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TECHNICAL REPORT

N,N-Dihaloamine Explosives as Harmful Agent Defeat Materials

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14. ABSTRACT

Sporicidal efficacy against anthrax surrogate spores was determined for detonation products from two specific classes of novel energetic materials based on the general class of N,N-dihaloamine derivatives: organic N,N-difluoramine (NF2) and N,N-dichloramine (NCl2) derivatives. Sporicidal efficacies from an N,N-difluoramine explosive, HNFX, were determined over a wide range of exposure times, ranging from milliseconds to seconds to hours. High killing efficacy ($\gtrsim$9 log-reductions) of anthrax surrogate spores was achieved by practically brief exposures (5 seconds or more) to gaseous products from detonations of HNFX. This agent defeat by HNFX was not due to heat or pressure of explosions but to harsh conditions of exposure to biocidal detonation products. The active sporicide was originally proposed conceptually to be hydrogen fluoride (HF), but other transient more-reactive halogen species may be involved. In comparison, a conventional nonhalogenated explosive of similar explosive power, HMX, showed only ~0.2 log-reduction of Bacillus spores following an even longer exposure (0.4 hour) to its detonation products. A powerful new elemental-chlorine-generating explosive in the class of N,N-dichloramines [1,3,5-benzenetris(N,N-dichlorosulfonamide)] was not nearly as effective against Bt spores as HNFX was. Another N,N-dichloramine, hexachloromelamine, was insufficiently explosive to detonate.

15. SUBJECT TERMS

Dihaloamine; difluoramine; dichloramine; HNFX; sporicide; octafluoropentaerythrityltetramine; hexachloromelamine; benzenetris(N,N-dichlorosulfonamide)

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CONVERSION TABLE

Conversion Factors for U.S. Customary to metric (SI) units of measurement.

MULTIPLY →	BY →	TO GET
TO GET ←	BY ←	DIVIDE
angstrom	1.000 000 x E -10	meters (m)
atmosphere (normal)	1.013 25 x E +2	kilo pascal (kPa)
bar	1.000 000 x E +2	kilo pascal (kPa)
barn	1.000 000 x E -28	meter^2 (m^2)
British thermal unit (thermochemical)	1.054 350 x E +3	joule (J)
calorie (thermochemical)	4.184 000	joule (J)
cal (thermochemical/cm^2)	4.184 000 x E -2	mega joule/m^2 (MJ/m^2)
curie	3.700 000 x E +1	*giga bacquerel (GBq)
degree (angle)	1.745 329 x E -2	radian (rad)
degree Fahrenheit	$t_k = (t^\circ f + 459.67)/1.8$	degree kelvin (K)
electron volt	1.602 19 x E -19	joule (J)
erg	1.000 000 x E -7	joule (J)
erg/second	1.000 000 x E -7	watt (W)
foot	3.048 000 x E -1	meter (m)
foot-pound-force	1.355 818	joule (J)
gallon (U.S. liquid)	3.785 412 x E -3	meter^3 (m^3)
inch	2.540 000 x E -2	meter (m)
jerk	1.000 000 x E +9	joule (J)
joule/kilogram (J/kg) radiation dose absorbed	1.000 000	Gray (Gy)
kilotons	4.183	terajoules
kip (1000 lbf)	4.448 222 x E +3	newton (N)
kip/$inch^2$ (ksi)	6.894 757 x E +3	kilo pascal (kPa)
ktap	1.000 000 x E +2	newton-second/m^2 ($N-s/m^2$)
micron	1.000 000 x E -6	meter (m)
mil	2.540 000 x E -5	meter (m)
mile (international)	1.609 344 x E +3	meter (m)
ounce	2.834 952 x E -2	kilogram (kg)
pound-force (lbs avoirdupois)	4.448 222	newton (N)
pound-force inch	1.129 848 x E -1	newton-meter (N-m)
pound-force/inch	1.751 268 x E +2	newton/meter (N/m)
pound-force/$foot^2$	4.788 026 x E -2	kilo pascal (kPa)
pound-force/$inch^2$ (psi)	6.894 757	kilo pascal (kPa)
pound-mass (lbm avoirdupois)	4.535 924 x E -1	kilogram (kg)
pound-mass-$foot^2$ (moment of inertia)	4.214 011 x E -2	kilogram-meter^2 ($kg-m^2$)
pound-mass/$foot^3$	1.601 846 x E +1	kilogram-meter^3 (kg/m^3)
rad (radiation dose absorbed)	1.000 000 x E -2	**Gray (Gy)
roentgen	2.579 760 x E -4	coulomb/kilogram (C/kg)
shake	1.000 000 x E -8	second (s)
slug	1.459 390 x E +1	kilogram (kg)
torr (mm Hg, 0^0 C)	1.333 22 x E -1	kilo pascal (kPa)

*The bacquerel (Bq) is the SI unit of radioactivity; 1 Bq = 1 event/s.

**The Gray (GY) is the SI unit of absorbed radiation.

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EXECUTIVE SUMMARY

We undertook to synthesize and experimentally determine the sporicidal efficacy against anthrax surrogate spores of two specific classes of novel energetic materials based on the general class of *N,N*-dihaloamine derivatives: organic *N,N*-difluoramine (NF_2) derivatives and *N,N*-dichloramine (NCl_2) derivatives.

Sporicidal efficacies of gaseous detonation products from an *N,N*-difluoramine explosive, HNFX, were determined over a wide range of exposure times, ranging from milliseconds to seconds to hours. For exposure times $\lesssim$1 second, observed $\log_{10}$-reductions of *Bt* were $\lesssim$3. However, high killing efficacy ($\gtrsim$9 log-reductions) of anthrax surrogate spores was achieved by practically brief exposures (5 seconds or more) to gaseous products from detonations of HNFX.

This agent defeat by HNFX was not due to heat or pressure of explosions but to harsh conditions of exposure to biocidal detonation products. The active sporicide was originally proposed conceptually to be hydrogen fluoride (HF), but other transient more-reactive halogen species may be involved. An example may be atomic fluorine or its by-products of reaction with spore materiel. Such transient reactive species may be unique to the class of *N,N*-difluoramines.

Glycerol acts as an apparent protective agent against the sporicidal product(s) produced by HNFX detonation. This is inconsistent with hydrogen fluoride as the sole sporicide (as glycerol is a known inert solvent for HF) but is consistent with more-reactive transient intermediates getting scavenged by oxidizable glycerol.

In comparison, a conventional nonhalogenated explosive of similar explosive power, HMX, showed only ~0.2 log-reduction of *Bacillus* spores following an even longer exposure (0.4 hour) to its detonation products.

One powerful elemental-chlorine-generating explosive in the class of *N,N*-dichloramines (BTD) was not nearly as effective against *Bt* spores as HNFX was. Another *N,N*-dichloramine, hexachloromelamine, was insufficiently explosive by itself to detonate. Residual solid by-products from HCM explosions were sporicidal, but gaseous products of such explosions were not very efficacious against *Bt* following $\gtrsim$5 seconds of exposure.

This project's results have proven the feasibility of the proposed general approach to defeat biological harmful agents using novel *N*-halogenated explosives that produce biocidal detonation products, such as hydrogen fluoride (or transient reactive fluorine species), under harsh conditions that rapidly kill anthrax surrogate spores in relatively short exposure times.

OBJECTIVES

Objectives of this project included the preparation and characterization of novel chemical explosive compounds capable of producing biocidal products of explosion (such as hydrogen fluoride or elemental chlorine) and a determination of their feasibility as effective harmful agent defeat weapon components.

BACKGROUND, PREMISE, AND GENERAL APPROACH

The Defense Threat Reduction Agency's "Basic Research for Combating Weapons of Mass Destruction" program (HDTRA1-07-BRCWMD) called for novel energetic materials to produce biocidal reaction products from explosive events that destroy structures or containers involving harmful chemical or biological agents, thus neutralizing the agents post-blast.

Some past agent defeat weapon development projects have utilized hydrogen chloride as a chemical neutralizer for biological agent simulants, e.g., Lockheed Martin's "Agent Defeat Warhead Device":[1] "These propellants produce gas-phase water and hydrogen chloride that combine to form very reactive hot hydrochloric acid; and as the reactants cool the cooled hydrochloric acid remains in the bunker and may act to continue neutralization of bunker contents for many days… Thus, incendiary agents based on standard composite rocket propellant technology are logical choices for the application described herein." However, it has long been known that hydrogen chloride has among the poorest bactericidal disinfecting activities of common acid species.[2] In contrast, hydrogen fluoride has long been recognized as an efficient antibacterial, antimicrobial biocide, with greater activity than HCl.[3] At a concentration of only 200 ppm, dilute aqueous hydrogen fluoride showed complete destruction of *Pseudomonas aeruginosa* and *Saccharomyces diastaticus* in five minutes.[4] Even concentrations of 1–100 ppm are capable of sterilizing water lines.[5] Hydrogen fluoride offers a further advantage over higher-valent halogen oxidizers in avoiding volatile, noxious by-products—such as chloramines—upon reaction with organics, so that environmental remediation of a decontaminated facility might be achieved merely by neutralizing the residual hydrogen fluoride with a volatile base (perhaps ammonia) and washing away the neutral salt. In contrast, oxidizing chemical neutralizers like chlorine have a tendency to linger somewhat after deployment. Thus, cleanly combusting organics that produce volatile neutralizers, as proposed here, offer environmental advantages over less-volatile, inorganic sources of such neutralizers, which sources may linger long-term and pose a continuing hazard.

Based on the attractiveness of hydrogen fluoride as a superior agent defeat by-product—from its known biocidal activity—a class of energetic ingredient that appeared particularly promising for incorporation into explosive formulations for the specific application of agent defeat weapons is the class of energetic difluoramines. Though hundreds of compounds in this class were developed under the aegis of Project Principia in the 1960s,[6,7] more-practical and attractive candidates in this class have been getting developed only in the last 10–20 years.[8] Energetic difluoramines are kinetically capable of producing expected thermodynamic products

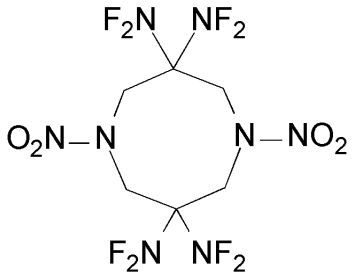
---

[1] Jones, J.W. US Patent 6382105 (2002).
[2] Paul, T.; Birstein, G.; Reuss, A. *Biochemische Zeitschrift* **1911**, *29*, 202.
[3] Lockemann, G.; Lucius, F. *Desinfektion* **1913**, *5*, 261.
[4] Bessems, E.; Junger, R. GB Patent 1584845 (1981).
[5] Tatsuno, T.; Miyamoto, M.; Ohta, Y.; Sawada, K. US Patent 5147605 (1992).
[6] Davenas, A *J. Propul. Power* **2003**, *19*, 1108.
[7] Castellano, J. *Amer. Scientist* **2008**, *96*, 489.
[8] Chapman, R.D. *Structure & Bonding* **2007**, *125*, 123.

from thermochemical processes such as detonation. Experimental measurements of detonation behavior have shown that performance is superior when HF is a fluorine product—as observed from 1,2-bis(difluoramino)propane, for example—rather than C–F species.[9]

We therefore undertook to synthesize and experimentally determine the sporicidal efficacy of two specific classes of novel energetic materials based on the general class of *N,N*-dihaloamine derivatives: organic *N,N*-difluoramine (NF_2) derivatives and *N,N*-dichloramine (NCl_2) derivatives. We expected that the former class of ingredient, difluoramine derivatives, should take higher priority, as their behavior as energetic materials is better understood, they have richer feasible synthetic methodology, and their biocidal by-product of detonation, hydrogen fluoride, has some technological advantages in field deployment over the expected by-product from the dichloramines, elemental chlorine.

A potentially attractive difluoramine derivative is 3,3,7,7-tetrakis(difluoramino)octahydro-1,5-dinitro-1,5-diazocine (HNFX).[10]



HNFX

HNFX has so far been produced in a crystallographic form that has significantly lower density than that theoretically predicted. This would limit its adoption as a large-scale replacement for a less expensive explosive such as HMX; however, as a candidate for a specific application such as hydrogen fluoride generation in an agent defeat weapon, it is still attractive. Cheetah code calculations show that it would generate 7.99 moles of HF per mole of HNFX upon detonation. It therefore has >39 wt% content of biocide (HF)-generating capacity.

A particularly attractive alternative candidate for an HF-generating explosive composition was envisioned: octafluoropentaerythrityltetramine or tetrakis(difluoramino)neopentane, $C(CH_2NF_2)_4$, was an "NF_2 analog of PETN" and an unknown target compound that may be feasible from direct fluorination of the known[11] corresponding free amine, $C(CH_2NH_2)_4$, or a protected derivative of it. The target compound $C(CH_2NF_2)_4$ would produce 58 wt% HF upon detonation.

---

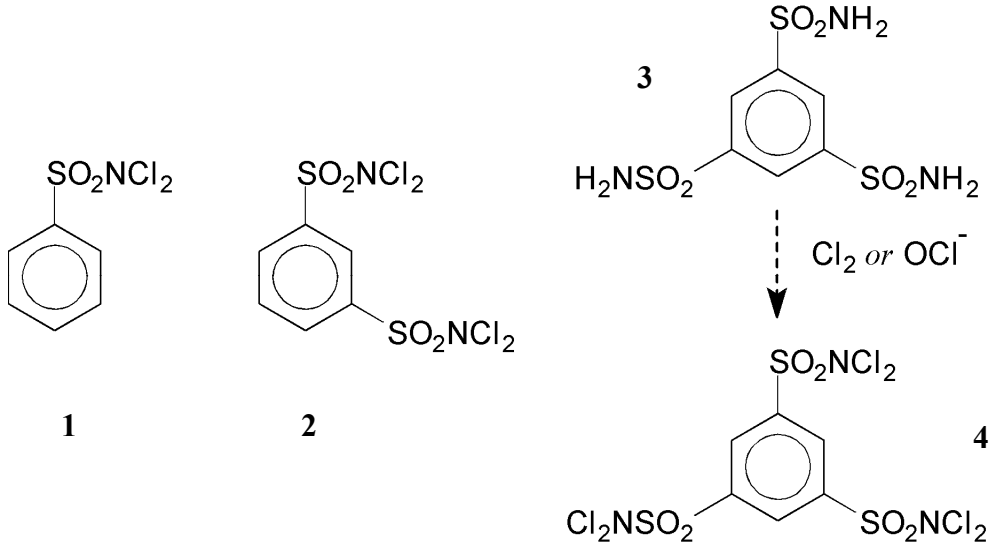
[9] McGuire, R.R.; Ornellas, D.L.; Helm, F.H.; Coon, C.L.; Finger, M. *Proc. Seventh Symp. (Internat.) Detonation* **1981**, 940.

[10] Chapman, R.D.; Gilardi, R.D.; Welker, M.F.; Kreutzberger, C.B. *J. Org. Chem.* **1999**, *64*, 960.

[11] Adil, K.; Goreshnik, E.; Courant, S.; Dujardin, G.; Leblanc, M.; Maisonneuve, V. *Solid State Sciences* **2004**, *6*, 1229.

By our second approach, elemental chlorine (Cl_2)—which is also an effective biocide and has been employed in recent agent defeat concepts, such as the Agent Defeat Warhead (ADW)[12]—would be generated by suitable *N,N*-dichloramine derivatives, which are generally straightforwardly prepared by simple chlorination of the corresponding amines with chlorine or hypochlorite.[13] Certain dichloramines can be explosive energetic materials. A known compound that has some desirable properties for this effect is octachloropentaerythrityltetramine or tetrakis-(dichloramino)neopentane, $C(CH_2NCl_2)_4$, a "very powerful explosive" that has been shown to generate elemental chlorine—not hydrogen chloride—upon explosion[14] and contains 70% chlorine by weight. Unfortunately, this specific compound is probably not suitable for practical application, as it has insufficient stability on long-term storage. However, other known, stable dichloramine derivatives suggest alternative candidates.

