Are we focusing on the right toxicants for assessing the toxicological properties of smokeless tobacco products?

John H. Lauterbach, PhD, DABT

Lauterbach & Associates, LLC

Macon, GA 31210-4708

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Background – 1

- There are numerous types of smokeless tobacco products (STP) in use worldwide
- They differ in composition, manufacturing method, additives, and contaminant levels
- They also differ in route of ingestion and typical amounts ingested per day
- For the purposes of this presentation, we will consider ***only*** contemporary STP made by manufacturers in USA and EU

Background – 2

- There is major public health debate on whether such contemporary STP are a safe alternative to smoking for those unable or unwilling to stop tobacco use
- Some points of contention
  - NRT readily available, so why need STP
  - Toxicant and toxin levels in contemporary STP
  - Little governmental oversight of STP composition and marketing
  - Concurrent use of other tobacco products

Background – 3

- Toxicant of most concern in STP is the carcinogen, NNK
  - NNK levels in most all STP higher than those in NRT (Stepanov *et al*., 2006)
  - Daily dosage from STP can be higher than from cigarettes (Hecht *et al*., 2007)
  - Main target organs believed to be oral cavity, esophagus, pancreas (Boffetta *et al*., 2008)
- Other toxicants of concern include B[a]P, NNN, VNA, metals, unidentified mutagens, agrochemicals, as well as bacterial toxins

Background – 4

- Some health experts say STP meeting GothiaTek® standard is okay to use

Component	Limit	Content 2007	Component	Limit	Content 2007
Nitrite (mg/kg)	3.5	0.9 (<0.5 - 1.7)	Cadmium (mg/kg)	0.5	0.3 (0.2 - 0.4)
TSNA (mg/kg)	5	0.7 (0.5 - 1.0)	Lead (mg/kg)	1	0.1 (0.08 - 0.2)
NDMA (µg/kg)	5	0.6 (<0.5 - 0.8)	Arsenic (mg/kg)	0.25	0.05 (<0.03 - 0.11)
BaP (µg/kg)	10	0.8 (<0.5 - 1.5)	Nickel (mg/kg)	2.25	0.8 (0.3 - 1.2)
Pesticides	According to the Swedish Match pesticide policy		Chromium (mg/kg)	1.5	0.5 (0.3 - 1.2)

Source: http://www.gothiatek.com/templates/start.aspx?page_id=84 accessed July 31, 2008

But, one issue is TSNA intake

- If usage is one can (~34 g)/day, total TSNA intake could be ~170 µg/day
- For typical moist snuff, intake could be:
  - NNN ~ 95 µg/day – NAT ~ 50 µg/day
  - NNK ~ 20 µg/day – NAB ~ 5 µg/day
- NNK intake is ~10^{15} molecules/kg-body-wt/day at one can/day; NNK threshold dosage in male F344 rats is ~10^{17} molecules/kg-body-wt/day; intake may be >10^{18} molecules/kg-body-wt/day for toombak users (Lauterbach, 2006)

What if NNK, NNN were BDL?

Component	Limit	Component	Limit
Nitrite (mg/kg)	3.5	Cadmium (mg/kg)	0.5
NAT & NAB (mg/kg)	5	Lead (mg/kg)	1
NNN (µg/kg)	< 0.01	Arsenic (mg/kg)	0.25
NNK (µg/kg)	< 0.01	Nickel (mg/kg)	2.25
NDMA (µg/kg)	5	Chromium (mg/kg)	1.5
BaP (µg/kg)	10	Pesticides	According to the Swedish Match pesticide policy

- If TSNA content is below level of concern, what else do we need to worry about that is not a worry with NRT?

