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Measuring toxic gases generated from reaction of guanidine isothiocyanate-containing reagents with bleach

By Samuel Paik,
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INTRODUCTION

We conducted this study in response to concerns regarding the safe handling and disposal of guanidine isothiocyanate (GITC)-containing reagents. GITC is commonly used in nucleic acid testing (NAT) applications in clinical laboratories as an agent for denaturing proteins and lysing cells or microorganisms.[1–4] Since biological materials may be present with the GITC-containing reagents in these laboratories, the most commonly used disinfectant, bleach (i.e., sodium hypochlorite in solution) may be used to treat them during spill cleanup or

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waste disposal, as it is well known that bleach is very effective in disintegrating nucleic acids and preventing laboratory contamination. Upon close analysis of the molecular structure of GITC and bleach, however, it becomes apparent that GITC can potentially react with bleach and generate toxic gases at dangerously high concentrations. Some of the gases that may result from this reaction, based purely on the molecular structure of these compounds, are nitrogen oxide (NO), nitrogen dioxide (NO_2), hydrogen chloride (HCl), hydrogen cyanide (HCN), chlorine (Cl_2), and carbon monoxide (CO). Another potential concern when handling GITC-containing reagents is their possible reaction with acid and base solutions since these solutions are also commonly used for treatment of most other biological wastes. With this in mind, the purpose of this study was to determine what gases may potentially arise, and at what concentrations, from the reaction of GITC-containing reagents with bleach, hydrogen chloride or sodium hydroxide (NaOH) solution.

EXPERIMENTAL

In order to test the various reaction scenarios, we fabricated a reaction chamber which would allow us to introduce reaction reagents to the chamber and collect gases generated from the chamber. We placed test solutions containing GITC into a 100 mL beaker and placed the beaker on the floor of a 4.5 L cylindrical glass cham-

ber. To sample the gases as completely as possible, we sealed the chamber by a chamber lid that had three outlets at its top. We used the first outlet to add the bleach, acid, or base solutions (mixing solutions) into the test solution in the beaker. We used the second outlet to collect the gases resulting from the reaction using appropriate collection media or a real-time measurement device. Finally, we used the third outlet to introduce replacement air into the chamber as the gases within the chamber were being sampled. By attaching a Tedlar bag at the tip of the tubing leading from the third outlet into the chamber, we collected the gases as products from the reaction without dilution by the makeup air or internal pressure reduction. A schematic of the test chamber is shown in Figure 1. We placed the entire chamber on a shaker to facilitate mixing of the chemicals. As soon as we added the mixing solution (e.g., bleach) to the 100 mL beaker in the chamber through the funnel, we removed the funnel, sealed the third outlet, and turned on the shaker table and sampling pumps. We allowed sampling to proceed for an appropriate duration by pumping out the gases in the chamber through the second outlet. We varied the sampling duration according to the prescribed flow rate of air through the pump. The process simultaneously inflated the Tedlar bag by up to approximately 3 L of air from the third outlet (open to the ambient atmosphere). Correspondingly, regardless of the gas collected and the reagent tested, we collected approximately 3 L of gases (about

Table 1. Target Gases and Their Sampling/Analytical Methods

Target gas	Validated method		Limit of detection
	Sampling media	Analysis	
Cl_2	Impinger (0.1% sulfamic acid)	Colorimetric (OSHA ID-101)[5]	2.5 µg
NO, NO_2	Adsorbent tube (TEA impregnated sieve with oxidizer)	Spectrophotometer (NIOSH 6014)[6]	1.3 µg
CO	Direct reading electrochemical sensor (NIOSH 6604)[6]		3 ppm
HCl	Adsorbent tube (acid washed silica gel)	Ion chromatography (NIOSH 7903)[6]	1.0 µg
HCN	Soda lime tube	Ion chromatography (NIOSH 6010)[6]	1.0 µg

67% of the total gas volume inside the chamber) from the chamber. After sampling was complete, we removed the sampling media and prepared them for sample analysis. We repeated this procedure for each gas for each mixing scenario.