Dichloramines can be stabilized as sulfonamide derivatives; for example, commercial product dichloramine-B is *N,N*-dichlorobenzenesulfonamide **1** (m.p. 76 °C), and it is capable of exploding "feebly" upon rapid thermolysis,[15] presumably due to its low explosophore content. In comparison, the doubly substituted derivative *N,N,N′,N′*-tetrachloro-1,3-benzenedisulfonamide **2** (m.p. 128 °C) explodes "with violence."[15] An extrapolation of these observations suggested that hexa-*N*-chloro-1,3,5-benzenetrisulfonamide (**4**)—hypothetically prepared (Scheme 1) from the known[16] corresponding simple trisulfonamide (**3**)—would be still more energetic, and it contains 41% chlorine by weight.



Scheme 1. Known (**1**–**2**) and proposed (**4**) explosive *N,N*-dichlorosulfonamide derivatives

[12] "Agent Defeat Weapon; Agent Defeat Warhead (ADW)"; http://www.globalsecurity.org/military/systems/munitions/adw.htm

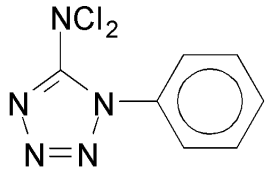
[13] Hanefeld, W. "N,N-Dihalogen-amine," in: Klamann, D. (ed) "Methoden der organischen chemie (Houben-Weyl), band E16a, organische stickstoff-verbindungen I teil 2," Georg Thieme, Stuttgart, 1990; p 926.

[14] Gryszkiewicz-Trochimowski, E.; Gryszkiewicz-Trochimowski, O.; Levy, R.S. *Memorial des Poudres* **1958**, *40*, 109.

[15] Chattaway, F.D. *J. Chem. Soc. Trans.* **1905**, *87*, 145.

[16] Manousek, O.; Exner, O.; Zuman, P. *Coll. Czech. Chem. Commun.* **1968**, *33*, 4000.

Another class of known, explosive dichloramines could be based on 5-(*N,N*-dichloramino)tetrazoles, certain examples of which can be stable and relatively high-melting. For example, even an energetically diluted structure, 5-(*N,N*-dichloramino)-1-phenyltetrazole (**5**), was reported as explosive.[17] Suitable energetic derivatives of this specific class were considered as targets for this project. Yet another potentially attractive material was hexa-*N*-chloromelamine (**6**),[18] which has 64 wt% chlorine and by itself has desirable biocidal activity.[19]



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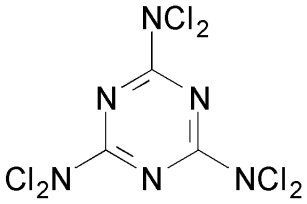


6

A stainless steel detonation chamber (8.5 liters in volume) was employed—with various internal hardware (e.g., Fig. 1) over the course of the project to accommodate biological samples—to test the feasibility of novel explosives as effective agent defeat weapon components, allowing exposure to generated biocidal products of explosion.

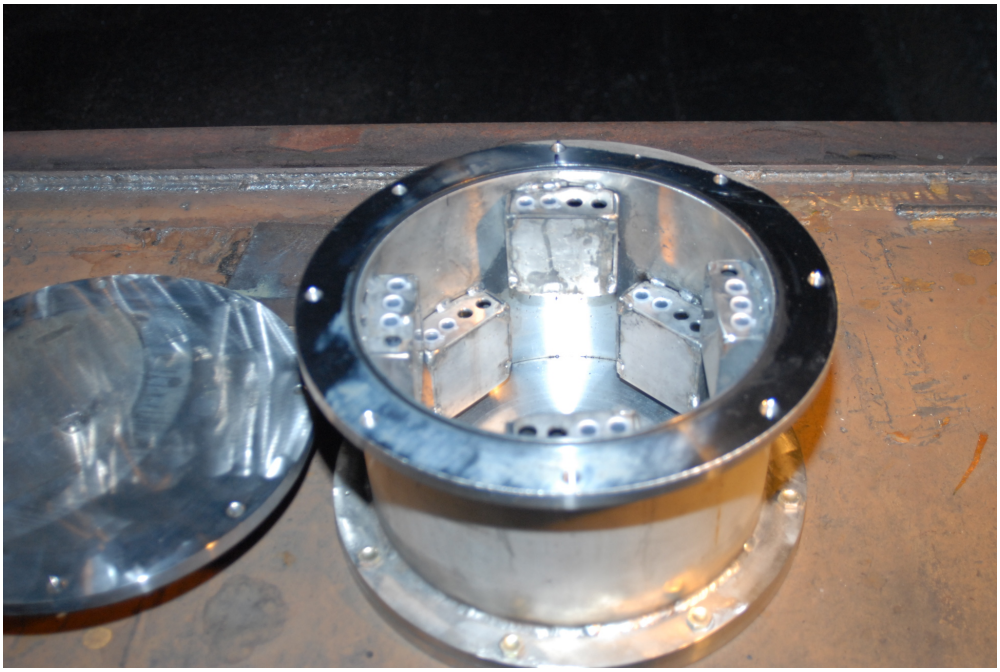


Figure 1. Stainless steel detonation chamber

[17] Stollé, R. *J. Prakt. Chem.* **1933**, *138*, 1.
[18] Arsem, W.C. US Patent 2472361 (1949).
[19] African Explosives & Chemical Industries Ltd., GB Patent 1092994 (1967).

SPECIFIC APPROACHES AND RESULTS

I. DIFLUORAMINE EXPLOSIVES

1. HNFX

HNFX was on hand from previous preparations.[10,20]

a. Long exposures (hours)

Three separate detonations of HNFX were carried out in the chamber in order to allow three different exposure times of the analytes to biocidal products of detonation of HNFX, which particularly include hydrogen fluoride. Biological analytes were surrogates of anthrax (*Bacillus anthracis*) spores. Spores of *Bacillus subtilis strain ATCC 6633* and *Bacillus thuringiensis kurstaki (BGSC 4D1)* were used as test organisms. *B. subtilis*, a commonly occurring organism, is designated by the Environmental Protection Agency as the surrogate organism for *B. anthracis* in controlled testing environments. *B. thuringiensis* is genetically very closely related to *B. anthracis* and has also been used as a surrogate for *B. anthracis*. Both strains can be used under standard laboratory conditions with minimal risks. *B. subtilis* was acquired from the American Type Culture Collection (Manassas, VA), and *B. thuringiensis* was from the Bacillus Genetic Stock Center (BGSC, Ohio State University, Columbus, OH). Spore preparations were made according to published methods.[21] *Bacillus* spores were aliquoted into GeneMate® 1.7-mL poly-propylene microcentrifuge tubes[22] (Fig. 2) and allowed to air dry overnight. Microcentrifuge tubes were chosen as containers for the spores in order to support them behind metal shields built into the chamber, which protect them from the direct blast of the explosive charges.

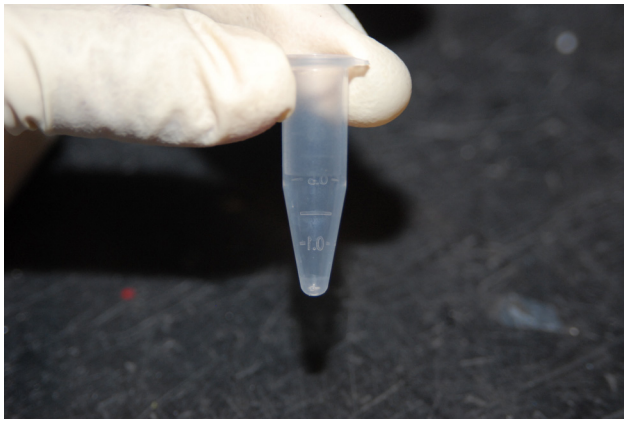


Figure 2. Microcentrifuge tube for *Bacillus* spores

[20] Chapman, R.D.; Groshens, T.J. US Patent 7632943 (2009).

[21] (a) Aronson, A.I.; Angelo, N.; Holt, S.C. *J Bacteriol.* **1971**, *106*, 1016; (b) Leighton, T.J.; Doi, R.H. *J. Biol. Chem.* **1971**, *246*, 3189.

[22] http://www.bioexpress.com/index.html?wscdet_show=000000000450458000458070

Quadruplicate tubes of each *Bacillus* species spore were placed at two different levels in the explosion chamber (Fig. 3). Tubes containing dehydrated spores but not placed in the explosion chamber were used as controls.

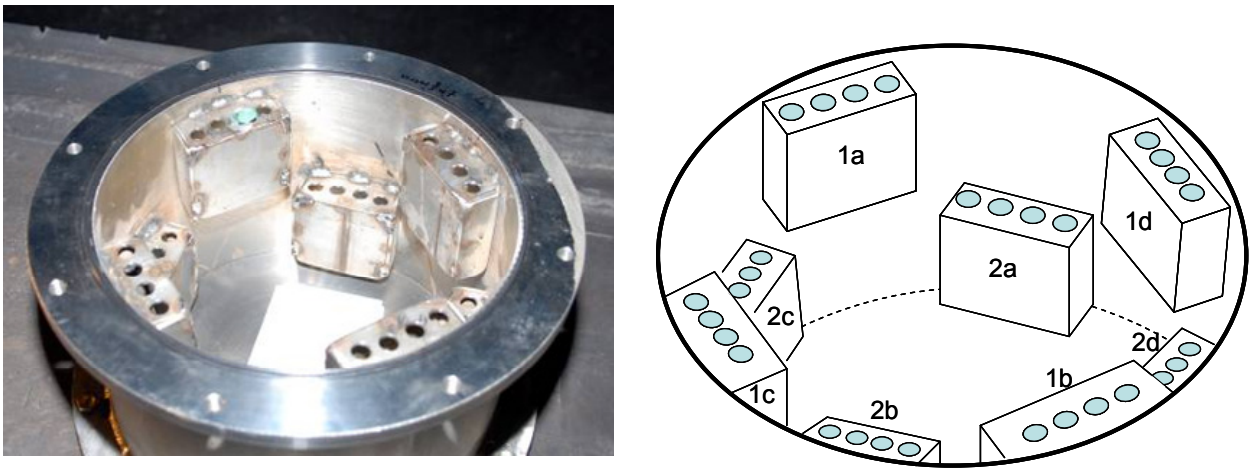


Figure 3. (left) Detonation chamber used for detonation; (right) the relative locations where *Bacillus* spore samples were placed for testing. Quadruplicate samples of two different *Bacillus* spores (*B. subtilis* and *B. thuringiensis*) at two different location levels (positions 1 and 2) were tested during each detonation.

HNFX explosive charges were 2.7 ± 0.2 grams of pure HNFX with 3% FC-43 Fluorinert™ Electronic Liquid[23] (3M Co.) additive—to reduce electrostatic sensitivity—contained in black conductive polypropylene vials (Emerald Plastics #EP145) with a volume of 4 cm^3 (Fig. 4). This proportion of explosive charge to chamber volume simulates the action of a warhead with a 50-pound explosive fill deployed in a structure of dimensions 16′ × 16′ × 10′. In these initial tests in the project, the charge was initiated by an RP-3 miniature exploding bridgewire detonator (Teledyne RISI).[24]

Transient overpressures produced by the detonations were discharged via the chamber's initiation wire port, which remained open though constricted. The success of detonation (as opposed to deflagration) of each HNFX sample was confirmed by witness denting of the chamber's lid caused by impact of the charge-containing vial (Fig. 5).

Without any precedent literature of the sporicidal efficacy of the detonation products from this class of compound at hand, product exposure durations were arbitrarily chosen to be convenient times of 0.4 hour, 2.9 hours, and 24.0 hours. At a selected time following each detonation, the chamber's lid was cracked open to allow venting of possible fumes for about 10 min before the lid was removed for spore sample retrieval. Exposure of spores to product gases was deemed to be terminated during this 10-min venting period (Fig. 6).

[23] http://multimedia.3m.com/mws/mediawebserver?66666UuZjcFSLXTtnxTEo8z6EVuQEcuZgVs6EVs6E666666--

[24] http://www.teledynerisi.com/productpdf/page22.pdf

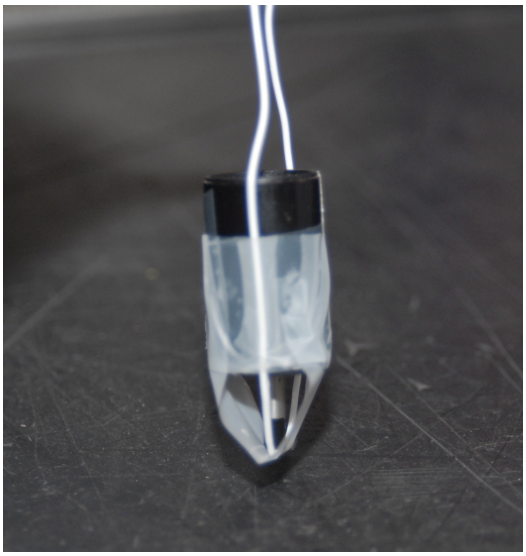


Figure 4. HNFX explosive charge with RP-3 detonator

Figure 5. Denting above the explosive charge at the initiation wire port

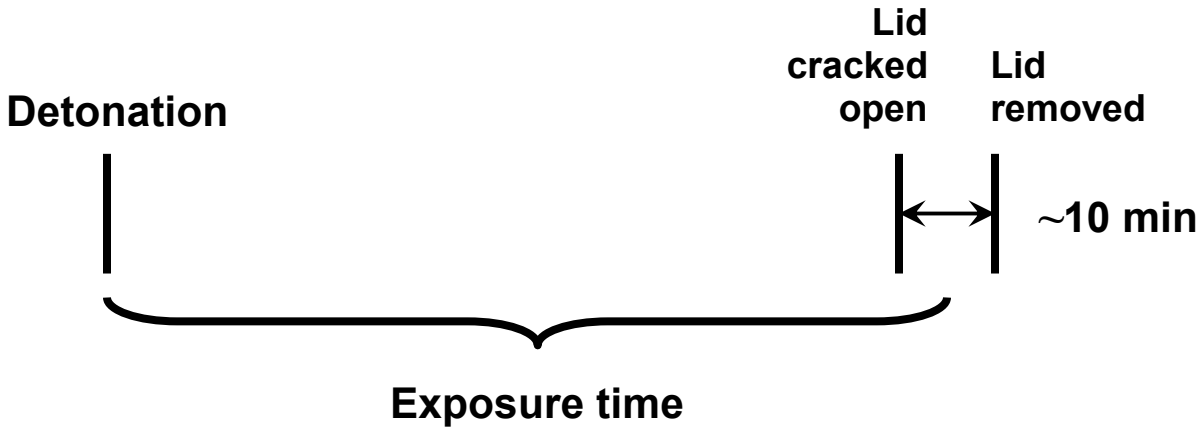


Figure 6. Schematic diagram of exposure times following detonation

To assess cross-contamination of the two spore types during detonation, the chamber was wiped with sterile filter paper and swabs cultured for bacterial growth. Between each detonation, the chamber was cleaned by wiping first with acetone, followed by an aqueous anionic detergent (Dawn® by Procter & Gamble) and a nonionic detergent (Alcojet® by Alconox Laboratory Cleaning Supplies), and then sterilized with 2% bleach solution. The chamber was finally rinsed with distilled water and with 91% isopropanol.

Spore survivability assessments were carried out at the facilities of Sun BioMedical Technologies (Ridgecrest, CA). Spore survivability after detonation was determined by measuring the number of viable spores in both detonation-exposed tubes and control tubes. Spores from each tube were extracted and resuspended using sterile dilution buffer (10% ethanol containing 0.05% Tween 20), serially diluted with buffer and plated onto Nutrient Broth agar plates. Agar plates were incubated at 37 °C for 24 h, and the numbers of colony forming units (CFU) were determined using Quantity One software (Bio-Rad Laboratories). For filter paper swabs, 3 mL of dilution buffer was added to swabs and agitated for 1 min using a vortex shaker. Aliquots of suspension were both plated onto Nutrient Broth agar plates and grown in liquid culture. For confirmation, all extracted spore suspensions were incubated in liquid cultures using Luria-Bertani broth with constant agitation at 37 °C for 24 h. The results obtained from each tube (Table 1) showed that no bacterial colonies grew on Nutrient Broth agar plates for any detonation-exposed samples, even when the incubation time was extended for 48 h.

