



Abstract

The recent rise in popularity and diversity of modern oral products has brought greater attention to this category from consumers and regulators alike. The U.S. Food and Drug Administration (FDA) has published an abbreviated list of 9 harmful and potentially harmful constituents (HPHCs) for smokeless product reporting. We analyzed select brands in this market for these 9 HPHCs and other compounds of interest. In order to ensure method robustness, products were chosen based on market volume and major variables, such as nicotine content, ingredients (flavors and sugars), and base matrices for the product category. Samples were analyzed for nicotine, minor alkaloids, nicotine degradants, tobacco-specific nitrosamines (TSNAs), benzo[a]pyrene (B[a]p), carbonyls, trace metals, aflatoxins, water activity, and water. Results show that aflatoxin levels (B1, B2, G1, G2) and ochratoxin were below quantitation limits (4.00 ng/pouch), consistent with the low water activity levels. Surprisingly, Karl Fisher titrations revealed that the water levels varied more than expected. Also, there were challenging matrix affects to overcome. As expected, HPHC levels were substantially lower in modern oral products compared to a reference tobacco pouch product and traditional reference cigarettes.

Samples

Sample	Type	Description
Pouch A	Tobacco-free nicotine pouch	Free nicotine; mint flavor
Pouch B	Tobacco-free nicotine pouch	Nicotine salt; unflavored flavor
Pouch C	Tobacco-free nicotine pouch	Free nicotine; Spearmint flavor
Pouch D	Tobacco-free nicotine pouch	Nicotine salt; Unflavored flavor
CRP 1.1	CORESTA reference pouch	Free nicotine; snus smokeless tobacco
1R6F filler	Tobacco filler from 1R6F cigarette	Free nicotine; tobacco

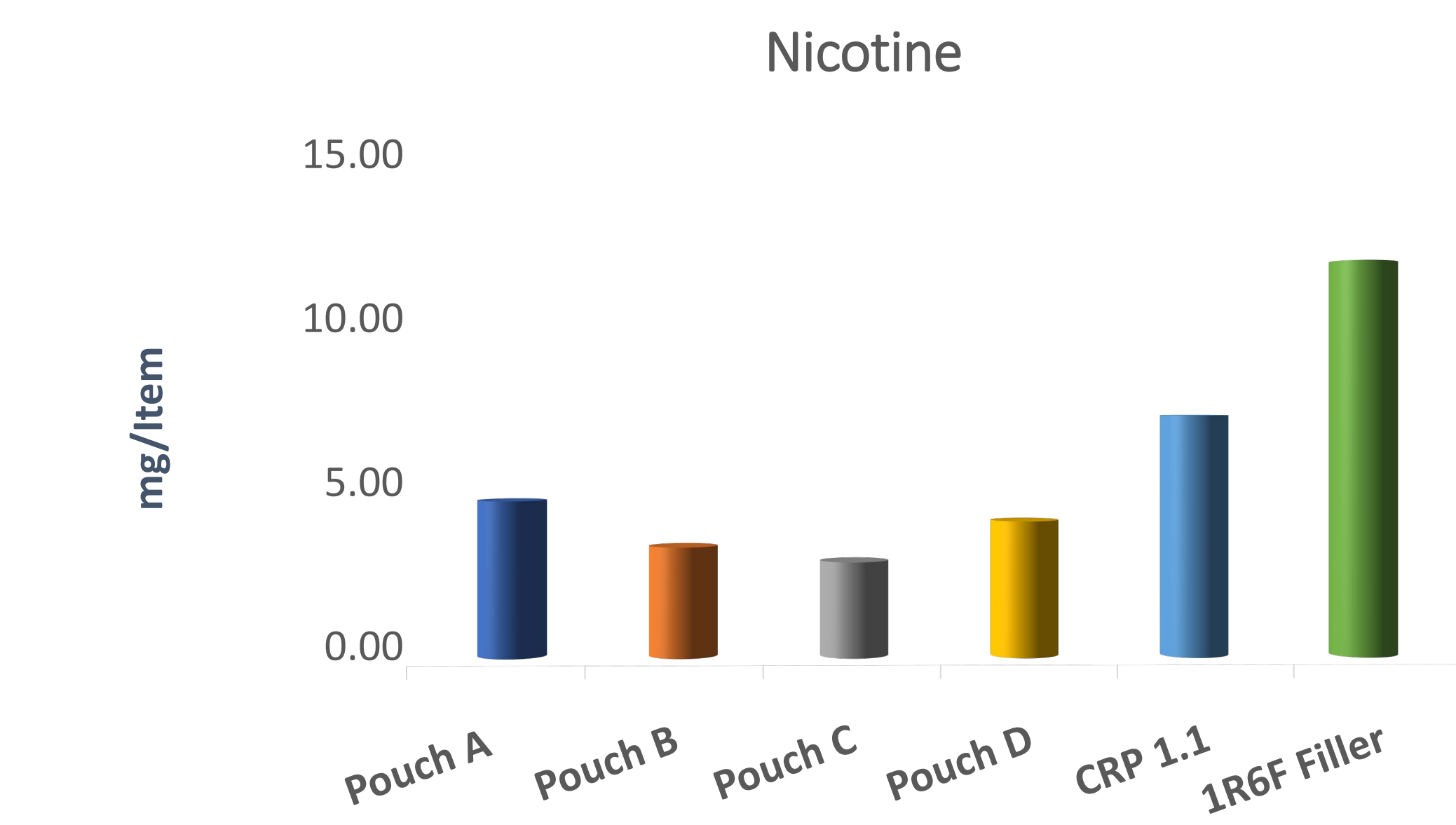
❖ Tobacco-free pouches were selected based on popular brands, availability, and nicotine type