To collect the gases of interest, we connected the sampling medium to an air sampling pump (Gilian Model HFS 513A or Gilian Model LFS 113D, Sensidyne, Clearwater, FA). We pre-calibrated and post-calibrated the pump using a frictionless piston primary flow calibrator (BIOS DryCal, SKC Inc., Eighty-Four, PA) at the flow rate prescribed by the sampling method.

Table 1 shows the different sampling and analytical methods used to collect and analyze the gases of interest. Only methods validated by Occupational Safety and Health Administration (OSHA) and National Institute of Occupational Safety and Health (NIOSH) were used in this study. All sampling media were obtained from Broadspire Inc. (Lake Zurich, IL).

For measuring CO, we utilized a direct-reading probe instrument with an electrochemical sensor (IAQ-CALC, Model 8762, TSI Inc., St. Paul, MN). We placed the monitor probe inside the chamber with the lid slightly ajar. We monitored carbon monoxide for 30 minutes after the mixing solution was completely added to the beaker, and we recorded the peak CO reading.

We tested three test solutions which had the following amounts of GITC:

Reagent A: 4.7 M
Reagent B: 3.5 M
Waste solution: 4.0 M

In addition to GITC, these test solutions also contained Tween 20 (5–10%), Tris buffer (0–100 mM) and potassium acetate buffer (0–50 mM). However, based on their molecular structures and relatively small presence in the test solutions, these compounds were not expected to contribute significantly to the gas products tested nor affect the reactivities of GITC with the bleach, acid and base solutions.[#1]

We tested all three test solutions with bleach. We additionally tested the waste solution for its reaction with acid and base solutions. The amounts of bleach, acid and base reagents tested were based on estimates of how much of them would be typically used during a clean-up or biohazard waste inactivation. The bleach consisted of 5% sodium hypochlorite in solution. The five different mixing scenarios were as follows:

45 mL Reagent A + 5 mL bleach
45 mL Reagent B + 5 mL bleach
45 mL waste solution + 5 mL bleach
40 mL waste solution + 10 mL 5N HCl
40 mL waste solution + 10 mL 5N NaOH

[#1] A separate test using reagents containing Tween 20 and reagents not containing Tween 20 confirmed that the presence of Tween 20 did not affect the reactivity of GITC with bleach, acid and base solutions.
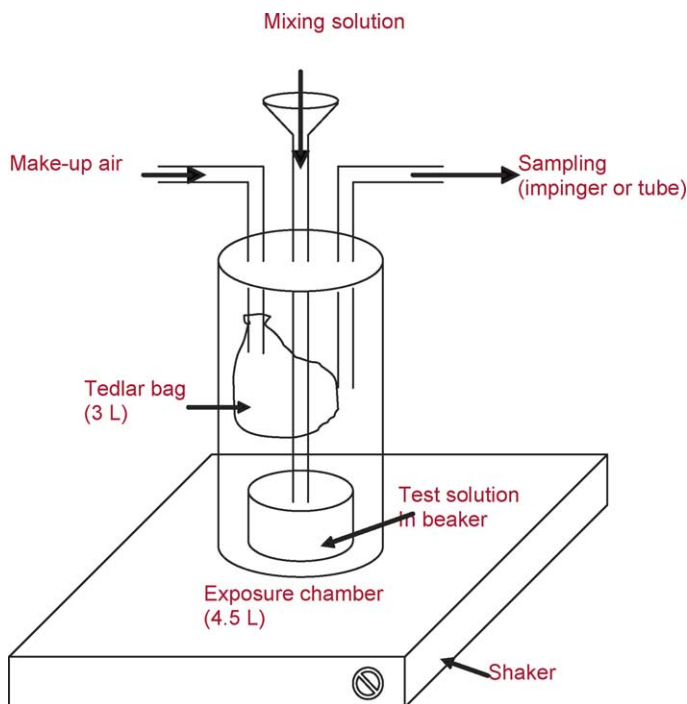

Figure 1. Test chamber used for mixing reaction components and gas collection.

