adsorption technology was applied to the larger scale decontamination. As shown in Fig. 7, lactose (45.5 mg) was immobilized to the silica monolith disc (20 mm φ × 5 mm long). Fifty three mg of ricin could be trapped on the disc during the constant flow of the ricin solution. About 4% concentration level of ricin was passed through the disc. This non-adsorption fraction was proven to be the same molecular structure of ricin but show lower ricin activities (ribonuclease, cytotoxic, and galactose binding activities), indicating that only active ricin binds strongly to this adsorbent and denatured ricin binds weakly.

Fullerenes can be utilized as decontaminants by immobilization of lactose chains to provide molecular-recognition function and raise hydrophilicity. Newly synthesized lactose-fullerenes can be easily converted to pseudo-liposomal hypermolecules. This organic nanocapsules (diameter: 40–50 nm) was examined for elimination capability for ricin in water. Under the high salt conditions (more than 100 mM sodium chloride), more than 95% of ricin was precipitated during the incubation at 25°C for 20 min by adding 363 µM lactose-fullerene into the ricin solution (100 µg/ml).
Organic polymers can be also used as adsorbent support. Lactose was immobilized to polyacrylamide (18–65% lactose density). The resulting glycopolymers effectively interfered with the ricin-lactoside adhesion event.\textsuperscript{109}

Antibody having high binding affinity and specificity toward macromolecules is a candidate of the molecular-recognition device, but in utilizing antibody we should consider the fundamental problems, that it cannot be supplied with long period and not stable under the hard field conditions. So, we tried to design the artificial peptides or proteins using phage display technology.\textsuperscript{110} SEB is a research target because it does not show specific binding to the carbohydrate chain. Fragments antigen-binding (Fab fragments) and single-chain variable fragments (scFv) against SEB were produced by phage display technology as shown in the following steps.\textsuperscript{111} SEB epitopes were first identified by phage display approach using the commercial anti-SEB monoclonal antibody ab53981 (Abcam, Cambridge, U.K.) as the target. Heptamer and dodecamer mimotope peptides recognized by ab53981 were screened from Ph.D-7 or Ph.D-12 random peptide phage libraries (New England Biolabs, Ipswich, MA, U.S.A.), providing a common sequence homologous to \textsuperscript{8}PDELHK\textsuperscript{14}S in the amino-acid sequence of SEB. The N-terminal 15-mer peptide was assessed to be an epitope. After immunization in mice with maltose-binding-protein-tagged N-terminal 15-mer peptide, a phage display Fab library was constructed using cDNA prepared from the mRNA of spleen cells. After three rounds of panning, one phage was obtained which produced a soluble Fab fragment from the transformed cells, and the fragment was converted into single-chain variable fragment. SPR analysis showed that the dissociation constants of the produced molecular recognition proteins with SEB were almost compatible to that of the antibody ab53981.

Development of Decomposing Technology Using Photocatalysis

The toxicity of ordinary oxidative reagents such as hypochlorite is high to harm not only human but also equipments and environment. So, lowering of the toxicity of the decontaminants is desirable. Photocatalysis has emerged as safe and permanently working technology for cleaning pollutants, and research levels on both basic science and application in Japan is prominent.\textsuperscript{112} The author’s group determined to utilize the photocatalytic technology to BCWAs decontamination. At first, we verified the usefulness of photocatalysis in decontamination of BCWAs. Dry decontamination of volatile CWAs was examined using ordinary photocatalytic material, titanium oxide. GB, GA and HD were vaporized, and introduced into the reaction chamber of photocatalysis without and with UV light irradiation, and the periodically sampled gas was analyzed by GC-MS. As shown in Fig. 8, without UV light irradiation some portion of the introduced GB and HD were adsorbed to the titanium oxide film to be the plateau concentrations within several decade minutes, and then after initiation of UV light irradiation the concentrations of the vapor CWAs were decreased exponentially. We can calculate the photocatalytic efficiency by analyzing the disappearance curves. As a result, the efficient dry decomposition of GB, GA and HD was ascertained. In addition, by the analysis of the infra-red spectroscopic change using attenuated total reflection Fourier transform infrared spectroscopy, adsorption and degradation of GB on the photocatalyst was observed, providing photocatalytic mechanism.\textsuperscript{113} GB was transformed to isopropylmethylphosphonate in the adsorption step, and alkoxyl function was cleaved in the photocatalytic step. In contrast, HD was not decomposed in the absorption step, but in the photocatalytic step. We also examined the wet decomposition of non-volatile VX and nitrogen mustards by the experiment where UV light was irradiated to the closed glass tubes containing the titanium oxide powder and CWAs in water and periodically sampled inner solution was extracted with dichloromethane and analyzed by GC-MS. We could ascertained the efficient photocatalytic decontamination of VX and nitrogen mustards. Besides, we examined the dry decontamination of virus stimulant MS2 coliphage (UV light resistant) and anthrax stimulant \textit{Bacillus subtilis} and \textit{Bacillus anthracis} spores by the experiment where UV light was irradiated to the stained BWAs on the titanium oxide powder coated glass cover and periodically sampled BWAs powder was subjected to plaque or colony forming test. We could ascertained the adequate photocatalytic decontamination of MS2 coliphage and \textit{Bacillus subtilis} spores. Kau et al. report the reduction of anthrax spore-induced mortality by visible light-activated photocatalyst by \textit{in vivo} experiment.\textsuperscript{114}