Neither was any bacterial growth observed after 24 h in liquid Luria-Bertani culture at 37 °C (Fig. 7). The total absence of any viable spores following detonation exposure indicated that no *Bacillus* spores survived exposures to detonation product gases.

Following the detonations, the explosion chamber and the tubes were covered with black soot (Fig. 8). Solid carbon as a product of detonation is predicted by Cheetah code calculations on HNFX, which show the following predominant final products of detonation (moles per mole of HNFX at 50% of theoretical maximum density): HF, 7.99; N_2, 4.00; CO, 3.99; carbon (solid), 2.00. Thus, the average sample weight of HNFX, 2.7 grams, should produce about 0.16 gram of carbon. However, a majority of the soot was probably due to the black polypropylene containers for HNFX, which weigh about 1.6 grams each and may not be expected to completely combust to gaseous products. Some black soot mixed with a known number of both *B. subtilis* and *B. thuringiensis* spores and incubated on Nutrient Broth agar plates at 37 °C for 24 h demonstrated that black soot material was not toxic to spore outgrowth.

Table 1. Viability of *Bacillus* spores following detonations [a]		
Samples	**Organism**	**Colony forming units (CFU)**
Bs Control	*B. subtilis*	$(1.13 \pm 0.27) \times 10^6$
Bt Control	*B. thuringiensis*	$(1.24 \pm 0.19) \times 10^6$
Detonation 1 (0.4 h)		
Location 1	*B. subtilis*	0
Location 2	*B. subtilis*	0
Location 1	*B. thuringiensis*	0
Location 2	*B. thuringiensis*	0
Swabs	—	0
Detonation 2 (2.9 h)		
Location 1	*B. subtilis*	0
Location 2	*B. subtilis*	0
Location 1	*B. thuringiensis*	0
Location 2	*B. thuringiensis*	0
Swabs	—	0
Detonation 3 (24.0 h)		
Location 1	*B. subtilis*	0
Location 2	*B. subtilis*	0
Location 1	*B. thuringiensis*	0
Location 2	*B. thuringiensis*	0
Swabs	—	0

[a] Control spores (*Bs* Control and *Bt* Control) were similarly prepared spore samples that were not exposed to detonation. Spore locations (Locations 1, 2) in the explosion chamber are indicated in Fig. 3. Exposure times of spores to detonation product gases are shown. Swabs were filter paper wipes taken on the chamber walls after each detonation to assess for bacterial cross-contamination. Results shown are from quadruplicate samples. For the control samples, mean ± standard deviation (SD) are shown.

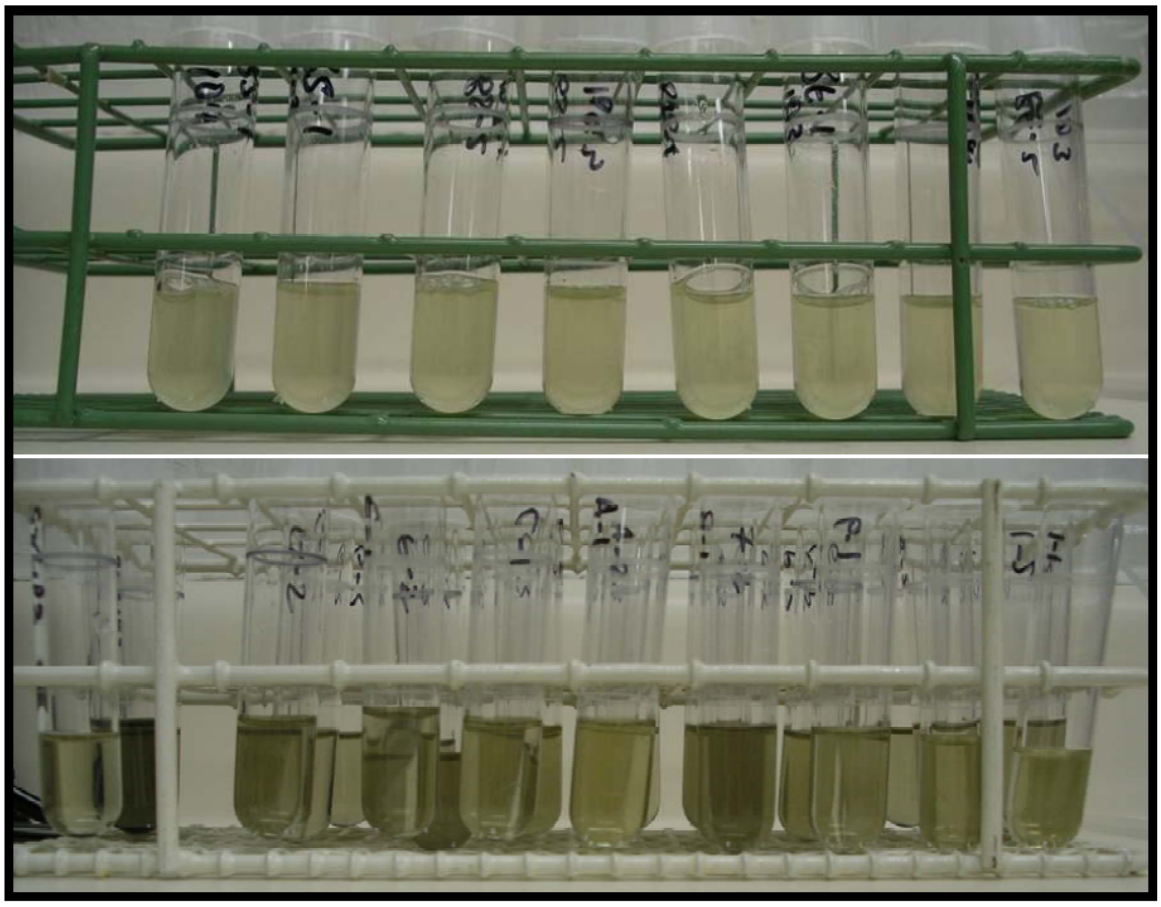


Figure 7. Liquid cultures of control *B. subtilis* (tubes 1–4 in top panel), *B. thuringiensis* (tubes 5–8 in top panel) spores, and the detonation-exposed test spore samples (bottom panel) in Luria-Bertani broth. Presence of bacterial growth is indicated by the turbid cultures in the control tubes. Absence of growth in the detonation-exposed test spore samples is indicated by the clear Luria-Bertani broth. Cultures were incubated at 37 °C for 24 h with constant orbital agitation.

Figure 8. Test spore tubes recovered from explosion chamber after detonation

Bacillus anthracis and its valid surrogates, such as *B. subtilis* and *B. thuringiensis*, are known to be resistant to high temperatures. For example, *B. anthracis* and *B. anthracoides* spores have been reported to survive exposure to a temperature of 400 °C for as long as 30 seconds.[25]

The tube-contained spores were shielded from direct blast by steel plates (which remained undamaged), and the relatively large detonation chamber was an effective heat sink that prevented significant internal heating of its contents, as no undue heat was observed upon inspecting the chamber within a minute or so of detonation. To further assess whether spore materials were still present in the spore tubes following detonation, extracted suspensions from representative tubes were observed under light microscopy. For comparison, *B. subtilis* and *B. thuringiensis* spores were similarly prepared and stained for light microscopy observation. As shown in Fig. 9, spore/membrane debris indicative of spore presence were seen in representative detonation-exposed samples. The cell/membrane debris demonstrated light refraction under light microscopy typical of bacillus spores.

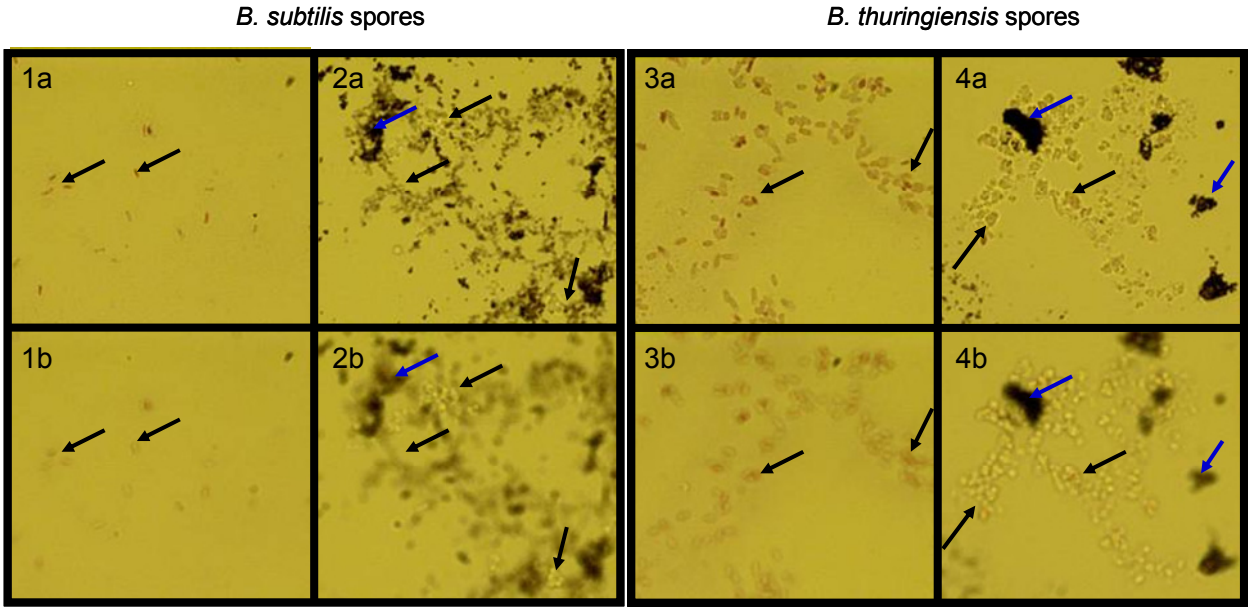


Figure 9. Light microscopy of *B. subtilis* and *B. thuringiensis* spores in non-detonated, control samples (panels 1a, 1b, 3a, 3b) and in test, detonation-exposed samples (panels 2a, 2b, 4a, 4b). Spores are indicated by black arrows (➞) and black soot by blue arrows (➞). Bottom panels (1b, 2b, 3b, 4b) are slightly off-focus frames of the corresponding top panels' shots to indicate the refractive property of the spores.

Results from spore assays indicated that neither *B. subtilis* nor *B. thuringiensis* spores survived conditions in the explosion chamber. No viable spores were recovered. With total sample sizes of $(1.896 \pm 0.264) \times 10^7$ colony forming units (CFUs) in the chamber—in eight tubes each of *Bs* and *Bt*—killing efficiency was therefore >7.27 $\log_{10}$ orders of magnitude.

[25] Oag, R.K. *J. Pathol. Bacteriol.* **1940**, *51*, 137.

Killing efficiency of *Bacillus* spores is expressed as $\log_{10}$-reduction and is calculated as

$$\boldsymbol{R} \equiv \log_{10}\text{-reduction} = \log[(N_0 + 1)/(N_E + 1)]$$

where N_0 is the mean number of viable spores recovered from the control (inoculated, unexposed) micro tubes, and N_E is the number of viable spores after exposure to detonation. A factor of 1 is added to the values to allow calculations based on $N = 0$.[26] The results from the initial three detonations are summarized in Table 2.

Table 2. HNFX Results (Long Exposures)

Exposure time:	**Detonation #1 (0.4 h)**		**Detonation #2 (2.9 h)**		**Detonation #3 (24.0 h)**	
Sample:	***B. subtilis***	***B. thurin-giensis***	***B. subtilis***	***B. thurin-giensis***	***B. subtilis***	***B. thurin-giensis***
$\frac{\textbf{Control}}{\log(N_0 + 1)}$	6.956 ± 0.104	6.997 ± 0.067	6.956 ± 0.104	6.997 ± 0.067	6.956 ± 0.104	6.997 ± 0.067
$\frac{\textbf{Exposed}}{\log(N_E + 1)}$	0	0	0	0	0	0
***R*(exposed)**	≥6.96	≥7.00	≥6.96	≥7.00	≥6.96	≥7.00
<*R*>(exposed)	**≥7.278 ± 0.060**		**≥7.278 ± 0.060**		**≥7.278 ± 0.060**	

The expectation value **<*R*>** treats all *Bacillus* spores collectively as surrogates of *B. anthracis* to estimate the log-reduction in survivability caused by detonation products.

Since the spores were protected from direct blast effects and are known to have significant dry heat resistance, the observed total loss of spore viability following detonations in this experiment is assessed to be due to spores' reactions to biocidal gases and products released during the explosion of HNFX.

Because of this unexpectedly efficient sporicidal activity, it became desired to achieve a measurable nonzero survival rate of anthrax surrogate spores following exposure to HNFX detonation products under different conditions. Exposure times much shorter than ~10 minutes would be impractical to achieve using the experimental protocol employed in the first HNFX test series. Therefore, modifications to this protocol were implemented; specific changes are described below

[26] Rose, L.J. et al. *Appl. Environ. Microbiol.* **2005**, *71*, 566.

b. Short exposures (milliseconds)

In subsequent tests of HNFX detonations, biological assays utilized spore sample sizes on the order of 10^9 CFUs so that killing efficiencies of this magnitude—a specified objective of DTRA's[27]—can be demonstrated, and spores were limited to *Bacillus thuringiensis*. Also in later tests, at the suggestion of the sponsor, the biological samples were utilized as thinner layers of spores spread onto stainless steel coupons (Fig. 10), in some experiments covered by strips of stainless steel mesh (to prevent possible dislodgement of spores and protect them from explosion debris), though the mesh was soon discovered to be unnecessary. This spore sample configuration was intended to alleviate any complication of hindered diffusion of detonation products into the microcentrifuge tubes previously used. The possible effect of moisture on killing efficiency by detonation product gases was tested by wetting half of the spore samples with 10 μL of glycerol (a less-volatile simulant of water) in each coupon. Glycerol has been demonstrated to be a good inert solvent toward hydrogen fluoride (the presumed sporicidal detonation product in these experiments), being capable of absorbing up to 382% of its weight in hydrogen fluoride under ambient conditions.[28] Reaction between the two components occurs only upon heating to 150–160 °C.

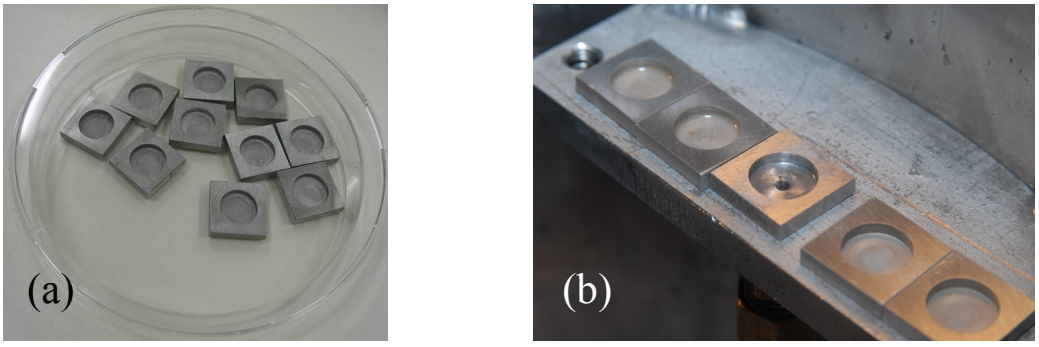


Figure 10. (a) Stainless steel coupons for spore samples; (b) spore sample coupons in place, "wet" (two left) and "dry" (two right); a coupon to accommodate an optional thermocouple is positioned in the center.