Hypothetical pouched moist snuff recipe

Ingredient	Function	QNE (%)
Water		50.0
Tobacco		37.0
Nontobacco material	Pouch material, FDA approved for tea-bags	5.0
Propylene glycol, FCC	Humectant	4.0
Sodium chloride, FCC	Taste enhancer	2.0
Sodium carbonate, FCC	Acidity requlator	1.0
Ethanol (SDA4)	Processing aid/solvent	0.50
Peppermint oil (FEMA, FCC)	Flavor	0.48
Spearmint oil (FEMA/GRAS)	Flavor	0.02
Total		100.0

- Safe if ultra-low TSNA and meets GothiaTek?

Maybe or maybe not

- Numerous reports of non-neoplastic oral disease and use of contemporary STP
  - Recently reviewed by Kallischnigg *et al.*, 2008
  - Snuff-induced lesions (SIL) associated with snus and moist snuff use, mostly reversible
    - Increase with nicotine content and pH
    - Decrease if switched from loose to pouched product
- Causes of SIL are still not clear
  - Unmeasured inorganics (Pappas *et al.*, 2008)
  - Polyphenols, others (Kew *et al*., 1998, 1999)
  - Microbial (Rubinstein and Pedersen, 2002)

Polyphenols/nitrate in STP not new

TABLE 2.—*Chemical analytical data for leading moist and dry snuff brands in the United States—1985–86: Other tobacco components*[a]

Snuff factor	Moist snuff[b]					Dry snuff		
	A	B	C	D	E	F	G	H
Moisture, %	50	51	45	49	20	4.7	5.6	5.4
pH[c]	7.8	7.8	7.9	8.2	6.0	5.9	6.1	6.0
Nitrate, %	3.2	2.7	3.1	2.5	0.7	1.5	3.9	4.7
Alkaloids								
Nicotine, mg/g	20.7	30.7	25.1	14.6	5.7	12.5	15.6	12.4
Nornicotine, mg/g	1.8	1.7	1.1	0.8	0.13	0.01	0.05	0.04
Anabasine, μg/g	20	20	20	20	5	2.4	7.1	6.8
Anatabine, μg/g	350	530	640	150	260	54	124	180
Polyphenols								
Chlorogenic acid, %	ND	ND	ND	ND	0.98	1.21	2.22	2.28
Scopoletin, %	0.21	0.21	0.21	0.16	0.25	1.66	0.43	0.61
Rutin, %	0.98	0.98	0.30	1.29	2.59	0.79	2.90	3.91
Kaempferol, %	1.02	1.25	0.17	0.58	1.85	2.02	4.20	4.54
Volatile carbonyls								
Acrolein, μg/g	0.5	0.6	0.3	0.4	0.3	0.3	0.07	0.1
Acetone, μg/g	11	6.1	11	20	9.4	16	19	8.5
Inorganics								
Lead, μg/g	1.93	2.53	2.96	1.37	0.86	0.34	0.27	0.34
Cadmium, μg/g	1.44	1.36	1.23	1.58	0.45	1.06	0.89	0.73
Selenium, μg/g	0.06	0.06	0.09	0.12	0.11	0.09	0.12	0.10