Ordinary titanium oxide powder used in photocatalysis has the advantage in semi-permanently working, but the catalytic power is too low to realize the rapid decomposition of disseminated BCWAs.
GB and HD were vaporized in 500 ml glass sampling container, and introduced into the glass reaction vessel (200 ml) containing glass slide coated with 1.25 mg P25 photocatalitic particle (Nippon Aerojil, Tokyo, Japan) per 5 cm² at room temperature. After 35 min, the ultraviolet light (360 nm) was irradiated (6 mW/cm², left figure). The inner air (0.4 ml) was periodically sampled and subjected to the analysis of fast gas-chromatograph mass spectrometer using Custom DB-5MS column (Agilent Technologies, Palo Alto, CA, U.S.A., 0.18 mm × 10 m, film thickness 0.18 µm), temperature program [40°C (0.2 min) to 290°C by 150°C/min, then 1 min hold] with 0.5 ml helium/min flow and electron ionization (m/z 40–300). The vapor concentrations of GB (closed circle) and HD (open square) in the reaction vessel were plotted against the reaction time (min).

in the terrorism filed. We tried to raise the catalytic power by improving the material construction methods, and succeeded in manufacture of constant sized and high-activity materials composed of peroxo titanium oxide. We also tried to build up the automated continuous decomposition system adopting photocatalysis under UV light irradiation, and ascertained the continuous decomposition of GB simulant dimethylmethylphosphonate by the repeated-batch reaction experiment. This type of automatic air purification equipment can be used in the hot zone to diminish the air level of dangerous CWAs.

Finally, we tried to develop the technology of coating photocatalytic materials on the protective suits. Photocatalyst-coated suits should work efficiently in the countermeasure work of the first responders in the hot zone. One piece of fluoride-impregnated butyl lubber protective suit material was coated with titanium oxide power in the presence of fluoroethylene-vinylether-copolyer and ethyleneglycol after hydrophilic surface treatment of alkaline immersion. We ascertained the efficient material coating which provided resistance to mechanical abrasion and dry decontamination against GB and GD.

**CONCLUSION**

In the field disseminated with BCWAs, it is necessary to decontaminate hazardous BCWAs safely, efficiently and effectively for minimization of the terrorism damage and field restoration. However, the present decontamination methods are used only empirically, and so it is urgently required to establish the decontamination system fulfilling the on-site mission for realization of safe and secure society. By utilizing at-front scientific technologies, the author’s group has established the evaluation methods using the authentic BCWAs, verified the present decontamination methods, and developed both novel specific adsorption technologies and photocatalytic technologies. Figure 9 shows the outline of the recommended scheme devoted to the on-site decontamination, which are composed of the present methods and our developing technologies, and both are verified for the field performance. This system is not still perfect, and in the near future R&D should be performed to reinforce the decontamination system fulfilling the on-site needs.

**Safety Consideration** Chemical warfare agents are highly toxic; these compounds should be carefully handled using protective clothing within a fume hood, and destroyed with sodium hypochlorite after experiments. Usage and synthesis of chem-
Fig. 9. Recommended Decontamination System Composed of the Existing and Newly Developed Technologies

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