The detonation chamber's component hardware was also modified in order to achieve nonzero survival rates (Fig. 11): spacers of variable lengths (¼-inch to 1-inch) placed around the bolts that secure the chamber's base to its body allowed more rapid venting of detonation products through a larger gap above the chamber floor, enabling much shorter exposure times between spores and detonation products. This gap would also allow high-speed video to monitor evolution of visible products throughout the detonation event. Within the chamber, the spore sample coupons are supported on stainless steel racks welded into a staircase pattern around the chamber wall.

[27] (a) Wilson, B. DTRA Basic Research Technical Review, 12 Nov 2008; (b) Peiris, S. DTRA Basic Research Technical Review, 13 Nov 2008.
[28] Matuszak, M. US Patent 2520947 (1950).

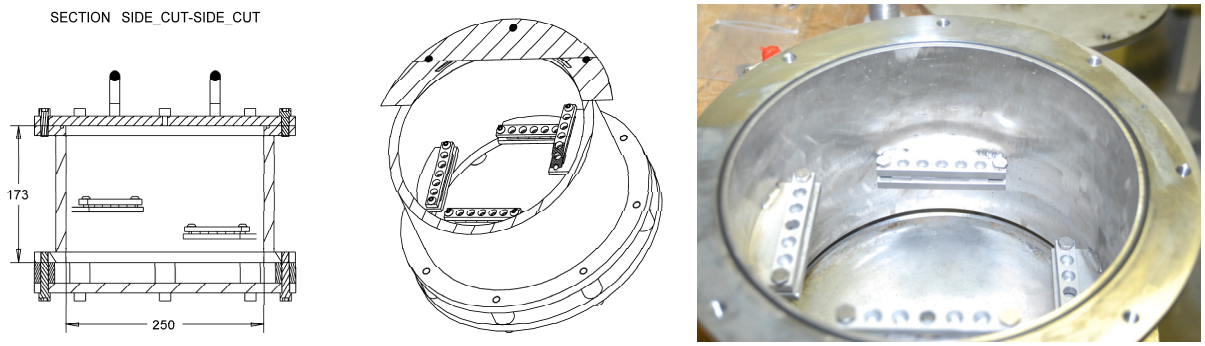


Figure 11. Modified detonation chamber design

Ports in the chamber's lid allowed attachment of pressure transducers to monitor pressure changes during an event; however, these were not successfully operated in the initial experiments. Helium purge lines were also attached via the chamber lid, allowing purging (via remote-controlled solenoid control) of any residual product gases at desired times. Fig. 12 is an external photo of the modified chamber configuration.

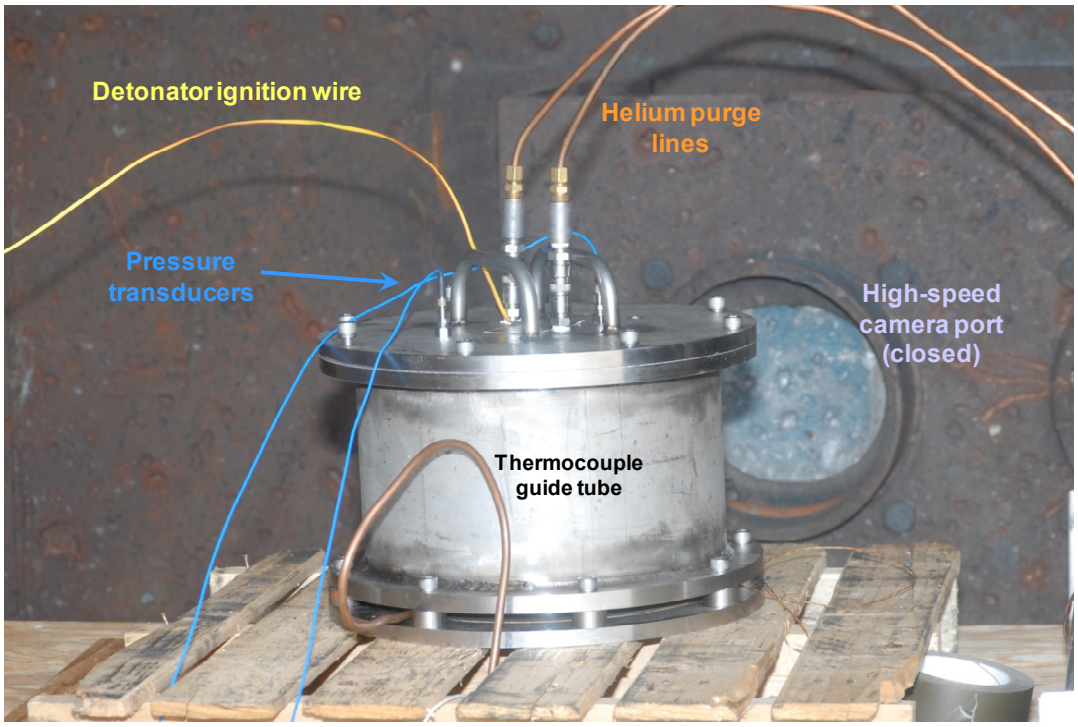


Figure 12. Modified detonation chamber configuration for short exposures (assembled)

Using the modified detonation chamber hardware, three detonations of HNFX charges (2.37 ± 0.28 grams) were conducted in the presence of *Bt* spores (>10^9 CFUs per detonation), using the various spacer lengths available (¼″–1″) and various delays before the chamber was purged with helium, ranging from essentially "immediate" (but in which experiment the purge

line coupling disengaged so purging was incomplete) to 180 seconds following detonation. High-speed video (500 frames per second) was acquired for HNFX Detonation #2 but was generally not informative due to uninterpretable light effects during the event. Following three tests with HNFX, a similar test was conducted using a charge of HMX (2.67 grams). Results of biological analysis from these four tests are summarized in Table 3.

Table 3. Modified Detonation Chamber Results for HNFX (Short Exposures)

	HNFX Detonation #1		**HNFX Detonation #2**		**HNFX Detonation #3**		**HMX Detonation**	
Explosive charge size	2.64 g		2.38 g		2.09 g		2.67 g	
Chamber spacer	½″		1″		¼″		¼″	
Delay before He purge	"immediate" (but partial purge)		100 sec		180 sec		180 sec	
***Bt* sample state**	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Control $\overline{\log(N_0+1)}$	9.472	9.511	9.520	9.571	9.502	9.623	9.575	9.651
Exposed $\overline{\log(N_E+1)}$	8.459	8.624	8.490	8.669	8.246	8.588	9.491	9.599
***R*(exposed)**	1.013	0.887	1.030	0.902	1.256	1.035	0.084	.052
<*R*>(exposed)	**0.945**		**0.958**		**1.109**		**0.064**	

The expectation value **<*R*>** treats all *Bt* spores collectively as surrogates of *B. anthracis* to estimate the log-reduction in survivability caused by detonation products.

These results from the modified detonation chamber show that there is very little effect of the chamber spacer length on the killing efficiency and also only a small effect of the additional exposure time (to any residual products present after expulsion by detonation) allowed by longer delays up to 180 seconds before purging by helium. Most significantly, the killing efficiencies were much lower—being only about one order of magnitude (1.027 ± 0.082)—than those seen when product gases were mostly contained within the chamber following detonation except for the portions that escaped via the ignition wire port due to equilibrium overpressure (Table 2). The killing efficiency caused by a conventional explosive charge (HMX) was even poorer, with 86% of spores surviving the detonation. Despite the poorer magnitude of killing efficiency achieved by both compounds, the difference between the two classes of explosive (~0.94 order of magnitude) might have been attributable to a real chemical effect even during the very short duration of the exposure prior to expulsion of biocidal gases. This hypothesis suggested another test that was performed: a detonation was performed with a similar charge of HMX (2.67 grams)

in the chamber essentially closed (no spacers), reproducing the chamber conditions that were utilized in the initial HNFX experiments (Table 2). Results from this test of HMX are summarized in Table 4.

Table 4. HMX Detonation in "Sealed Chamber" (No Spacers)		
Explosive charge size	2.67 grams	
Delay before opening	0.4 hour	
***Bt* sample state**	Dry	Wet
Control $\overline{\log(N_0+1)}$	9.681	9.699
Exposed $\overline{\log(N_E+1)}$	9.487	9.504
***R*(exposed)**	0.194	0.195
<*R*>(exposed)	**0.195**	
The expectation value **<*R*>** treats all *Bt* spores collectively as surrogates of *B. anthracis* to estimate the log-reduction in survivability caused by detonation products.		

The results show that even with the chamber essentially closed (except for the ignition wire port), killing efficiency was quite low and dramatically poorer than that observed from similar detonations of HNFX charges. This result corroborates the early tentative conclusion that high killing efficiency achieved by HNFX detonations was a genuine chemical effect and not due to extreme temperature or pressure conditions that occurred in those experiments.

Additionally, there was negative evidence of a biocidally significant thermal environment during the early tests: the polypropylene microcentrifuge tubes showed no evidence of melting, so the immediate surroundings of the spores (i.e., the tubes) did not experience an elevated temperature as high as 160~165 °C for any significant duration, even seconds. *Bacillus anthracis* and its valid surrogates, such as *B. subtilis* and *B. thuringiensis*, are known to be resistant to high temperatures (*vide supra*): *B. anthracis* and *B. anthracoides* spores have been reported to survive exposure to a temperature of 400 °C for as long as 30 seconds.[25]

Additionally, the effect of extreme pressure blasts on *Bacillus* spores has been determined by Horneck et al. (German Aerospace Center DLR),[29] who measured survivability of *B. subtilis* after subjection of spores to direct shocks of 32 GPa from explosive charges of Composition B. These pressures effected $\log_{10}$-reductions of spores in the range of 5.20 ± 0.20 (tentatively attributed to incomplete recovery of spores) to 3.98 ± 0.17, the latter figure being a likely accurate estimate of the effect. The poor killing efficiency seen in the last HMX test (Table 4) is evidence that the detonation chamber configuration did not allow extreme direct pressure blasts on the spore samples, while the significantly higher killing efficiencies seen in the first HNFX experiments (Table 2) indicate that killing must have been due to a chemical effect of one or more biocidal products of detonation from this class of compound, difluoramines.

[29] Horneck, G.; Stöffler, D.; Eschweiler, U.; Hornemann, U. *Icarus* **2001**, *149*, 285.

c. Intermediate exposures (seconds)

The sporicidal product exposure times achieved by the chamber with spacers in line—allowing an effectively open chamber—were clearly too short to achieve high-orders-of-magnitude killing efficiency consistent with DTRA's objectives. Exposure times therefore need to be longer than the millisecond range allowed by expulsion from an "open" chamber and would preferably (for the purpose of realistically deploying agent defeat weapons based on this chemical system) be less than the durations of ≥0.4 hour employed in the early HNFX experiments. At the suggestion of the sponsor, exposure durations in the range of 1 to 5 seconds were chosen as a desirable condition to test.

Therefore, another modification was introduced into the experimental design of the detonation hardware. A specially constructed vacuum chamber ~81 liters in volume (i.e., about ten times the volume of the detonation chamber) was attached to the detonation chamber, so that at any desired time following detonation, two copper lines connecting the two chambers could be opened via remote-controlled solenoid valves (Asco RedHat II #8215G020) in order to rapidly evacuate most of the atmosphere from the detonation chamber into the vacuum chamber (Fig. 13). In the new configuration, the chamber's ignition wire port was sealed with a metal plate and silicone gasket material in order to contain the overpressure exerted by detonations. The chamber was also fitted with a piezoresistive pressure transducer (Omega Pressure Engineering #PX409-500A5V) capable of measuring 0–500 psia, with a porous metallic pressure snubber (Omega #PS-8) in line in order to alleviate possible damage by a detonation shock wave.

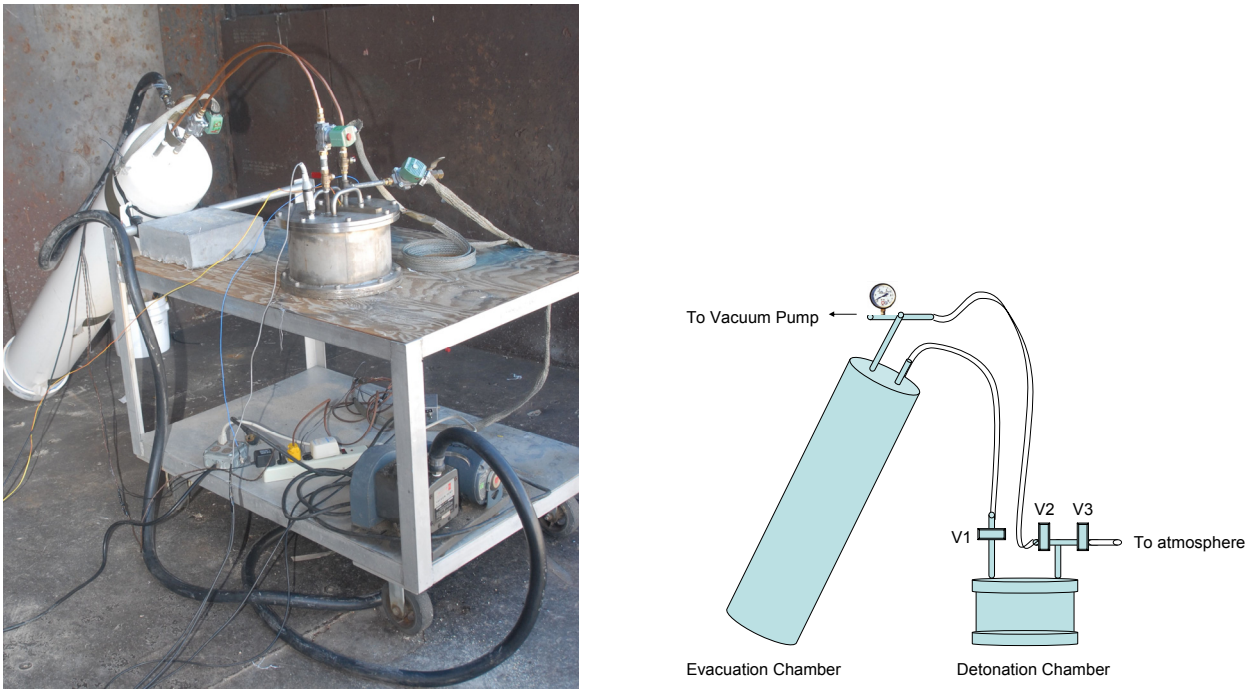


Figure 13. Dual-chamber system connecting vacuum chamber (81 liters) to detonation chamber (8.5 liters) to atmosphere via remote-controlled solenoid valves

In the series of tests in the intermediate-duration range, seven specific tests were conducted: one at a nominal exposure time (that is, opening of the vacuum solenoids at chosen times following the detonation, followed by purging the evacuated detonation chamber to atmosphere upon equilibration of vacuum) of 5 seconds and duplicate tests at exposure times of 2.5 sec, 1.25 sec, and 0.5 sec. (The cost of testing according to our experimental procedures *precluded multiple testing beyond these duplicates*.) In each test, *Bt* spores were used half dry and half wet with 10 µL glycerol (as in the short-exposure tests). The HNFX charge was 2.90 grams in all tests, but 4-mL polypropylene vials (Waters Corp. #WU-98814-30) were used as their containers in this series.

The results of sporicidal efficacy of HNFX detonation products at various exposure times are summarized in Table 5.