Mixing solution

Make-up air

Sampling (impinger or tube)

Tedlar bag (3 L)

Test solution in beaker

Exposure chamber (4.5 L)

Shaker

Table 2. Resulting Gases from Reactions Between Test Solutions and Mixing Solutions

Test solution + mixing solution	HCl	HCN	Cl_2	NO	NO_2	CO
Reagent A + bleach	O	O	X	X	O	O
Reagent B + bleach	O	O	X	X	O	O
Waste + bleach	O	O	X	O	X	O
Waste + acid	X	O	X	O	O	O
Waste + base	X	X	X	X	O	X

X: not detected; O: observed.

RESULTS AND DISCUSSION

Table 2 shows a summary of experiments on the five different mixing scenarios. We detected hydrogen cyanide, HCl, and CO from all three mixing scenarios wherein we mixed a test solution with the bleach solution.

Reagent A (45 mL) + bleach (5 mL)

We generated hydrogen cyanide, HCl, NO_2, and CO when we mixed Reagent A with bleach solution. However, we did not detect Cl_2 or NO. The results are shown in Table 3. We collected two samples of HCl from two independent reactions using adsorbent tubes. The sampling durations were 8.5 and 11 minutes for the two samples, respectively. The amounts of HCl we collected were 104 and 81 μg, respectively. For simplicity, we assumed that the reactions were complete by the time each sample was collected and since the chamber was completely sealed from the outside, we assumed the sampled amounts represented the maximum amount of gas released from the reaction. Based on the amount of HCl analyzed on the samples and the volume of air collected, we calculated concentrations of 27 and 16 ppm. These concentrations represent a situation when a technician is exposed to the atmosphere that has all the resulting gases emitted from a reaction mixture when the reagent–space ratio is about 1:90 (based on reagent-bleach volume of 50 mL and chamber volume of 4.5 L). Compared to the OSHA Permissible Exposure Limit-Ceiling (PEL-Ceiling) of 5 ppm, these expected concentrations would be significant enough to cause adverse health effects. However, considering the expansive nature of chemical vapors, a realistic spill cleanup scenario would assume that the vapors emitted from the spill would fill a much larger volume of, say, $1\,m \times 1\,m \times 1\,m$ ($=1\,m^3$), i.e., the reagent–space ratio would be about 1:20,000. Based on this assumption, the projected concentra-

tion of HCl in $1\,m^3$ would be 0.12 and 0.074 ppm, respectively. Compared to the OSHA PEL-Ceiling of 5 ppm, these expected concentrations would not be significant enough to cause adverse health effects.

Similarly for HCN, the worst-case concentration was 20 ppm. In the corresponding less severe scenario, the concentration was 0.09 ppm, which is below the OSHA PEL of 10 ppm. We discarded one of the two samples for HCN because of an error in collecting the sample. We measured negligible amounts of CO, NO and NO_2 from the reaction as they were either less than their corresponding OSHA PEL or not detectable.

Reagent B (45 mL) mixed with bleach (5 mL)

We observed hydrogen cyanide, HCl, CO, and NO_2 from the reaction between Reagent B and bleach. Their concentrations and the other gas concentrations found in this test were very similar to those found with the Reagent A solution. As in the case with Reagent A, the projected concentrations of these gases did not exceed their corresponding OSHA PELs. The results are shown in Table 4.

Waste solution (45 mL) mixed with bleach (5 mL)

We observed hydrogen cyanide, HCl, CO, and NO from the reaction

Table 3. Results of Reagent A Mixed with Bleach

					Concentration (ppm)	
Test gas (OSHA PEL)	Run	Sampling time (min)	Sampling volume (L)	Amount (μg)	Experimental	Projected (over $1\,m^3$ volume)
Chlorine (1 ppm)	A	3.0	3.1	<2.5[a]	<0.28[a]	<0.0012[a]
	B	3.0	3.1	<2.5[a]	<0.28[a]	<0.0012[a]
HCl (5 ppm, ceiling)	A	8.5	2.6	104	27	0.12
	B	11.0	3.3	81	16	0.074
HCN (10 ppm)	A[b]	NA	NA	NA	NA	NA
	B	13.2	2.6	58	20	0.091
NO (25 ppm)	A	106	2.8	<1.3[a]	<0.38[a]	<0.0017[a]
	B	106	2.7	<1.3[a]	<0.39[a]	<0.0018[a]
NO_2 (5 ppm, ceiling)	A	106	2.8	1.7	0.32	0.0015
	B	106	2.7	<1.3[a]	<0.26[a]	<0.0012[a]
CO (50 ppm)					3–4 ppm	

