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Testing the API CA-61, HM-34 and AJ-05 Poison Detectors

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TESTING SERVICES AGREEMENT

Signed Jonathan C. Allen
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Executive Summary.

The objectives of this validation effort were to test the detectors for poisons in food developed by Appealing Products Inc., API, according to the instructions provided and to determine if the detectors respond according to the specifications posted by API. Over sixty tests were conducted using six different foods with variable properties that were laced with nine different poisons. The poisons were selected from the groups of poisons that a terrorist might use to poison a dignitary or a VIP and included cyanides, arsenic compounds, azides, chromates, thallium, mercury, lead, cadmium and sulfides.

Each serving of food, as defined by the FDA, was laced by an amount of poison equal to 25 times the LD_{50} for that poison. This corresponds to half the amount that will affect in three days a small person that weighs only 50 Kg. Each laced food sample was diluted with clean water as per API's instructions and the aqueous solution was tested for the presence of the poison. Each of API's Poison Detection Kits, (The KT-05 and the KT-06) includes three detectors: the CA card detects the anionic poisons cyanides, azides, chromates and sulfides; the HM detector detects the heavy metals cations of thallium, cadmium, lead and mercury; and the AJ pouch is a mini-reactor that detects arsine. All arsenic compounds are reduced in the AJ pouch to produce arsine, which is subsequently detected using a card that changes its color in response to arsine.

The sample preparation and the execution of all the tests for poisons were very easy and the results were obtained very quickly, usually within 30 to 40 seconds. Detecting the poison took in one case only 5 seconds and in one case 480 seconds. Detecting that there was a poison in the sample conformed to the detection parameters stated by API.

Detecting arsenic compounds using the AJ pouch was very obvious even though the amount of arsenic added to the food was only 10 % of the amount calculated by the above procedure. In all the tests for anionic poisons using the CA card, there was no false positive or false negative and the particular poison was readily identifiable. When testing for heavy metals using the HM card, the lacing metals were detected in all but a single case. When testing for mercury in chicken from a can that contained also phosphate brine, the mercury was not detected. Mercury that was added before or after cooking fresh chicken, without brine, was readily detected. It appears that the phosphates used in the brine for preserving the chicken precipitated mercury phosphate and thus the mercury ions were not detected. It is well known that the toxicity of water-insoluble salts, such as mercury phosphate, is not high, because they are not readily assimilated physiologically and are often excreted from the body rapidly.

Colored foods did not significantly stain the control cards with no added poisons, except for a light brown stain from the cola and a pale yellow from orange juice. This did not interfere with the detection. When considering the reactions on the two lanes of the HM card, color difference did not normally prevent identification of the specific poison. Identifying the specific metallic ion poison was easy when the metal was thallium or cadmium, but it was more difficult to differentiate between mercury and lead.

Very viscous samples such as beef stew required dilution by a factor of three to achieve fast detection speed at the 0.5 D_{50} level. Under these conditions, all added poisons at the 0.5 D_{50} level of addition to the foods were still detectable by the cards.

Our overall opinion is that API's detectors for poison in food readily detect dangerous levels of poison yet they respond very rapidly and are easy to use even by a layman.

1. Objectives.

The main objective of the project was to perform INDEPENDENT VALIDATION tests on API's Poison Detectors using API's published procedure for food sample preparation.

2. Approach.

Appealing Products Inc. (API) has developed a set of cards and a pouch mini-reactor for detecting toxic chemicals that could be intentionally added to foods to cause acute toxicity when ingested (poisons). The detectors were designed to detect the poisons that a terrorist could have access to and which could be readily used for poisoning foods. The 30 or so most commonly available and used poisons were identified and detectors were developed for them. In API's analytical method, foods are divided into THREE CLASSES, based on the way they interact with water. Once a food is classified into a group, it is prepared in a way compatible with the food class. Prepared food samples can be analyzed using the same analytical procedure, regardless of the class it belonged to.

Our goal was to test foods from all three classes with different poisons that should be detected by each of the three types of detectors.