Table 5. HNFX Results (Intermediate Exposures)

***Bt* sample state:**	**Dry**		
Delay before evacuation	**$N_0 = \sum\log(n_0 + 1)$ (Control)**	**$N_E = \sum\log(n_E + 1)$ (Exposed)**	***R*(exposed)**
5.00 sec	9.58	0	**≥9.58**
2.50 sec (#1)	9.63	6.83	**2.80**
2.50 sec (#2)	9.61	6.80	**2.82**
1.25 sec (#1)	9.82	6.46	**3.36**
1.25 sec (#2)	9.83	7.73	**2.10**
0.5 sec (#1)	9.56	5.89	**3.67**
0.5 sec (#2)	8.75	7.71	**1.04**
***Bt* sample state:**	**Wet (glycerol)**		
Delay before evacuation	**$N_0 = \sum\log(n_0 + 1)$ (Control)**	**$N_E = \sum\log(n_E + 1)$ (Exposed)**	***R*(exposed)**
5.00 sec	9.50	6.60	**2.89**
2.50 sec (#1)	9.34	8.13	**1.21**
2.50 sec (#2)	9.66	8.18	**1.49**
1.25 sec (#1)	9.84	8.14	**1.70**
1.25 sec (#2)	9.82	8.13	**1.21**
0.5 sec (#1)	9.30	8.36	**0.95**
0.5 sec (#2)	8.73	7.64	**1.08**

With 5.00 sec exposure prior to evacuation, *total kill* of over 10^9 CFUs was seen for the dry spores. However, with these shorter exposures (compared to those of Table 2), there is seen a significant effect of the "wetness," as the glycerol-wet spores show significantly greater survival

than the dry spores, which brings into question the validity of glycerol as an inert simulant of water. As glycerol is an organic compound susceptible to oxidation, it might therefore be a scavenger of the most reactive biocidal species formed in the detonations, particularly ones more reactive than equilibrium product hydrogen fluoride: hypothetically, these could be transient fluorine species that are predicted by Cheetah code to form. Thus, predictions of explosive performance of HNFX at 50% of theoretical maximum density (TMD)—an approximation of the packing density used in these tests—show a variety of very reactive transient chemical species formed at the Chapman–Jouguet condition (as excerpted in Table 6), including atomic fluorine (F), formyl fluoride (CHOF), and carbonyl fluoride radical (CFO); at higher packing densities, trifluoromethyl hypofluorite (CF_3OF) is also predicted to form. If such species are the most active sporicidal products from HNFX detonation, our original premise that glycerol is an inert matrix to simulate water in order to wet spores may not be valid, even if it is a suitable solvent for the ultimate equilibrium product hydrogen fluoride. The 10 μL of glycerol used to wet each coupon is estimated to be nearly twenty times the aggregate volume of the spores it wetted, i.e., sufficient to coat and possibly chemically protect the spores to some extent.

Table 6. The Chapman–Jouguet Condition for HNFX (50% TMD) Predicted by Cheetah

```
                              The C-J condition

The shock velocity    =   5.10140e+003 m/s
The particle velocity =   1.43428e+003 m/s
The speed of sound    =   3.66712e+003 m/s

P0 =       1 atm, V0 =  1.10681 cc/gm, E0 = -164.15346 cal/gm


                          Reference state = reactants
              H(R) = H--164.14, E(R) = E--164.15, S(R) = S- 0.00


          P            V          T        H(R)       E(R)       S(R)      VGS
        (ATM)       (CC/GM)      (K)     (CAL/GM)   (CAL/GM) (CAL/K/GM) (CC/GM)
 1.)   65244.0       0.7956    4924.8    1502.99     245.85     1.930     0.7751

Product concentrations
          Name      Phase  (mol/kg) (mol gas/mol explosive)
            hf        Gas   1.881e+001  7.677e+000
            n2        Gas   9.757e+000  3.983e+000
            co        Gas   9.062e+000  3.699e+000
          chfo        Gas   2.163e-001  8.827e-002
           co2        Gas   1.386e-001  5.656e-002
           cfo        Gas   1.364e-001  5.569e-002
             f        Gas   1.081e-001  4.411e-002
```

In the two runs with 2.50 seconds exposure, fairly reproducible poorer killing efficiency (relative to that at 5.00 sec) is seen for both dry and wet spores. At even shorter exposures, 0.50–1.25 sec, there seems to be some variability among the log-reductions. One value from a nominal 0.50-sec exposure appears quite out of line, but generally, the log-reductions go distinctly down

with exposures shorter than five seconds. The log-reductions vs. nominal exposure times are plotted in Fig. 14. Linear regressions are shown with and without statistical weighting by the variances of each point; however, such weighting might not be justified because of the small number of samples.[30] The regressions do not exhibit a high correlation, but the data show reasonably consistent trends. These are consistent with the relatively poor efficacy ($\boldsymbol{R} \lesssim 1$) seen in the tests with very short exposures (Table 3).

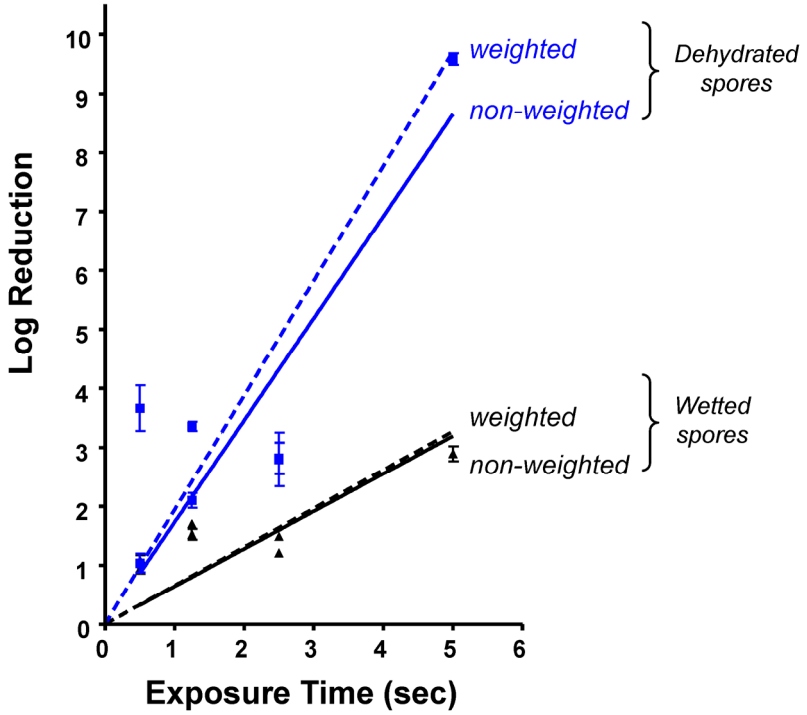


Figure 14. Log-reductions caused by HNFX vs. nominal post-detonation exposure times

In order better to correlate sporicidal efficacy of HNFX detonation products as a function of realistic exposures to such products, we considered the complex behavior of the detonation chamber pressure throughout each test, since it was expected that the *amount* of sporicidal atmosphere in contact with spores was changing immediately after the detonation (manifested as pressure changes in the chamber). A record from the chamber's pressure transducer for one test (2.50-sec nominal exposure time) is shown in Fig. 15. Note how the initial pressure decay looks like a double exponential decay. Following a chosen exposure time of 2.50 sec, two solenoids open lines to the vacuum chamber, and evacuation takes a couple more seconds, followed by purging with atmosphere, which takes a couple more seconds to reach equilibrium.

[30] Motulsky, H.; Christopoulos, A. "Fitting Models to Biological Data Using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting," Oxford University Press, New York, 2004; Chapter 14.

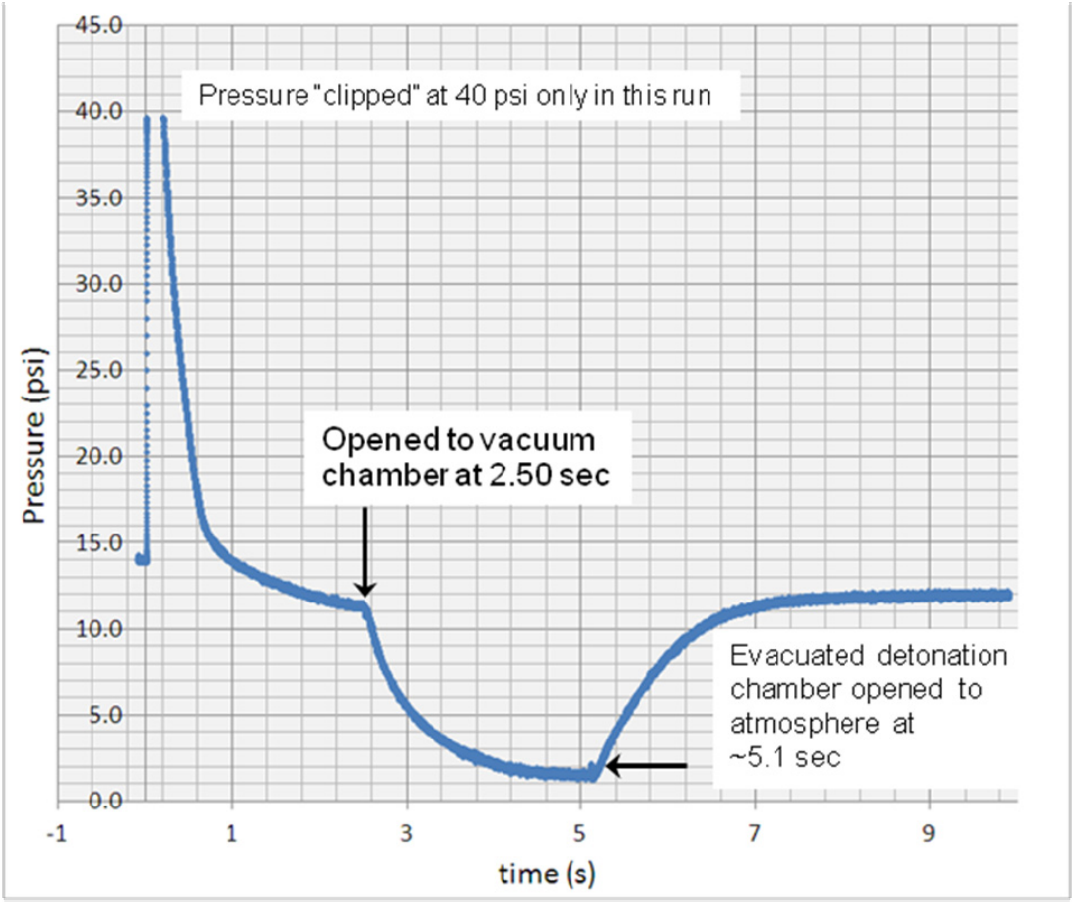


Figure 15. Sample pressure vs. time profile (HNFX, 2.50-sec nominal exposure)

The pressure vs. time curve reflects two processes that occurred in each test: an expected relaxation for the quasistatic overpressure caused by the blast (it may be speculated that the timescale of that measurement might be influenced by the porous metallic pressure snubber that was used to protect the transducer from the detonation); and then a slower release of excess equilibrium overpressure (due to conversion of the solid explosive to gaseous products) through a leak that we believed to be via the "atmosphere solenoid" (Fig. 13), because of its unidirectional nature, as it was designed for holding vacuum in the chamber but briefly held an overpressure above atmospheric.

Consistent with the behavior observed for one of these processes, it has been demonstrated that the relaxation of quasistatic overpressure following detonation in a *closed vessel* follows a generally exponential decay, as reported in a couple of examples. Weibull[31] monitored pressure relaxation following detonation of 5 kg of trinitrotoluene (TNT) in a 0.41-m^3 sphere (Fig. 16).

[31] Weibull, H.R.W. *Ann. N.Y. Acad. Sci.* **1968**, *152*, 357.

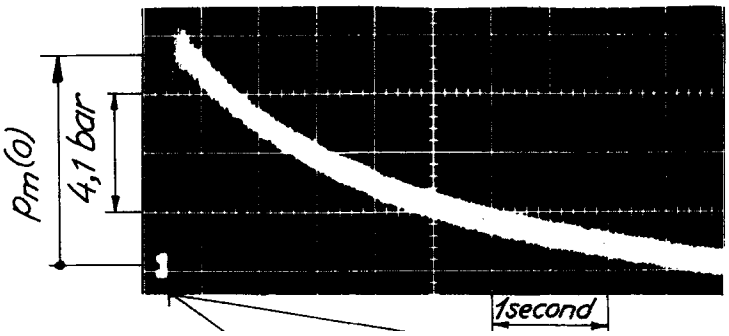


Figure 16. Pressure relaxation following detonation of 5 kg of TNT in a 0.41-m^3 sphere[31]

Proctor and Filler[32] also measured pressure vs. time responses for *confined* explosions of RDX in air (Fig. 17).

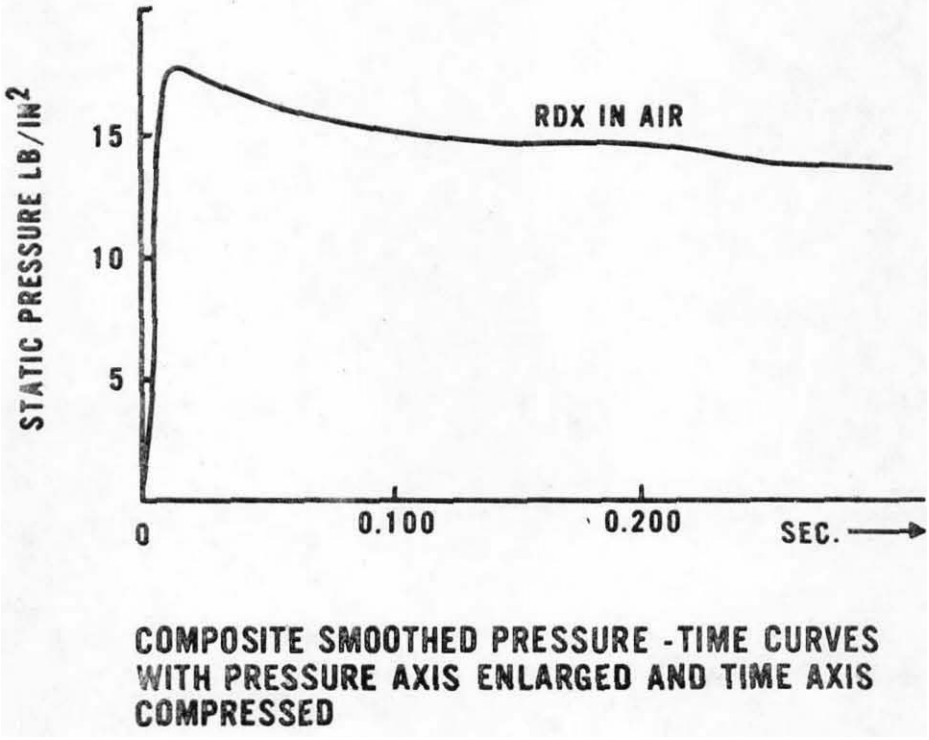


Figure 17. Typical pressure vs. time record for a confined explosion of RDX in air[32]

[32] Proctor, J.F.; Filler, W.S. *Minutes of the 14th Annual Explosives Safety Seminar*, New Orleans, LA, 8–10 November 1972; p. 99.

Regarding the second process that occurred in our test configuration, it has been demonstrated that release of excess pressure through a leak in a vessel also generally follows an exponential decay, the Kinney–Sewell equation:[33]

$$\log P = \log P_{\max} - 0.315\left[\frac{\text{Area(vent)}}{\text{Volume}}\right]t_{\text{ms}}$$

Kinney et al. have also stated,[34] "The slow pressure-decay rates for internal explosions lead to relatively long overpressure duration times that are perhaps as long as a major fraction of a second."

In light of the precedent data related to the processes occurring in our test apparatus, the pressure vs. time data (Fig. 15) may be reevaluated, including the apparent double exponential decay in the initial part of the curve. The early data are indeed a nearly perfect fit to a double exponential (Fig. 18): a relatively faster decay, taking a major fraction of a second; and a slower decay, taking (probably coincidentally) about 2.5 sec to nearly reach an equilibrium of atmospheric pressure before the chamber is opened to vacuum, at which point another smooth exponential decay occurs until the system equilibrates at a partial vacuum. Then it is opened to the air, so the sporicidal atmosphere gets purged out of the detonation chamber.