[a] Concentrations indicated as "less than" amounts were non-detectable. "Less-than" concentrations are based on the limit of detection (LOD) of the analytical method. [b] Data from this run was discarded due to an error in sampling.

Table 4. Results of Reagent B Mixed with Bleach

Test gas (OSHA PEL)	Run	Sampling time (min)	Sampling volume (L)	Amount (μg)	Concentration (ppm) Experimental	Projected (over 1 m³ volume)
Chlorine (1 ppm)	A	3	3.1	<2.5	<0.28	<0.0012
	B	3	3.1	<2.5	<0.28	<0.0012
HCl (5 ppm, ceiling)	A	9.5	2.9	40	9	0.042
	B	10	3.0	82	18	0.082
HCN (10 ppm)	A	14	2.8	99	32	0.14
	B	14	2.8	110	36	0.16
NO (25 ppm)	A	121	3.1	<1.3	<0.28	<0.0015
	B	120	3.1	<1.3	<0.28	<0.0015
NO$_2$ (5 ppm, ceiling)	A	121	3.1	1.6	0.27	0.0012
	B	120	3.1	<1.3	<0.22	<0.0010
CO (50 ppm)						3–4 ppm

Refer to Table 3 for explanation of "less-than" amounts.

between waste solution and bleach. The concentrations found in this test were very similar to those found with Reagent A and Reagent B. As in the case with the reagents, the projected concentrations of these gases did not exceed corresponding OSHA PEL's. The results are shown in Table 5.

Waste solution (40 mL) mixed with acid solution (10 mL)

We detected hydrogen cyanide, NO, and NO$_2$ in very small quantities. The projected concentrations were well below the relevant PELs. The results are shown in Table 6.

Waste solution (40 mL) mixed with base solution (10 mL)

Only NO$_2$ was released from the mixing of waste solution with base solution, while the other gases were not detected. The results are shown in Table 7.

The results from all tests indicated that the only reactions of potential concern from an employee exposure standpoint are those between GITC-containing solutions and bleach. The reaction of GITC-containing wastes with acid or base solutions produced minimal amounts of NO, NO$_2$, and HCN, and were therefore not con-

sidered to be harmful in this application. When we mixed GITC-containing reagents (Reagents A and B and waste solution) with bleach, however, both HCl and HCN were released in potentially excessive amounts. While the projected concentrations of these gases were well below their respective PELs for the small spill cleanup scenarios, larger spills utilizing larger amounts of bleach can produce higher concentrations, potentially above their PELs. Based on the chemical composition of GITC and bleach, the following equation is proposed as the possible

Table 5. Results of Waste Solution Mixed with Bleach

Test gas (OSHA PEL)	Run	Sampling time (min)	Sampling volume (L)	Amount (μg)	Concentration (ppm) Experimental	Projected
Chlorine (1 ppm)	A	3	3.1	<2.5	<0.28	<0.0012
	B	3	3.1	<2.5	<0.28	<0.0012
HCl (5 ppm, Ceiling)	A	9.5	2.9	58	13	0.060
	B	10	3.0	16	4	0.016
HCN (10 ppm)	A	14	2.8	86	28	0.13
	B	14	2.8	120	39	0.17
NO (25 ppm)	A	110	2.8	1.5	0.4	0.0020
	B	115	2.9	<1.3	<0.37	<0.0016
NO$_2$ (5 ppm, ceiling)	A	110	2.8	<1.3	<0.25	<0.0011
	B	115	2.9	<1.3	<0.24	<0.0011
CO (50 ppm)						2–4 ppm

Refer to Table 3 for explanation of "less-than" amounts.