3. Methods.

3.1 Classes of Food

Foods are classified into three CLASSES:

A. Solids that DO NOT ABSORB WATER. I. Beef stew (Dinty Moore), and, II. Chicken breast (Bumble Bee) in cans were purchased from a local supermarket (Harris Teeter).
B. Solids and PASTES that ABSORB WATER. I. Yogurt (Activia), in a plastic container and, II. Apple sauce (store brand), in a glass jar were purchased from a local supermarket.
C. LIQUIDS. I Coca Cola, and, II. Orange juice (store brand). Bottles with these drinks were purchased from a local supermarket (Harris Teeter).

3.2 Food Adulterants for the study

The poisons used in the of the study are:

- **Group I**: 1. Potassium cyanide. 2. Sodium Azide. 3. Potassium Chromate, and, 4. Sodium Sulfide.
- **Group II:** 1. Cadmium chloride, 2. Lead acetate, 3. Mercuric nitrate, and, 4. Thallium sulfate.
- **Group III**: 1. Sodium arsinite.

The poisons were added to separate portions of each of the foods separately according to the dosage shown in Table 1. The amount of poison added to each food has been calculated so that if a person who weighs 50 kg (110 lbs), eats a serving of that food, as defined by the FDA, he would ingest:

$$\frac{1}{2}*LD_{50}*50 = \frac{1}{2}*D_{50} \text{ corresponding to the 50 kg bodyweight.}$$

When making artificially-laced foods, the amount of poison added was equivalent to ½*D_{50}, except for the arsenic compound where only 1/10 the D_{50} of arsenic compound was added.

				6. Food Class:	Class A	Class A	Class B	Class B	Class C	Class C
				7. Test Food:	Beef Stew	Chicken Breast	Yogurt	Apple Sauce	Coca-Cola	Orange Juice
				8. Serving Size (g):	200	200	60	30	100	100
1. Poison	**2. Formula**	**3. LD_{50} (g/kg)**	**4. D_{50} (g)**	**5. Testing Limit (g)**	**g Poison / 60 g Sample**	**g Poison / 60 g Sample**	**g Poison / 60 g Sample**	**g Poison / 60 g Sample**	**g Poison / 60 g Sample**	**g Poison / 60 g Sample**
Group I - Anionic Poisons				***@ 1/2 D_{50} (g)***						
Potassium Cyanide	KCN	0.005	0.25	0.125	0.038	0.038	0.125	0.250	0.075	0.075
Sodium Azide	NaN_3	0.027	1.35	0.675	0.203	0.203	0.675	1.350	0.405	0.405
Sodium Sulfide	Na_2S	0.208	10.40	5.2	1.560	1.560	5.200	10.400	3.120	3.120
Potassium Chromate	$KCrO_4$	0.180	9.00	4.5	1.350	1.350	4.500	9.000	2.700	2.700
Group II - Heavy Metals				***@ 1/2 D_{50} (g)***						
Mercury Nitrate	$Hg(NO_3)_2$	0.026	1.30	0.65	0.195	0.195	0.650	1.300	0.390	0.390
Thallium Sulfate	Tl_2SO_4	0.016	0.80	0.4	0.120	0.120	0.400	0.800	0.240	0.240
Lead Acetate	$Pb(C_2H_3O_2)_2$	0.200	10.00	5	1.500	1.500	5.000	10.000	3.000	3.000
Cadmium Chloride	$CdCl_2$	0.088	4.40	2.2	0.660	0.660	2.200	4.400	1.320	1.320
Group III - Arsenic Compounds				***@ 1/10 D_{50} (g)***						
Sodium Arsenite	$NaAsO_2$	0.041	2.05	0.205	0.062	0.062	0.205	0.410	0.123	0.123

Table 1. Dosage of various adulterants added to foods.

3.2 Food Preparation.

In each test, approximately 60 ml or about 60 grams of each food was diluted with water before analysis. The solid samples were minced before mixing with clean ordinary water.

3.4 Dilution Ratio.

Samples of Class A were mixed with an equal volume of water. Samples of Class B were mixed with TWICE their volume of water. The foods and water were thoroughly mixed, and the solids were pressed to the bottom of the containers so that an aqueous fraction, taken below any fat layers that formed, was taken from the mixture and applied to the opening on each of the detector cards. As per API's instructions, adding more water when the sample was too viscous was used to reduce the analysis time by reducing the viscosity of the prepared food sample. This approach was used in some of the Class B foods where up to an additional amount of water equal in volume to the food sample was added and mixed.