Methods

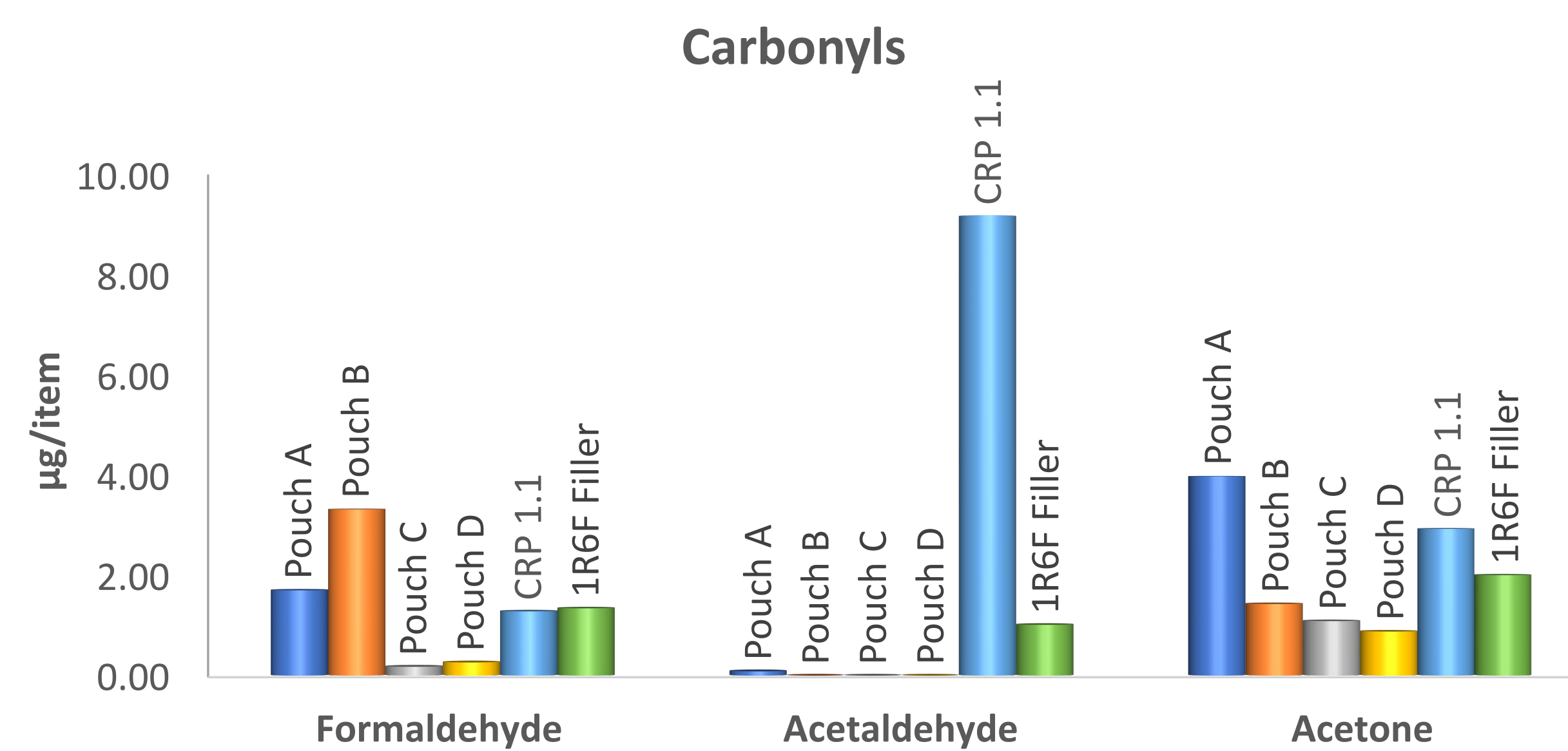
Sample preparation: Cut tobacco blend (1R6F filler) was weighed and extracted in the appropriate solvent. Whole pouches were cut in half (tobacco-free nicotine pouches & CRP 1.1) and the filler and pouch materials were extracted in the appropriate solvent. Sample extracts were diluted according to the internal test methods. The instruments used for the analysis for the different compound classes are listed below:

- GC-MS: Nicotine Degradants, Carbonyls
- LC-MSMS: Nicotine, TSNAs, Aflatoxins
- LC-FLD: B[a]P
- ICP-MS: Trace Metals
- Karl Fisher: Water

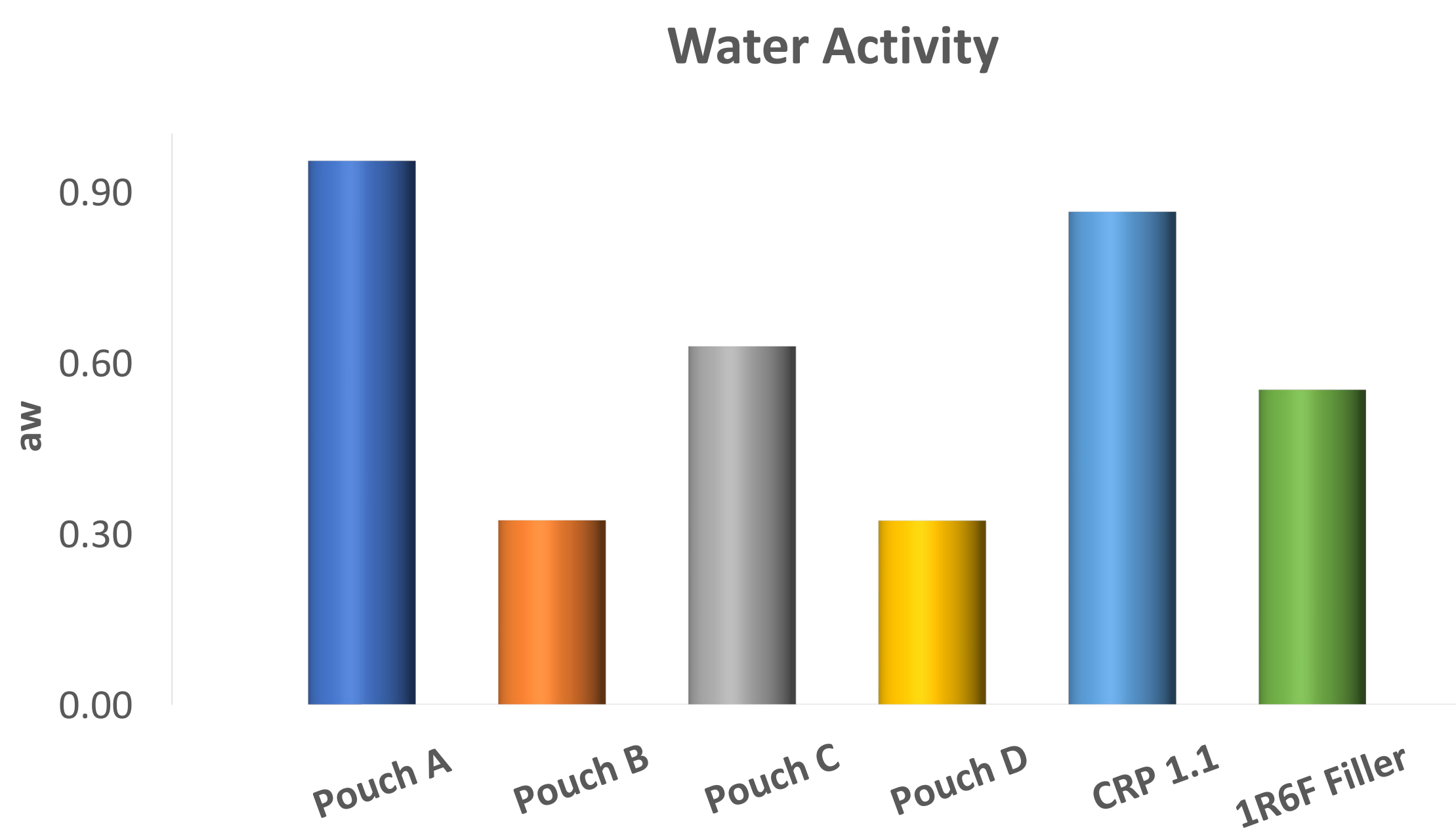
Results and Discussion



- ❖ Nicotine levels determined by internal methods are consistent with product labeling
- ❖ The tobacco-free pouches contain less nicotine than snus (CRP 1.1) and 1R6F filler