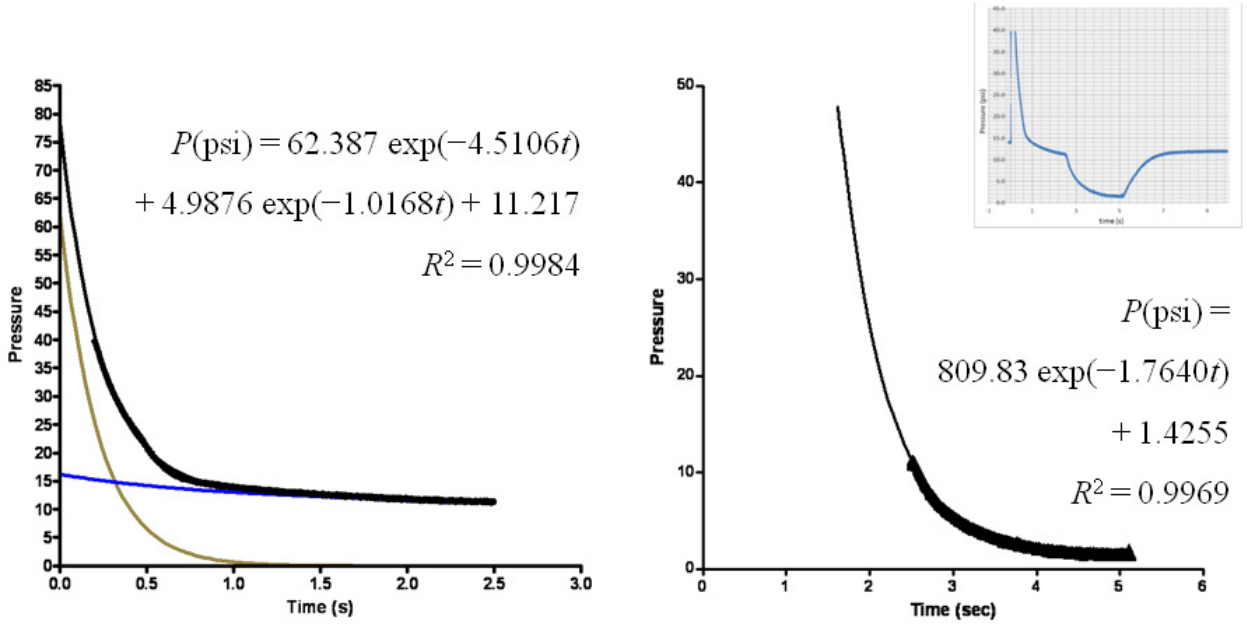


Figure 18. Pressure vs. time: Stages of exponential decays

From the complete pressure vs. time curves (prior to purging to atmosphere), a new parameter called "total exposure" (E) was calculated by integrating the data throughout the time when there was significant exposure: pressure (P_E) vs. time (t_E). However, this analysis uses only

[33] Kingery, C.; Schumacher, R.; Ewing, Jr, W. "Internal Pressure From Explosions in Suppressive Structures," ARBRL-MR-02848, June 1978; ADA059966.

[34] Kinney, G.F.; Sewell, R.G.S.; Graham, K.J. "Peak Overpressures For Internal Blast," NAWCWD TP 6089, June 1979; ADA071312.

the slower decay reflecting passage of the atmosphere out of the chamber through the solenoid and not the blast overpressure relaxation, which does not affect the presence of the sporicidal gas. Then the integration of the diminishing pressure during the evacuation stage is added to give a total value for exposure. A sample calculation for a nominal 2.50-sec exposure (Fig. 18) is given:

$$E(2.50\ \text{sec}) \equiv \sum P_{\text{E}} t_{\text{E}} \approx \int_{0}^{2.5} [4.9876 \exp(-1.1068t) + 11.217]\, \text{d}t + \int_{2.5}^{5.258} [809.83 \exp(-1.7640t) + 1.4255]\, \text{d}t = 42.030\ (\text{psia·sec})$$

Now the trend of log-reduction of *Bt* vs. the parameter "total exposure" (*E*) is tabulated and plotted in Fig. 19. Linear regression of these data seems to give a somewhat better correlation than the plot vs. nominal exposure time (Fig. 14). Unfortunately, the piezoresistive transducer was received and installed only in time for four of the seven tests, so only those results were available to analyze in this manner. There is apparently still one unexpectedly high log-reduction value (for one 1.25-sec nominal exposure), for which no technical explanation is available. Still, an interesting trend leading to attractive log-reductions in practical exposure times is demonstrated (Fig. 14 and Fig. 19) in this test series.

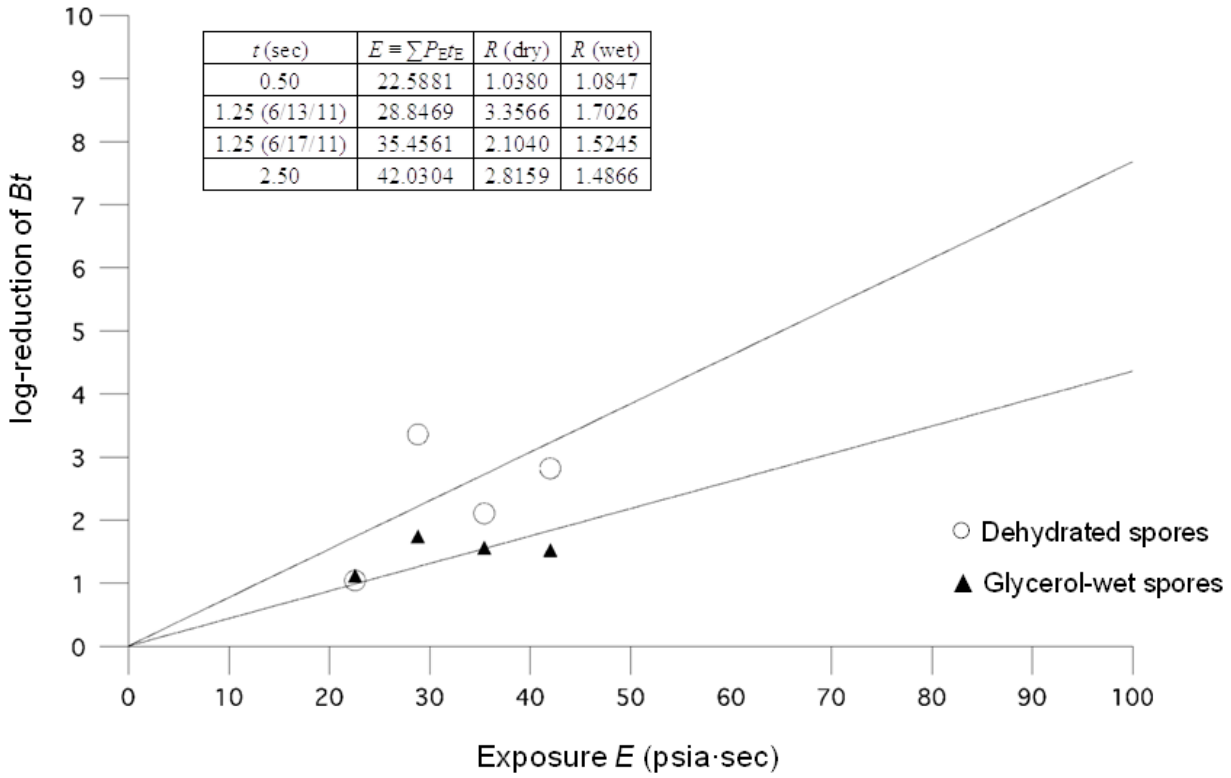
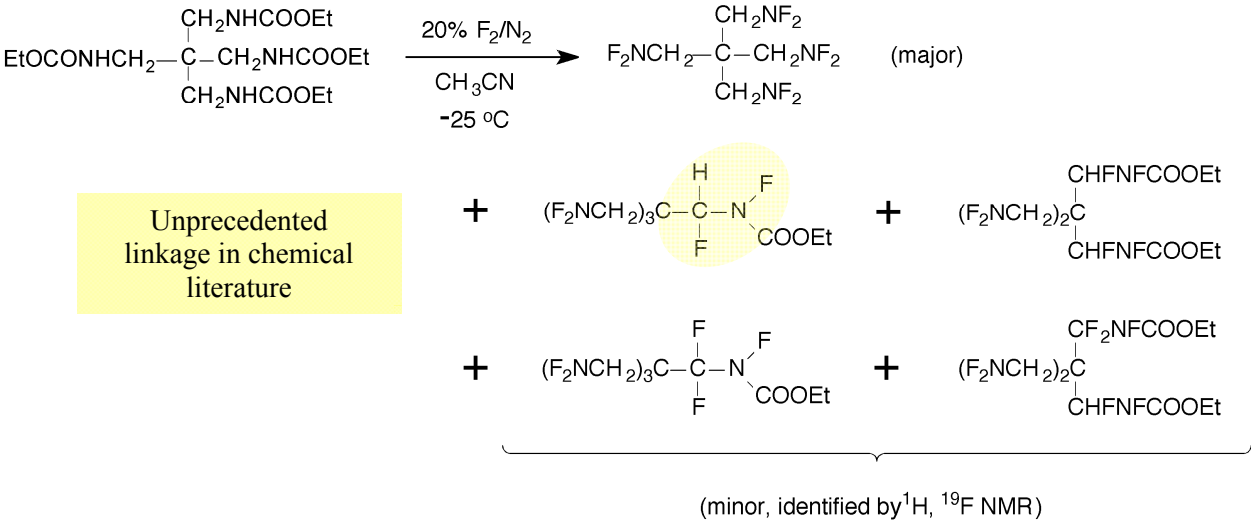


Figure 19. Log-reductions caused by HNFX vs. total exposure parameter *E*

2. Octafluoropentaerythrityltetramine (Octafluoro-PETA)

Octafluoro-PETA ("F_8-PETA") was prepared by direct fluorination of a new intermediate, tetraethyl pentaerythrityltetracarbamate [$C(CH_2NHCOOEt)_4$] (which has been subsequently independently reported by Joo and Shreeve[35]), with elemental fluorine (Scheme 2), involving transformations similar to those in the route used by Archibald and Manser to prepare 3,3-bis(difluoraminomethyl)oxetane.[36] Tetraethyl pentaerythrityltetracarbamate was made in one pot in nearly quantitative yield from PETA tetrahydrochloride, basified in aqueous solution by excess sodium hydroxide and treated with ethyl chloroformate, followed by extraction into dichloromethane. Fluorine (20% in nitrogen) was bubbled through the tetracarbamate in aceto-nitrile solvent at –25 °C until excess fluorine appeared in an aqueous iodide trap. The major product was the desired octafluoro-PETA, which could be purified chromatographically. Several by-products of fluorination of the methylene (CH_2) bridges of the pentaerythrityl backbone were identified (by multinuclear NMR) among the chromatographic fractions (Scheme 2) and are all new compounds. One minor by-product, as indicated, is interesting in containing a chemical linkage that is unprecedented in chemical literature: (CHF)(NF).



Scheme 2. Fluorination products from tetraethyl pentaerythrityltetracarbamate

Experimental:[37] Tetraethyl pentaerythrityltetracarbamate was made in one pot in quantitative yield from pentaerythrityltetramine tetrahydrochloride (prepared by the procedure of Adil et al.[11]). A solution of 2.78 g of pentaerythritylamine tetrahydrochloride (10 mmol) in 15 mL of water containing 4.0 g of sodium hydroxide (100 mmol) was cooled in an ice–salt bath, and 6.71 g of ethyl chloroformate (5.89 mL, 60 mmol) was then added slowly over 30 min. After the addition was complete, stirring with cooling was then continued for an additional 1.5 h. The

[35] Joo, Y.-H.; Shreeve, J.M. *Chem. Commun.* **2010**, *46*, 142.
[36] Archibald, T.G.; Manser, G.E. US Patent 5789617 (1998).
[37] Chapman, R.D.; Hollins, R.A. U.S. Patent 8008527 (2011).

mixture was brought to room temperature, and a solution of 1 mL of ethyl chloroformate in 10 mL of methylene chloride was added and stirring continued for an additional 30 minutes. The mixture was extracted with approximately 100 mL of chloroform and the separated organic layer was then dried over anhydrous magnesium sulfate and evaporated on a rotary evaporator giving 3.83 g of white solid (100% yield). Fluorine (20% in nitrogen) was bubbled through a solution of tetraethyl pentaerythrityltetracarbamate in acetonitrile solvent at –25 °C until excess fluorine appeared in an aqueous iodide trap. The major product was the desired octafluoro-PETA, which was purified chromatographically (silica gel/chloroform). ^{1}H NMR ($CDCl_3$): δ 3.88 (t. 27 Hz). ^{19}F NMR ($CDCl_3$): δ 62.13 (t, 27 Hz). ^{13}C NMR ($CDCl_3$): δ 38.17 (m), 66.95 (t, 8.8 Hz).

Octafluoro-PETA, upon isolation, exhibited unusual physical properties. Most relevant to its possible application as a munitions ingredient, it has an unexpectedly low melting point (40~42 °C) and quite high volatility; a sub-gram sample of crystals contained in a vial sublimed up through the vial after storage on top of an oven (~30 °C) overnight (Fig. 20).

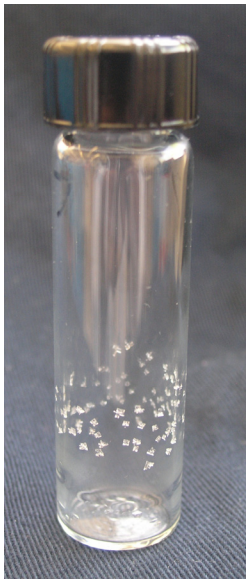


Figure 20. Sample of octafluoro-PETA after storage at ~30 °C overnight

By X-ray crystallographic analysis, the compound's unit cell volume could be determined—indicating a reasonable density of 1.70 g/cm^3—but extreme disorder in the crystal prevented complete solution of the molecular structure. Crystallographic disorder of NF_2 substituents is a common feature among difluoramine derivatives,[38] so octafluoro-PETA's physical properties are most likely attributable to the same phenomenon occurring in its four NF_2 substituents. For comparison, pentaerythrityl tetrafluoride [$C(CH_2F)_4$], a pentaerythrityl derivative with smaller substituents than octafluoro-PETA's, is also relatively volatile (m.p. 92 °C, b.p. 110 °C)[39] for a pentaerythrityl tetrahalide, but even its phase transformations occur at significantly higher temperatures. Octafluoro-PETA's physical properties probably limit its practical

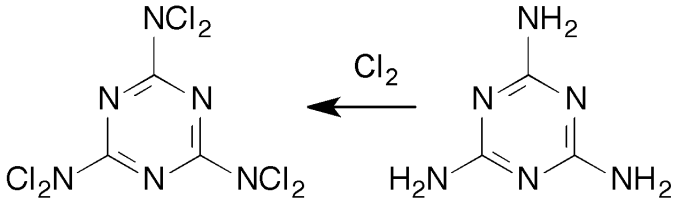
[38] Butcher, R.J.; Gilardi, R.; Baum, K.; Trivedi, N.J. *Thermochim. Acta* **2002**, *384*, 219.
[39] Gryszkiewicz-Trochimowski, E. *Bull. Soc. Chim. France* **1953**, 125.

applications in munitions formulations. For the specific purpose of biological agent defeat, it may still be attractive, but weapons hardware would need to accommodate its volatility and low melting point. Because of these complications, octafluoro-PETA was abandoned for further study in this project.