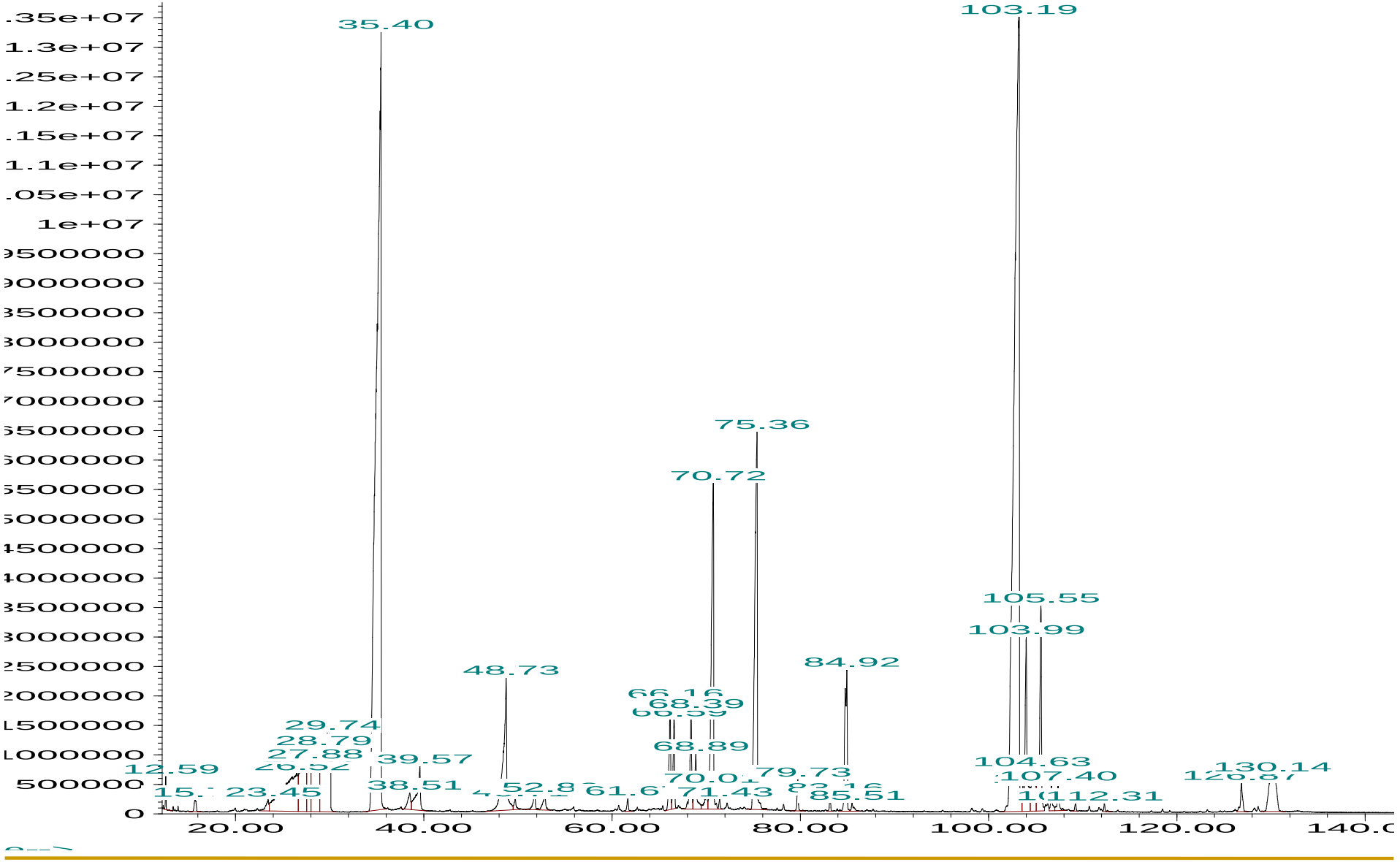
[a] All data are based on dry weight.
[b] ND = not detected, <0.02%.
[c] pH of a suspension of 1.0 g snuff in 10 ml distilled water.