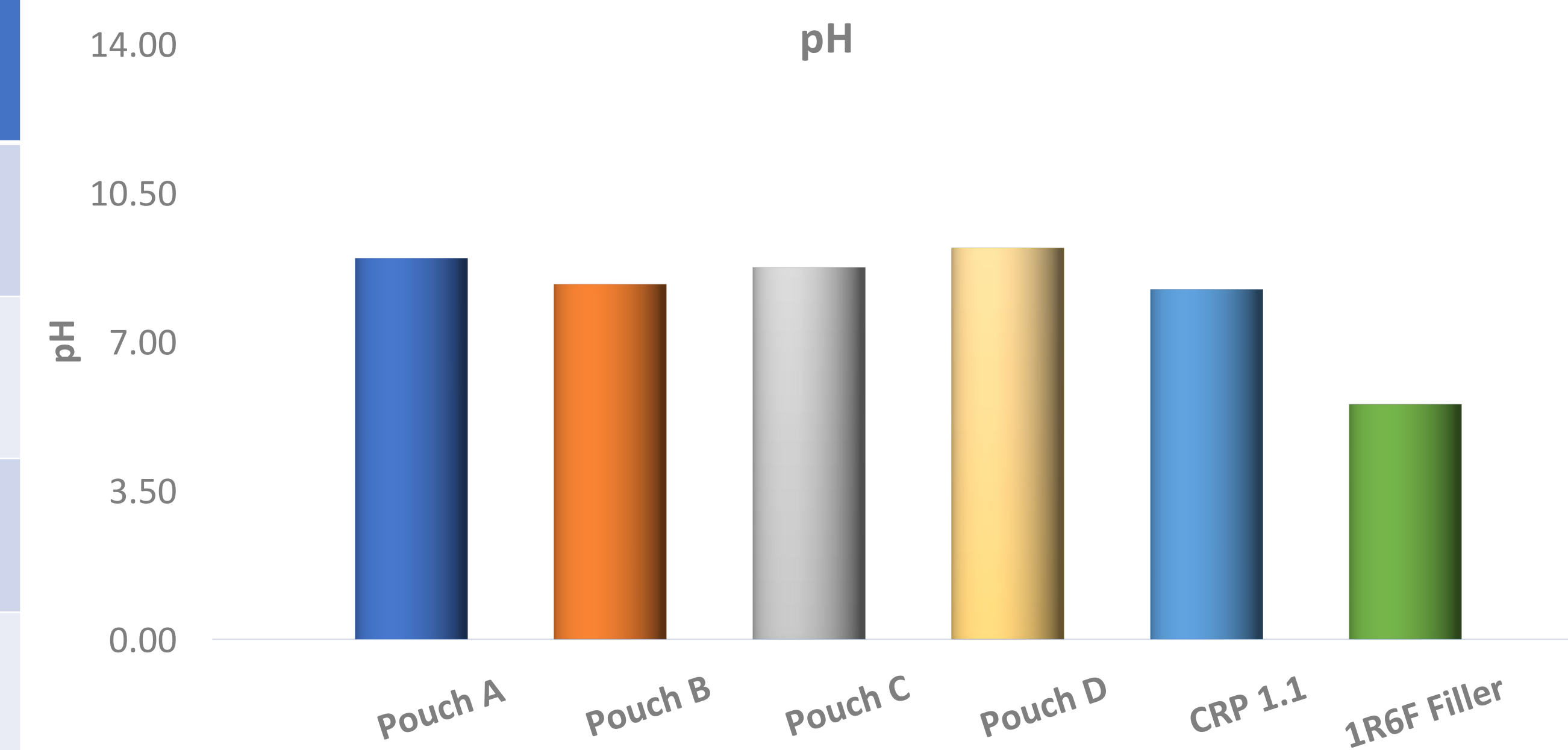


- ❖ Formaldehyde levels were higher in pouches A, and B compared to CRP 1.1 and 1R6F
- ❖ Pouch A had higher levels of acetone than CRP 1.1 and 1R6F