II. DICHLORAMINE EXPLOSIVES

1. Hexachloromelamine (HCM)

For this project, hexachloromelamine was prepared, by a literature procedure,[40] via direct chlorination of melamine (Scheme 3) in a biphasic solvent system of water and carbon tetrachloride. The crude product contained a minor amount of penta-*N*-chloromelamine ascertained by multinuclear NMR spectra of the impurity [^{1}H NMR ($CDCl_3$) δ 6.75; ^{13}C NMR ($CDCl_3$) δ 169.62, 173.52]. Recrystallization from CCl_4 afforded pure hexachloromelamine [^{13}C NMR ($CDCl_3$) δ 173.76]. Its identity was corroborated by X-ray crystallography, which also provided a previously unreported density of 2.006 g/cm^3 for the compound. Explosive sensitivity tests were performed on the product. (However, to our knowledge, hexachloromelamine has not been previously reported as being explosive.) Electrostatic discharge: 10/10 no-fires at 0.25 J. ABL friction: 10/10 no-fires at 1000 lbf. An apparent response of hexachloromelamine to impact was barely detectable by sound and odor and was certainly not dramatic. Impact (ERL modified Type 12 tool): $H_{50} \approx 9.2$ cm (RDX Class 2 $H_{50} \approx 18$ cm).



Scheme 3. Hexachloromelamine formed by direct chlorination of melamine

a. Long exposures (hours)

Three separate detonations of hexachloromelamine were carried out in the chamber—configured as in the first test series of HNFX (Section I.1.a)—in order to allow three different exposure times of the *Bacillus* analytes to presumed biocidal products of detonation, which were expected to include elemental chlorine.

This series of tests employed spore sample sizes on the order of 10^9 CFUs per test, so the spore pellets contained in the microcentrifuge tubes were significantly larger than in the first HNFX tests. In order to prevent dislodgement of spores from the tubes into the chamber during explosion, and to avoid explosion debris from contaminating the spores, a 2 cm × 2 cm square of

[40] Hamprecht, G.; Mohr, R. US Patent 3364214 (1968).

270-mesh (53-µm opening) stainless steel screen (Small Parts, Inc., Miramar, FL) was inserted into each of the spore tubes (Fig. 21).

Figure 21. (left) Stainless steel mesh (2 cm × 2 cm), as used; (right) tube containing the screen rolled into a cone, inserted in the tube.

Quadruplicate tubes of each *Bacillus* species spore were placed at two different levels in the explosion chamber (Fig. 22). These tubes were uncapped so that dehydrated spores would be exposed to biocidal gases that are released from the explosion. In addition, capped tubes containing dehydrated spores were placed in the explosion chamber to determine whether any spores are also killed by other effects, such as transient changes in temperature/pressure resulting from the explosion. Tubes containing dehydrated spores but not placed in the explosion chamber were used as controls.

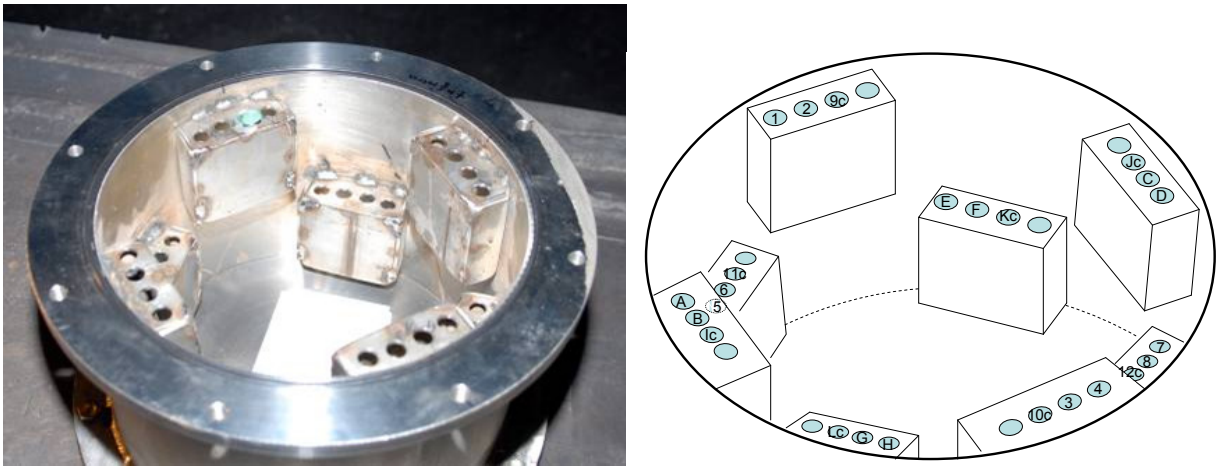


Figure 22. (left) Detonation chamber used for detonation; (right) placement positions of *B. subtilis* (positions 1–8, 9c–12c) and *B. thuringiensis* (positions A–H, Ic–Lc). Tubes at positions 1–8 and A–H were uncapped; tubes 9c–12c and Ic–Lc were fitted with manufacturer's caps.

Hexachloromelamine explosive charges were 2.80 ± 0.16 grams of pure hexachloromelamine contained in black conductive polyolefin vials (Emerald Plastics #EP145) with a volume of 4 cm^3, similar to those used in the previous HNFX experiments (Fig. 4). (This proportion of explosive charge to chamber volume simulates the action of a warhead with a 50-pound explosive fill deployed in a structure of dimensions 16′ × 16′ × 10′.) The charge for the first two detonations was initiated by an RP-3 miniature exploding bridgewire detonator (Teledyne RISI), which contains a total of ~29 mg of PETN.[24] Because a significant amount of light-colored, powdery residue—which was tentatively suspected of being unexploded hexachloromelamine—was left on the chamber floor following the first two detonations, a somewhat larger RP-2 exploding bridgewire detonator[41] (32 mg PETN + 18 mg RDX) was used for the third test in order to ensure maximal achievable initiation of hexachloromelamine detonation. Relative humidity on the day of assembly of the experiments was 17%.

Initial high pressures produced by the explosions were discharged via the chamber's open initiation wire port. Detonation product exposure times were chosen to be 0.4 hour, 3.0 hours, and 24.0 hours. At a selected time following each detonation, the chamber's lid was cracked open to allow venting of possible fumes for about 10 min before the lid was removed for spore sample retrieval. Exposure of spores to product gases was deemed to be terminated during this 10-min venting period (Fig. 6). (Unlike in the previous HNFX experiments, the tubes recovered from the chamber after all explosions were intact, and no sign of their physical damage or of dislodgement of dehydrated spores was apparent. This is further evidence of the poorer explosive performance of hexachloromelamine in comparison to HNFX.) Between each detonation, the chamber was cleaned by wiping first with acetone, followed by an aqueous anionic detergent (Dawn® by Procter & Gamble) and a nonionic detergent (Alcojet® by Alconox Laboratory Cleaning Supplies), and then sterilized with 2% bleach solution. The chamber was finally rinsed with distilled water and with 70% isopropanol.

Following the first detonation (exposure time of 0.4 h), a small amount of light-colored powder was observed on the lid of the chamber. Analysis of this powder by ^{13}C NMR confirmed it to be unreacted hexachloromelamine. The third detonation (with the more powerful RP-2 detonator) also left some grayish powdery residue on the chamber floor. Analysis of this powder (soluble in dimethylformamide-d_7) by NMR spectroscopy showed it *not* to be unreacted hexachloromelamine. Elemental analysis of the material was informative: carbon, 22.08%; hydrogen, 2.18%; nitrogen, 12.71%; chlorine, 13.66%. This analysis does not correspond to hexachloromelamine or likely simple products of thermolysis, such as extended azo derivatives formed via links between dichloramino substituents following chlorine elimination, such as have been observed in degradations of other (dichloramino)azines.[42] The observed mole ratio of chlorine to nitrogen in the residue is 0.42, compared to a ratio of 1.00 in the original compound. As hexachloromelamine is the only likely source of these two elements among the by-products of the explosion, significant degradation of hexachloromelamine but not complete detonation to elemental chlorine is indicated by this analysis. A noticeable but not strong odor of chlorine was also observed by technicians upon disassembly of the chamber following all detonations.

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[41] http://www.teledynerisi.com/productpdf/page21.pdf

[42] Banks, R.E.; Barlow, M.G.; Mamaghani, M. *J. Fluor. Chem.* **1981**, *17*, 197.

A statistical *summary* of the results from survivability assessments in this series of tests showed possible anomalies that called for inspection of the raw data from the measurements, which are shown in Table 7 below.

Several data points were suspected of being statistical outliers, so their possible rejection was tested and then justified based on standard statistical tests.[43] One such result was a nonzero value (7.60E+04) for tube D with *Bt* in Detonation #1, although all other tubes with *Bt* in Detonation #1 showed zero surviving spores. Statistically, this point could be rejected at a confidence level of 99% ($\alpha = 0.01$). A speculation can be offered about the reason for this apparent anomaly, although definitive proof is not at hand. Other evidence has been described (above) that hexachloromelamine behaved poorly as an explosive. All tubes contained some fine powder that had been dispersed in the detonations. (In Detonation #1, white residue on the chamber lid was analyzed as unreacted hexachloromelamine.) It is speculated that the stainless steel screen inserted into tube D of Detonation #1 became sufficiently blocked by powder that biocidal gases had restricted access to the *Bt* spores, resulting in incomplete killing in that tube, although all other uncapped tubes apparently allowed sufficient access of biocide to effect complete kills.

Among the *Bacillus* control samples, variability appeared more likely to occur when high spore counts were used (such as here) due to high viscosity of the spore suspensions and the resuspension of the dried spores for assay. Sonication (a standard procedure for suspending high-spore-count pellets) was then used to ensure that the spores were well suspended. This variability was apparent as one outlier in each of the sets of *Bs* and *Bt* controls. Each outlier could be rejected at a confidence level of 98% ($\alpha = 0.02$).[43]

The result that samples in "capped" tubes also showed some reduction in survivability suggests that biocidal detonation products must have leaked into the tubes at some time and may have remained in contact with spores throughout the exposure period. Only speculation can be offered to explain such leakage, and it is tentatively attributed to brief pressure changes during detonation, which may have deformed the tubes' caps and allowed ingress of small amounts of pressurized biocidal product gases into the transiently lower-pressure tubes. The high variability of the survival rates, none of which could be rejected as outliers by statistical tests, is consistent with the uncontrolled occurrence of unexpected leakage through the caps.

Detonation #2 seems the most anomalous overall, allowing survival of the largest number (small but not nonzero) of *B. subtilis* spores in four out of eight uncapped tubes and also the smallest number of surviving spores in "capped" tubes. Although *B. subtilis* has slightly greater resistance to chlorine-based chemical disinfecting agents (Clorox) than *B. thuringiensis*,[44] it is unexpected that *Bs* should survive to a greater extent after 3.0 hours' exposure than after 0.4 hour's exposure. Without evidence or presumption of error in the biological assays, this variability is also tentatively attributed to the poor explosive performance of hexachloromelamine, possibly resulting in uneven dispersal of solid by-products and uneven pressure fronts contributing to capped tubes' leakage.

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[43] (a) Dixon, W.J. *Biometrics* **1953**, *9*, 74. (b) Proschan, F. *Am. J. Phys.* **1953**, *21*, 520. (c) Dixon, W.J.; Massey, Jr., F.J. "Introduction to Statistical Analysis" (2nd ed.), McGraw-Hill, New York, 1957; pp. 275ff. (d) Grubbs, F.E. *Technometrics* **1969**, *11*, 1.

[44] Sagripanti, J.-L.; Carrera, M.; Insalaco, J.; Ziemski, M.; Rogers, J.; Zandomeni, R. *J. Appl. Microbiol.* **2007**, *102*, 11.

Table 7. Viability of *Bacillus* spores following hexachloromelamine detonations [a]						
	Detonation #1 (0.4 h) N_E		**Detonation #2 (3.0 h) N_E**		**Detonation #3 (24.0 h) N_E**	
Sample	***B. subtilis***	***B. thuringiensis***	***B. subtilis***	***B. thuringiensis***	***B. subtilis***	***B. thuringiensis***
Uncapped:						
1/A	0	0	3000	0	0	0
2/B	0	0	2900	0	0	0
3/C	0	0	0	0	0	0
4/D	0	~~7.60E+04~~	1520	0	0	0
5/E	0	0	0	0	0	0
6/F	0	0	0	0	0	0
7/G	0	0	3170	0	0	0
8/H	0	0	0	0	0	0
$\overline{\log(N_E+1)}$	**0**	**0**	**1.703 ± 1.823**	**0**	**0**	**0**
"Capped":						
9c/Ic	6.35E+06	1.00E+07	2.0E+01	0	3.20E+04	1.90E+04
10c/Jc	8.35E+06	7.70E+07	1.6E+03	3.80E+07	4.10E+04	1.70E+04
11c/Kc	1.09E+07	6.50E+06	8.6E+03	3.75E+06	2.40E+04	1.60E+04
12c/Lc	2.90E+06	3.87E+07	6.7E+03	6.80E+04	2.70E+04	4.70E+04
$\overline{\log(N_E+1)}$	**6.806 ± 0.248**	**7.322 ± 0.501**	**3.072 ± 1.210**	**4.747 ± 3.362**	**4.482 ± 0.101**	**4.346 ± 0.219**
	Control *B. sub*	**Control *B. thur***				
	2.9E+07	9.7E+08				
	2.8E+07	9.0E+08				
	~~1.0E+08~~	8.4E+08				
	2.4E+07	~~2.4E+07~~				
$\overline{\log(N_0+1)}$	**7.430 ± 0.044**	**8.955 ± 0.031**				
***R*("capped")**	**0.624 ± 0.252**	**1.633 ± 0.502**	**4.358 ± 1.211**	**4.208 ± 3.362**	**2.948 ± 0.110**	**4.609 ± 0.221**
***R*(uncapped)**	**>7.43**	**>8.95**	**5.727 ± 1.824**	**>8.95**	**>7.43**	**>8.95**
<*R*>(uncapped)	**>8.968 ± 0.054**		**7.265 ± 1.824**		**>8.968 ± 0.054**	

[a] Exposure times of spores to detonation products are shown. Control spores (Control *B. sub* and Control *B. thur*) were similarly prepared spore samples that were not exposed to detonation. Spore locations in the explosion chamber are indicated in Fig. 22. Uncertainties shown are standard deviations (s_{n-1}). ~~Strikeout~~ font indicates data that are rejected as statistical outliers (see text). $\boldsymbol{R} \equiv$ log-reduction $= \log[(N_0 + 1)/(N_E + 1)] = \log(N_0 + 1) - \log(N_E + 1)$. The expectation value $\langle \boldsymbol{R} \rangle$ treats all *Bacillus* spores collectively as surrogates of *B. anthracis* to estimate the log-reduction in survivability caused by detonation products.

b. Intermediate exposures (seconds)

Prior to testing HCM with a shorter exposure time of seconds, an alternative, more powerful dichloramine-based explosive, 1,3,5-benzenetris(*N,N*-dichlorosulfonamide) (BTD), was tested with longer exposure times (see results below) for a comparison to the corresponding results from HCM. Because of BTD's poor sporicidal performance even at long exposure times, HCM was further investigated. Using the modified chamber configuration (Fig. 13) to test exposures in the range of seconds, one test of HCM was carried out with a nominal exposure time of 5.00 sec and in this test a charge containing 5.45 grams of HCM (more tightly packed than in the first series using HCM). That test gave an *apparent* good result (though not as good as HNFX) of $\boldsymbol{R} = 5.53 \pm 1.56$ using 1.13×10^{10} total CFUs in the chamber. The uncertainty in that number arises from the 16 coupons showing a range of survivals: 12 with zero survivors, three in the range of 1100~1200, and one with 3×10^4 survivors.