4. Sample Analysis.

Three detection devices were used: The CA card for detecting toxic anions, the HM card for detecting toxic cations and the AJ pouch for detecting ALL arsenic compounds. The detectors were used according to the instructions supplied with the kits.

The aqueous food extracts were added to the openings on the detector cards with a dropper. The solution moves up the space containing the reactants by capillary action. As soon as a color reaction occurred indicating the presence of a reactive chemical in the food, an electronic image of the card was made with an optical scanner. The images were read by two investigators to determine the presence of poison and, if possible, label the probable poison based on the color reactions on the indicator card.

5. Results:

The cards were able to detect the poisons as indicated in the tables below. There were no false positive results with the control food sample that had not been laced with any chemical poison. Canned chicken prepared with a sodium phosphate broth laced with mercury nitrate did not react positively with the HM-34 detector card, presumably due to precipitation of poorly soluble mercury phosphate ($Hg_3(PO_4)_2$). Fresh cooked chicken without phosphate was then laced with mercury nitrate. A suspension made from this material produced a positive result on the HM card. Photos of the test results with fresh chicken and with chicken containing phosphate are also enclosed for comparison.

All alderated foods were tested for reactive arsenic with the AJ pouch system. The only indicator cards that showed a positive reaction were those exposed to the foods that were adulterated with sodium arsenite. All of the sodium arsenite-laced foods produced a brown discoloration on the indicator cards.

Food class A (solid food that do not absorb water).
Beef stew:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	Color*	Poison **	CA Card	HM Card	
1.(cyanide)	Blue[2]	CN		N	129s		
2.(azide)	Light Brown	Azide		N	133s		
3.(sulfide)	Black[1],	Sulfide		N	40.1s		
4.(chromate)	Yellow[1,2], Green[2]	Chro-mate		N	303s		
5.(mercury)		N	Orange[1], light gray[2]	Hg		129s	
6.(thallium)		N	Gray[2]	Tl		133s	
7.(lead)		N	Orange[1], Dark gray[2]	Pb		40.1s	
8.(cadmium)		N	Orange[1], Yellow[2]	Cd		303s	

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
** "N" means card shows no indication of poison.

Canned Chicken:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	HM Card	Poison**	CA Card	HM Card	
1.(cyanide)	Blue[2]	CN		N	21.2s		
2.(azide)	Brown[2]	Azide		N	17.8s		
3.(sulfide)	Black[1], Brown[2]	Sulfide		N	12.6s		
4.(chromate)	Yellow[1,2], Green[2]	Chro-mate		N	46.4s		
5.(mercury)		N		N			
6.(thallium)		N	Gray[2]	Tl		8.6s	
7.(lead)		N	Gray-brown[2]	Pb		15s	
8.(cadmium)		N	Orange[1], Yellow[2]	Cd			

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
**"N" means card shows no indication of poison

Food class B (solid foods that do absorb water).

Yogurt:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	HM Card	Poison **	CA Card	HM Card	
1.(cyanide)	Blue[2]	CN		N	47.5s		
2.(azide)	Brown	Azide		N	124s		
3.(sulfide)	Black[1], Brown[2]	Sulfide		N	33.4s		
4.(chromate)	Yellow[1,2], Green[2]	Chro-mate		N	480s		
5.(mercury)			Orange[1], Gray[2]	Y		15s	
6.(thallium)			Yellow[1], Gray[2]	Y		45s	
7.(lead)			Red[1], Gray[2]	Y		27s	
8.(cadmium)		N	Orange[1], Yellow[2]	Cd			

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
**“Y” means sample does have poison, but cannot document which poison. “N” means card shows no indication of poison

Apple Sauce:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	HM Card	Poison **	CA Card	HM Card	
1.(cyanide)	Blue[2]	CN		N	6.4s		
2.(azide)	Brown	Azide		N	19.6s		
3.(sulfide)	Black[1], Green[2]	Sulfide		N	8.7s		
4.(chromate)	Yellow[1,2], Brown-Green[2]	Chro-mate		N	20.2s		
5.(mercury)		N	Orange[1], gray[2]	Hg		24.5s	
6.(thallium)		N	Yellow[1], Gray[2]	Tl		6.1s	
7.(lead)		N	Red[1], Dark gray[2]	Pb		5.6s	
8.(cadmium)		N	Red[1], Yellow[2]	Cd			

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
**“N” means card shows no indication of poison.