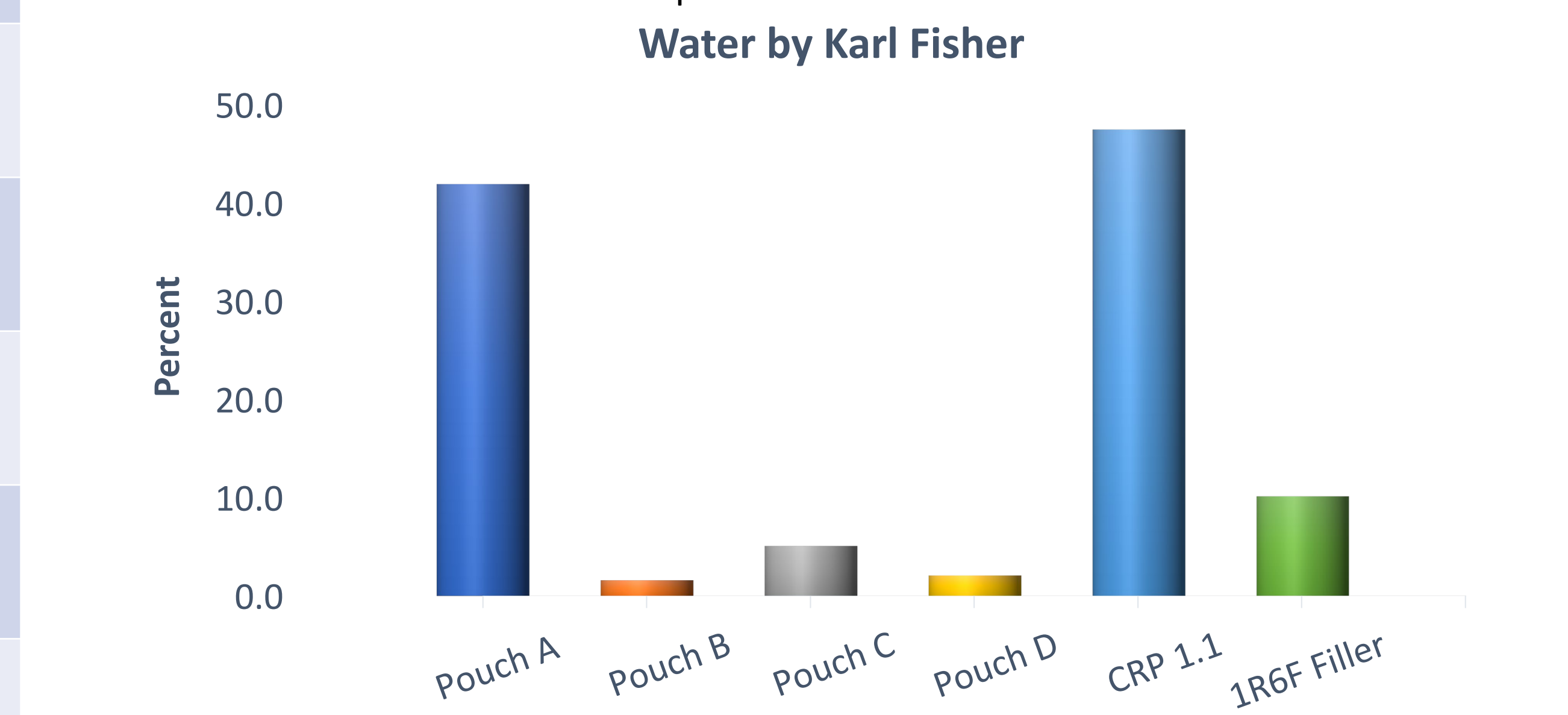


- ❖ All products had water activity of less than 0.95

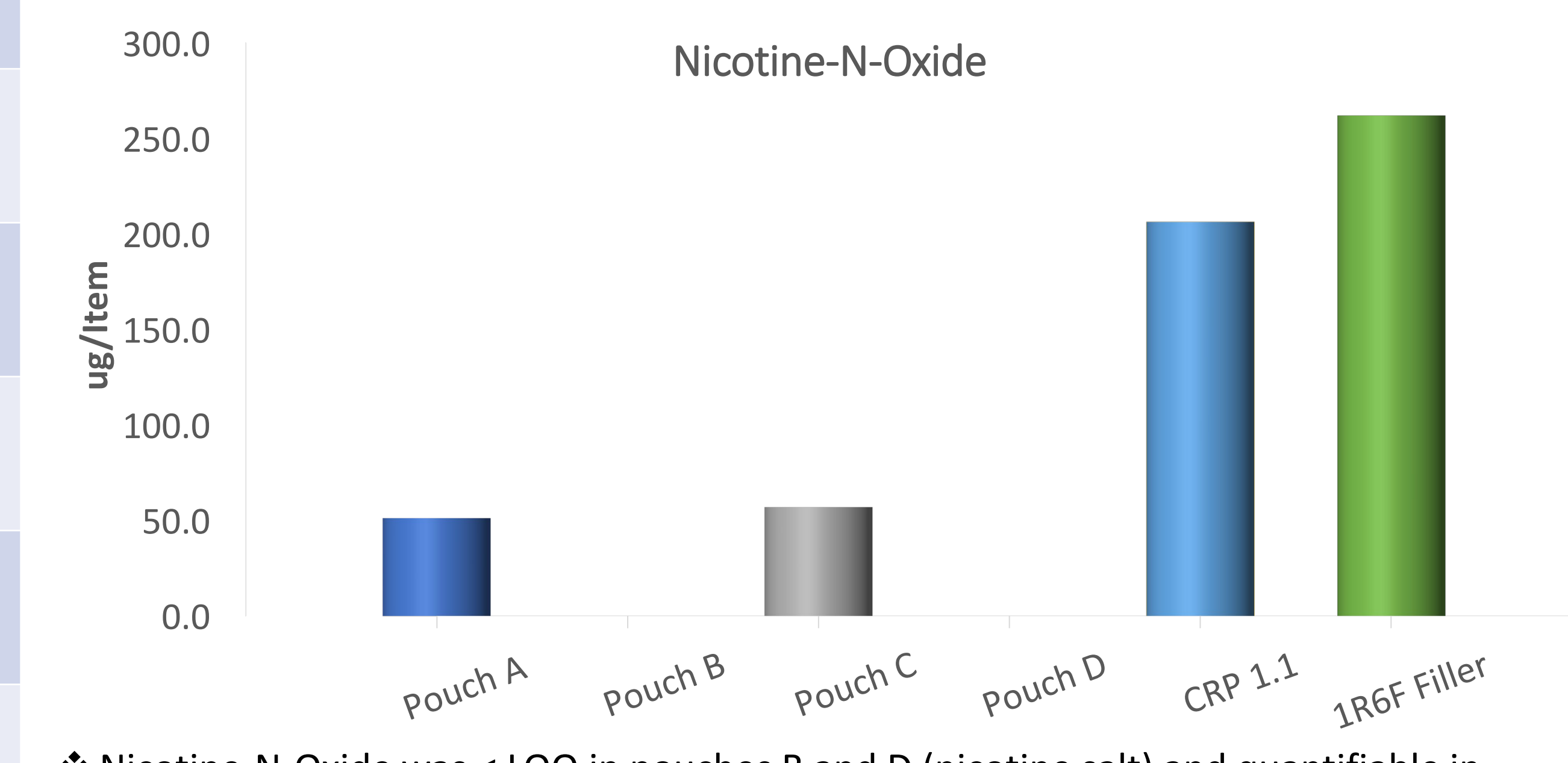
Analytes	Pouch A	Pouch B	Pouch C	Pouch D	CRP (Snus)	1R6F Filler
Nicotine (mg/item)	4.55	3.22	2.78	3.96	7.03	11.5
Formaldehyde (µg/item)	1.71	3.34	0.180	0.266	1.29	1.35
Acetaldehyde (µg/item)	0.09	< LOQ	< LOQ	< LOQ	9.22	1.02
Acrolein (µg/item)	< LOQ	< LOQ	< LOQ	0.08	< LOQ	< LOQ
Crotonaldehyde (µg/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
B[a]P (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	0.456	2.97
Arsenic (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	36.3	69.6
Cadmium (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	170	239
NNN (µg/item)	< LOQ	< LOQ	< LOQ	< LOQ	0.198	1.44
NNK (µg/item)	< LOQ	< LOQ	< LOQ	< LOQ	0.0485	0.438
Aflatoxin B1 (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Aflatoxin B2 (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Aflatoxin G1 (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Aflatoxin G2 (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ochratoxin (ng/item)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Yeast (CFU/g)	<10	<10	<10	<10	<10	-
Mold (CFU/g)	<10	<10	<10	<10	200	-