Source: Hoffmann *et al.*, JNCI, VOL. 79, NO. 6, DECEMBER 1987

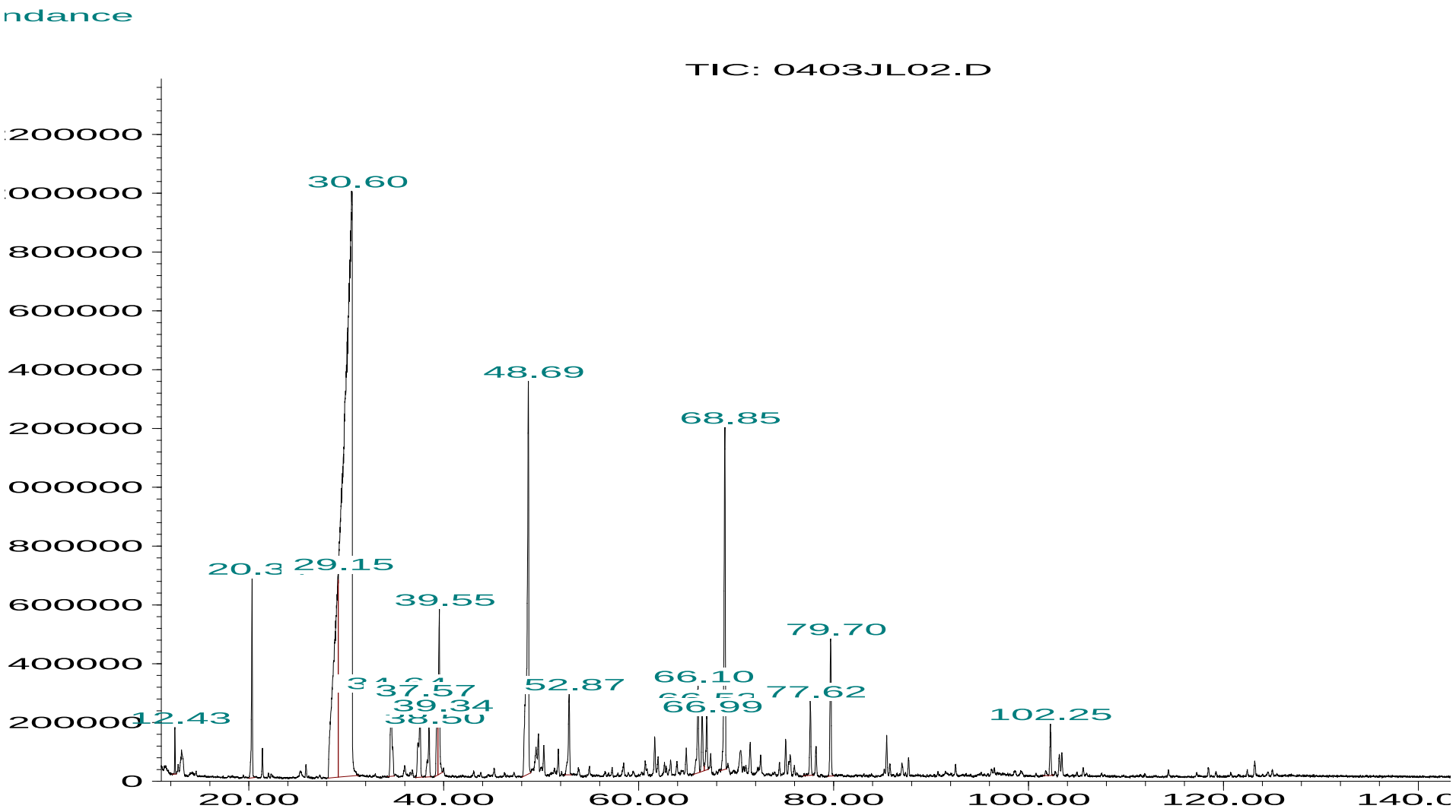
Are toxicological implications new?

- Most contemporary STP has high nitrate levels as made from air-cured tobaccos
- *In vitro* study showed *NO in PBS or saliva extract moist snuff (Lam *et al*., 2003)
- *In vivo* study with female S-D rats showed 1S3 PBS extract raised urinary MDA and liver DNA damage (Bagchi *et al*., 1994)
- *In vitro* study with DMSO/HC moist snuff extract and POII cells gave 3-NT, $ONOO^-$ injury, DNA damage (Barley *et al.*, 2004)
- Are there reactions with compounds in STP?