Because of a suspicion that residual *solid* present after all HCM "explosions" to date might be sporicidal and may therefore have contributed to the observed apparently high efficacy of HCM products (Table 7) via contact with the spores, the 5.00-sec test was repeated with an experimental design to alleviate any complication due to residual HCM or sporicidal chloramine by-product arising from HCM explosion. The 5.00-sec exposure was repeated (using an explosive charge of 5.73 grams HCM), but immediately upon opening the detonation chamber, each coupon was quenched into dilute aqueous sodium thiosulfate to neutralize any HCM (or *N*-chloro by-product) stuck to the coupon. Aqueous sodium thiosulfate has been demonstrated to be a feasible reagent for destroying excess chemical neutralizers such as sodium hypochlorite[45] and 1,3-dichloro-1,3,5-triazinetrione[46] (an *N*-chloro compound in the same general class as HCM) without adverse effects on *Bacillus* spores. Therefore, each coupon retrieved from the chamber following a nominal 5.0-sec exposure was immediately quenched into 5 mL of aqueous sodium thiosulfate containing 270 mg of $Na_2S_2O_3 \cdot 5H_2O$. Sodium thiosulfate is capable of neutralizing HCM (via reduction of NCl_2 to NH_2 of melamine) according to the overall reaction

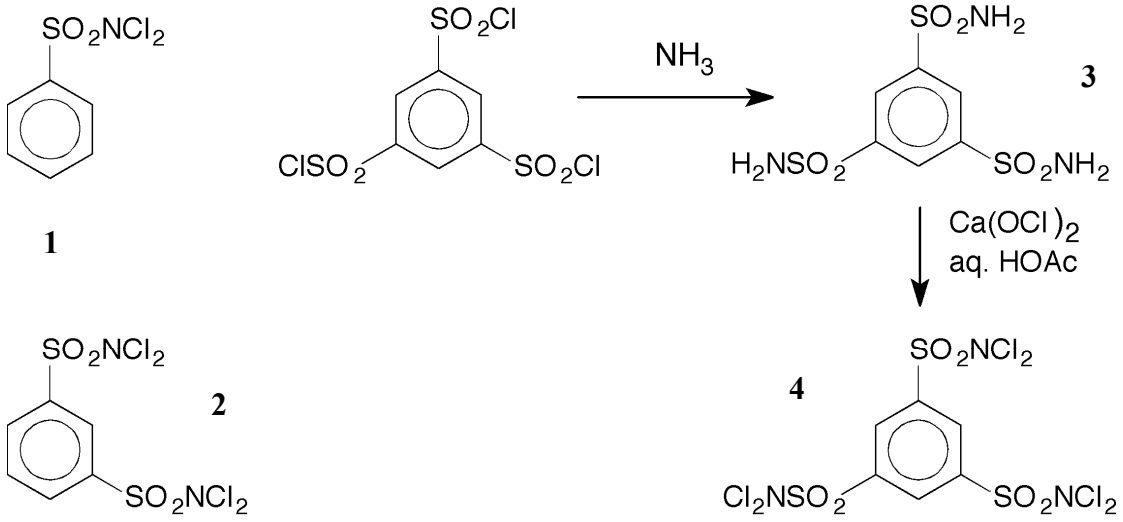
$$C_3N_6Cl_6 + 12Na_2S_2O_3 + 6H_2O = C_3N_6H_6 + 6NaCl + 6Na_2S_4O_6 + 6NaOH$$

so 10 mg of HCM requires 89.5 mg of $Na_2S_2O_3 \cdot 5H_2O$ to neutralize it. With that treatment to destroy any residual sporicidal solid by-product of HCM, a much lower log-reduction was observed: only ~0.17, essentially confirming that the apparent high sporicidal efficacy seen in all previous tests of HCM was likely due to such residual products of the explosions. HCM was therefore abandoned as a legitimate "dihaloamine explosive" for this application, at least as a pure compound, although formulations such as with booster charges might still make it a viable candidate for agent defeat.

[45] Rice, E.W.; Adcock, N.J.; Sivaganesan, M.; Rose, L.J. *Appl. Environ. Microbiol.* **2005**, *71*, 5587.
[46] Raber, E.; Burklund, A. *Appl. Environ. Microbiol.* **2010**, *76*, 6631.

2. 1,3,5-Benzenetris(*N,N*-dichlorosulfonamide) (BTD)

Previously unreported 1,3,5-benzenetris(*N,N*-dichlorosulfonamide) (**4**) was prepared by simple aqueous ammonolysis (by the procedure of Jackson[47]) of commercially available 1,3,5-benzenetrisulfonyl chloride followed by N-chlorination of benzenetrisulfonamide (**3**) with acidified calcium hypochlorite (Scheme 4), analogously to preparations of **1** and **2**.[15]



Scheme 4. Old (**1**–**2**) and new (**4**) explosive *N,N*-dichlorosulfonamide derivatives

Experimental: To a vigorously stirred suspension of calcium hypochlorite (18 g) in 600 mL of water, which eventually dissolved, was added 1,3,5-benzenetrisulfonamide (6 g, 19 mmol), and stirring was continued for 15 min. To this mixture was then added 18 mL of concentrated acetic acid, and the stirring continued for an additional 10 min. The mixture was extracted with 400 mL of ethanol-free chloroform followed by a second extraction with 300 mL of ethanol-free chloroform. The extracts were combined, dried ($MgSO_4$), and evaporated at reduced pressure (warm on a rotary evaporator). The obtained solid was then heated to boiling with 50 mL of ethanol-free chloroform and, while hot, 50 mL of hexanes was added with stirring. After cooling, the solid was filtered and washed with hexanes and then air-dried to yield 4.3 g of product 1,3,5-benzenetris(*N,N*-dichlorosulfonamide) (**4**) (65% yield). ^{1}H NMR ($CDCl_3$): δ 9.13 (s, CH). ^{13}C NMR ($CDCl_3$): δ 133.2, 139.2. The crude product contained ~7% impurity (^{1}H NMR δ 9.02). The identity of the product (BTD, **4**) was also corroborated by X-ray crystallography, which also provided a density of 1.973 g/cm^3 for the compound.

Explosive sensitivity tests were performed on the new product (**4**). Electrostatic discharge: 10/10 no-fires at 0.25 J. Friction (#1 ABL): F_{50} ~ 447 lbf (RDX Class 2 F_{50} ≈ 692 lbf). Impact (ERL modified Type 12 tool): H_{50} ~ 7.8 cm (RDX Class 2 H_{50} ≈ 18 cm). In contrast to hexachloromelamine, whose response to impact was detectable by sound and odor but was not

[47] Jackson, C.L. *Am. Chem. J.* **1887**, *9*, 325.

dramatic, that of **4** gave a sharp report and a heavy chlorine odor. Thus, **4** appears to perform as a more powerful explosive than hexachloromelamine and distinctly produces elemental chlorine as a detonation product.

Two tests of sporicidal efficacy of products of detonation by BTD were conducted, using exposure times of 0.4 h and 2.9 h in order to compare to the results from HCM "explosions" (Section II.1.a). The tests used only dehydrated spores (on 16 coupons): 9.89×10^9 CFUs for the shorter exposure and 5.12×10^9 CFUs for the longer exposure. The log-reductions measured were $\boldsymbol{R} \approx 1.26$ for 0.4 h and $\boldsymbol{R} \approx 1.33$ for 2.9 h exposure. Thus, although *N,N*-dichloramine BTD was a clearly superior explosive compared to HCM, and it generated elemental chlorine upon detonation, the conditions that prevailed in our test configuration were not sufficient to achieve sporicidal efficacy nearly as attractive as that shown by *N,N*-difluoramine HNFX. This was a result that prompted the reinvestigation of HCM with a procedure to eliminate the possible effect of residual solid by-products (Section II.1.b).

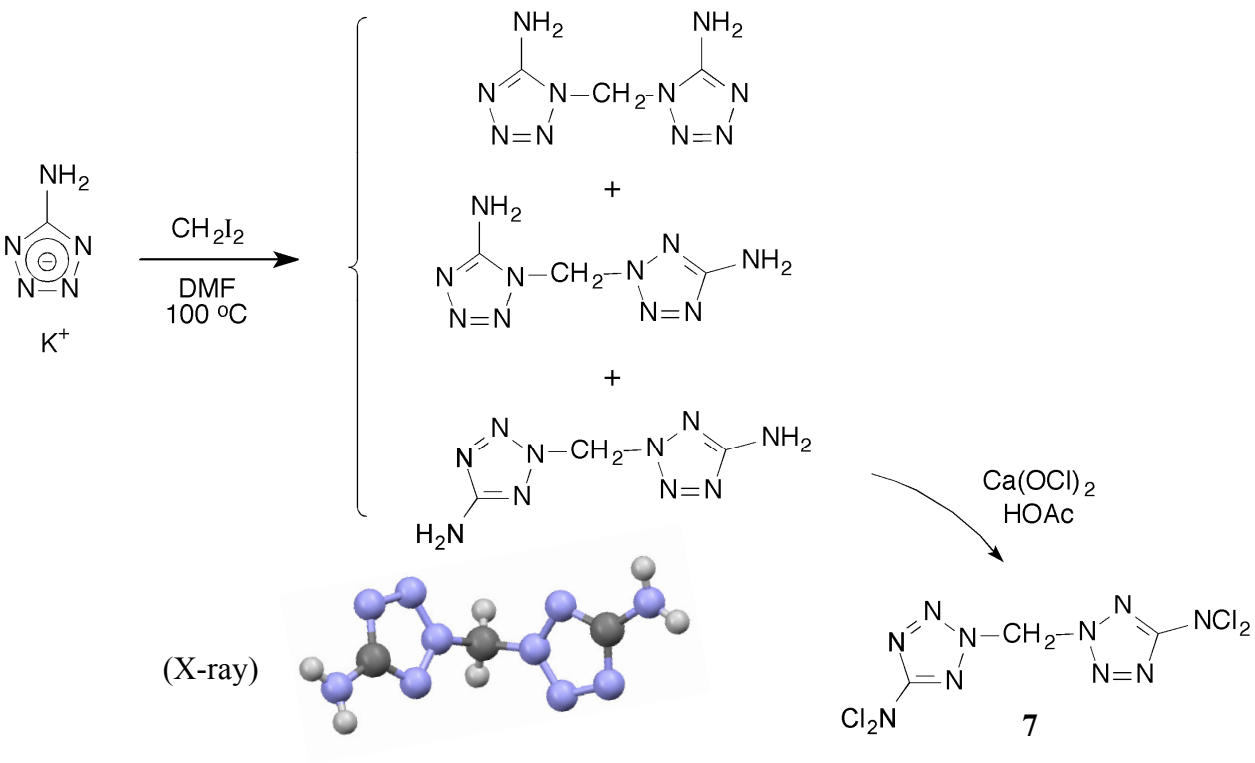
3. 5-(*N,N*-Dichloramino)tetrazoles

As an attractive new example of compound in this class that contains a high content of biocidal equivalent, we envisioned as a target compound methylenebis[5-(*N,N*-dichloramino)-tetrazole] (any feasible isomer linked between N^1 or N^2 ring nitrogens), which could generate >44 wt% Cl_2 upon detonation. The desired product(s) might be straightforwardly prepared by direct N-chlorination of any corresponding methylenebis(5-aminotetrazole), which was, however, also an unreported structure in the literature. An unsuccessful attempt to prepare 1,1′-methylenebis(5-aminotetrazole) was reported by Barmin et al.,[48] who used dibromomethane as an alkylating reagent toward 5-aminotetrazole potassium salt in refluxing acetone solvent but saw no formation of the desired product in 32–36 hours. Our synthesis of three isomers of methylenebis(5-aminotetrazole) was successfully carried out by using diiodomethane with 5-aminotetrazole potassium salt in dimethylformamide solvent at 100 °C (Scheme 5 below).

The formation of three isomers (1,1′, 1,2′, and 2,2′) was indicated by multinuclear (^{1}H, ^{13}C) NMR in comparison to reference 5-amino-1- and 2-methyltetrazoles,[49] but only the minor 2,2′ isomer was identified crystallographically, following its first isolation by column chromatography from the isomer mixture. 2,2′-Methylenebis(5-aminotetrazole) was chlorinated analogously to 1,3,5-benzenetrisulfonamide using calcium hypochlorite in aqueous acetic acid. The dichloromethane-d_2-soluble extract (solubility being consistent with a new dichloramine product and not with the starting material) exhibited a ^{1}H NMR spectrum consistent with containing the desired product, 2,2′-methylenebis[5-(*N,N*-dichloramino)tetrazole] (**7**); however, an overnight acquisition of ^{13}C NMR data provided evidence (spectral in addition to concomitant precipitation of insoluble solid) that the product was degrading even in dichloromethane solution overnight at 300 K. Thus, at least this isomer (2,2′) of the proposed target compounds, methylenebis[5-(*N,N*-dichloramino)tetrazoles], appears too unstable to be practical. Since other comparisons of 1-alkyl vs. 2-alkyl 5-energetically-substituted tetrazoles have shown greater *instability* of the 2-alkyl

[48] Barmin, M.I.; Gromova, S.A.; Mel'nikov, V.V. *Russ. J. Appl. Chem.* **2001**, *74*, 1156.
[49] Bocian, W.; Jaźwiński, J.; Koźmiński, W.; Stefaniak, L.; Webb, G.A. *J. Chem. Soc. Perkin Trans.* **1994**, 1327.

isomers,[50] the 1,1′ isomer of the new target structures may still offer promise as a practical agent defeat weapon ingredient, but it was not further pursued.



Scheme 5. Preparation of new tetrazole derivatives

[50] (a) Spear, R.J.; Elischer, P.P.; Bird, R. "1-Methyl-5-Nitrotetrazole and 2-Methyl-5-Nitrotetrazole. Part 2." Materials Research Labs (Ascot Vale, Australia) Report MRL-R-771, 1980; DTIC AD106280. (b) Schroeder, M.A.; Henry, R.A. "Quantum-Mechanical Studies on Chemical Reactivity and Ballistic Chemistry. VII." Army Ballistic Research Lab (Aberdeen Proving Ground, MD) Report ARBRL-TR-02371, 1981; DTIC AD A107288.

CONCLUSIONS

The following specific conclusions can be drawn from the results obtained in this project.

- High killing efficacy ($\gtrsim$9 $\log_{10}$-reductions) of anthrax surrogate spores has been achieved by exposures of 5 seconds or more to gaseous products from detonations of HNFX.

- This agent defeat by HNFX was not due to heat or pressure of explosions but to harsh conditions of exposure to biocidal detonation products.

- The active sporicide was originally proposed conceptually to be hydrogen fluoride (HF), but other transient more-reactive halogen species may be involved. An example may be atomic fluorine or its by-products of reaction with spore materiel. Such transient reactive species may be unique to the class of *N,N*-difluoramines.

- Glycerol acts as an apparent protective agent against the sporicidal product(s) produced by HNFX detonation. This is inconsistent with hydrogen fluoride as the sole sporicide (as glycerol is a known inert solvent for HF) but is consistent with more-reactive transient intermediates getting scavenged by oxidizable glycerol.

- In comparison, a conventional nonhalogenated explosive of similar explosive power, HMX, showed only ~0.2 log-reduction of *Bacillus* spores following an even longer exposure (0.4 hour) to its detonation products.

- Sporicidal efficacies of HNFX detonation products were determined at a wide range of exposure times, ranging from milliseconds to seconds to hours. For exposure times $\lesssim$1 second, observed log-reductions of *Bt* were $\lesssim$3.

- One powerful elemental-chlorine-generating explosive in the class of *N,N*-dichloramines (BTD) was not nearly as effective against *Bt* spores as HNFX was.

- Another *N,N*-dichloramine, hexachloromelamine, was insufficiently explosive by itself to detonate. Residual solid by-products from HCM explosions were sporicidal, but gaseous products of such explosions were not very efficacious against *Bt* following $\gtrsim$5 seconds of exposure.

This project's successful results have proven the feasibility of the proposed general approach to defeat biological harmful agents using novel *N*-halogenated explosives that produce biocidal detonation products, such as hydrogen fluoride (or transient reactive fluorine species), under harsh conditions that rapidly kill anthrax surrogate spores in relatively short exposure times. This effort supported the DTRA initiative *Advanced Energetic Materials for Agent Defeat* aimed at advancing the state of the science and body of knowledge of novel solid energetic

materials capable of both destroying the storage and production facilities of harmful agents and then neutralizing the biological agents therein.

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