- ❖ pH was similar for tobacco-free nicotine pouches and the CRP 1.1 (snus style pouch)
- ❖ 1R6F filler is more acidic than the pouches



- ❖ Water was highest in Pouch A and CRP 1.1 (snus)



- ❖ Nicotine-N-Oxide was < LOQ in pouches B and D (nicotine salt) and quantifiable in Pouches A and C, as well as CRP 1.1 and 1R6F filler (products containing free nicotine)
- ❖ Other nicotine degradants including anabasine, anatabine, beta-nicotryne, cotinine, myosmine, and nornicotine were found in 1R6F and CRP1.1, but not Pouches A - D

Summary & Future Work

Tobacco-free nicotine pouches (A – D) were compared to traditional tobacco reference products (CRP 1.1 and 1R6F filler). The nicotine levels are lower in the nicotine pouches than in CRP 1.1 and 1R6F filler. Carbonyls show that Pouch A had higher levels of formaldehyde and acetone compared to CRP 1.1 and 1R6F filler. Pouch B had the highest levels of formaldehyde for all the products tested but is still relatively low (at 3.34 µg) compared to other tobacco-related products. Pouch D was the only product with quantifiable levels of acrolein, and the nicotine pouches had less acetaldehyde than the reference materials tested. As expected, B[a]P, TSNAs (NNN and NNK) and metals (arsenic and cadmium) were not quantifiable in the nicotine pouches but were in the reference materials tested. Aflatoxins (B1, B2, G1, G2) and ochratoxin were < LOQ for all samples. Interestingly, nicotine-N-oxide was the only nicotine degradant observed above LOQ in products containing free nicotine [CRP 1.1, 1R6F filler, two pouches (A and C)]. Ongoing work includes comparing nicotine pouches to other tobacco-free modern oral products such as gum, lozenges, pastes, strips, and others.