Table 6. Results of Waste 2 Solution Mixed with Acid Solution

Test gas (OSHA PEL)	Run	Sampling time (min)	Sampling volume (L)	Amount (μg)	Concentration (ppm) Experimental	Projected
Chlorine (1 ppm)	A	3	3.1	<2.5	<0.28	<0.0012
	B	3	3.1	<2.5	<0.28	<0.0012
HCl (5 ppm, Ceiling)	A	10	3.0	<1	<0.22	<0.0010
	B	10		<1	<0.22	<0.0010
HCN (10 ppm)	A	12.5		<1	<0.36	<0.0016
	B	12.5		1.8	0.7	0.0029
NO (25 ppm)	A	127		2	0.5	0.0020
	B	110		2.6	0.7	0.0031
NO$_2$ (5 ppm, Ceiling)	A	127		<1.3	<0.19	<0.00086
	B	110		3.0	0.3	0.0014
CO (50 ppm)					<3 ppm	

Refer to Table 3 for explanation of "less-than" amounts.

Table 7. Results of Waste Solution Mixed with Base Solution

Test gas (OSHA PEL)	Run	Sampling time (min)	Sampling volume (L)	Amount (μg)	Concentration (ppm) Experimental	Projected
Chlorine (1 ppm)	A	3	3.1	<2.5	<0.28	<0.0012
	B	3	3.1	<2.5	<0.28	<0.0012
HCl (5 ppm, ceiling)	A	11.5	3.4	<1	<0.20	<0.00089
	B	10	3.0	<1	<0.22	<0.0010
HCN (10 ppm)	A	12.5	2.5	<1	<0.36	<0.0016
	B	12.5	2.5	<1	<0.36	<0.0016
NO (25 ppm)	A	112	3.2	<1	<0.25	<0.0011
	B	112	3.2	<1	<0.25	<0.0011
NO$_2$ (5 ppm, ceiling)	A	112	3.2	1.6	0.27	0.0012
	B	112	3.2	1.5	0.25	0.0011
CO (50 ppm)					<3 ppm	

Refer to Table 3 for explanation of "less-than" amounts.

reaction that took place.

$$SCN^+ + OCl^- + H_2O$$
$$\rightarrow \mathbf{HCN + HCl + S + O_2}$$

SCN$^+$ represents the thiocyanate ion formed by guanidine isothiocyanate in solution. OCl$^-$ represents the negative ion formed by sodium hypochlorite in solution. HCN and HCl are suggested products of the reaction. Both HCN, as a chemical asphyxiant, and HCl, as a caustic irritant, can be extremely hazardous to human health. While HCl has strong warning properties in the form of acute effects on the eyes, mucous membranes, and skin, even at concentrations as low as 5 ppm, HCN can cause rapid death without warning. It is therefore desirable to prevent the generation of these gases even at relatively low levels.

CONCLUSIONS

This study examined the reaction of GITC-containing reagents with bleach, a potential scenario during routine spill cleanup and waste decontamination procedures. The tests indicated that very little or non-detect-

This study examined the reaction of GITC-containing reagents with bleach, a potential scenario during routine spill cleanup and waste decontamination procedures.

able amounts of NO, NO_2, Cl_2, and CO were produced in these reactions. However, comparatively large amounts of gaseous HCN and HCl were released during these reactions. While the projected levels of HCN and HCl in the air were well below their respective regulatory limits (PELs) for the amounts that were tested, potentially excessive concentrations can be produced when larger amounts of reagent and bleach are combined. These gases are products of reaction between two very reactive components, thiocyanate ion from GITC and hypochlorite ion from bleach. It is therefore imperative upon manufacturers of these reagents and manufacturers of equipment that utilize such reagents that the end-users be notified of such hazards so that appropriate measures (e.g., local exhaust ventilation, respirators) can be taken to protect them.

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