Food class C (liquid food)

Coca cola:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	HM Card	Poison **	CA Card	HM Card	
1.(cyanide)	Light Blue[2]	CN		N	15.2s		All samples have some brown color (cola color)
2.(azide)	Light Brown	Azide		N	15.9s		
3.(sulfide)	Black[1], Green[2]	Sul-fide		N	14.1s		
4.(chromate)	Yellow[1,2], Brown-Green[2]	Chro-mate		N	18s		
5.(mercury)		N	Red[1], gray[2]	Hg		22.7s	
6.(thallium)		N	Yellow[1], Gray[2]	Tl		14.6s	
7.(lead)		N	Red[1], gray[2]	Pb		7.9s	
8.(cadmium)		N	Red[1], Yellow[2]	Cd			

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
**"N" means card shows no indication of poison.

Orange Juice:

	CA		HM		Time of Reaction (sec)		note
	Color*	Poison **	HM Card	Poison **	CA Card	HM Card	
1.(cyanide)	Light Blue[2]	CN		N	22s		
2.(azide)	Light Brown	Azide		N	19.1s		
3.(sulfide)	Black[1], Green[2]	Sul-fide		N	6.8s		Pale yellow in HM from Orange juice
4.(chromate)	Yellow[1,2], Brown-Green[2]	Chro-mate		N	14.3s		
5.(mercury)		N	Orange[1], Pale gray[2]	Hg		16.3s	
6.(thallium)		N	Gray[2]	Tl		4.9s	
7.(lead)		N	Brown[1], Green[2]	Pb		6.3s	
8.(cadmium)		N	Orange[1], Yellow[2]	Cd			

*Number following color indicates Sample lane 1 (Left) or lane 2 (Center)
**"N" means card shows no indication of poison.

6. Discussion:

Testing all the detectors with all the foods was easy to perform and the results were obtained in a short period of time. Detecting that there was a poison in the sample conformed with the stated detection parameters of Appealing Products, Inc. with a single exception: chicken breast which came from a can that contained phosphates as a brining solution did not show a positive reaction for mercury, although mercury that was added to fresh chicken was detected easily.

Detecting arsenic compounds using the AJ pouch was very consistent and no false positive or false negative results were observed during these tests. The arsenic tests were done using one-tenth of the D50 level. Since the detection was clear and successful at these levels, no additional tests at higher concentrations were performed. The AJ pouch is a successful and accurate detector to test for arsenic.

Very viscous samples such as beef stew required dilution by a factor of three to achieve fast detection speed at the 0.5 D50 level. Under these conditions, all added poisons at the 0.5 D50 level of addition to the foods were still detectable by the cards.

In most cases, the detector cards allowed the operator not only to detect that the food was poisoned but also to determine the specific poison present based on the color of reaction product. One case that was difficult to ascertain, before the investigators became practiced reading the chemical detectors to identify the specific metals, involved distinguishing the shades of gray on the HM card in response to thallium, mercury and lead. The cards often showed a black, grey, or brown color that was not the exact color printed on the card for the specific poison. Some color differences may have resulted from the different physical and chemical properties of different foods. However, the food colors did not significantly stain the control cards with no added poisons, except for a light brown stain from the cola and a pale yellow from orange juice. When considering the reactions on the two lanes of the HM card, color difference did not normally prevent identification of the specific poison.

Mercury that was added to canned chicken that contained brine was not detected by the HM Card. An identical quantity of mercury, added to fresh chicken which was cooked was detected readily. Adding nitric acid dropwise to the canned chicken did not enhance the detectability of mercury. The ingredient list on the canned chicken breast included the additive sodium "phosphate". We consider the precipitation of mercury phosphate to be the major interference with the detection of the toxic mercury in canned chicken breast, although we need more experiments to further define the impact of phosphate on mercury detection and toxicity.

The analysis time was in general in the range of 30-40 seconds but as short as 5 seconds. The analysis of two of the samples took a bit longer time, 303 seconds for cadmium in beef stew and 480 seconds for chromate in yogurt.