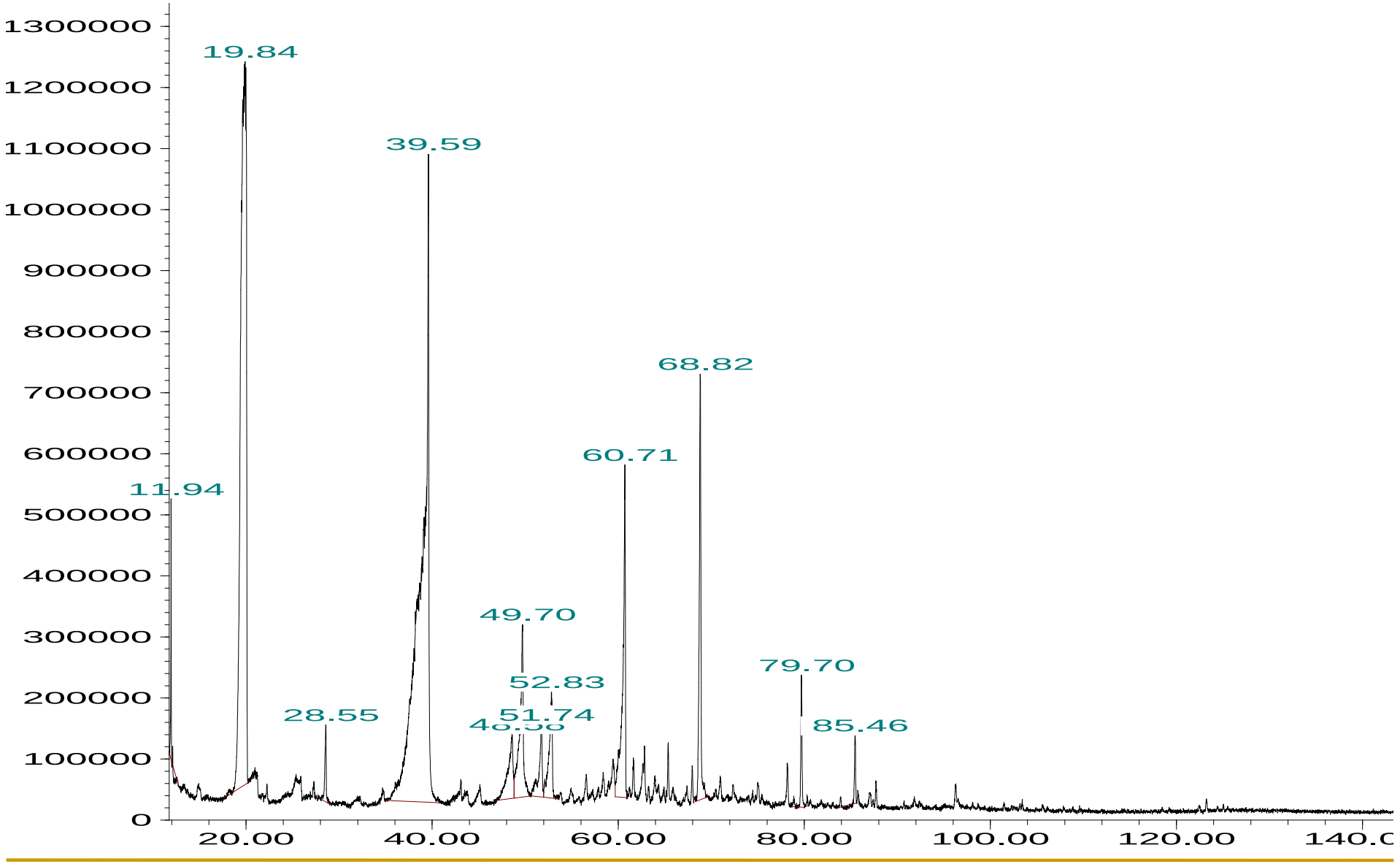
TMS derivatives 2S1 chewing tobacco



TMS derivatives 1S2 dry snuff



TMS derivatives 2S3 moist snuff



Conclusions

- There is more to STP toxicology than TSNAs and heavy metals
- Nitrate in STP and species derived from nitrate may be toxic agents
- Microbial contamination, other inorganics, and polyphenols may also impact toxicity
- Much work still needs to be done to identify the compounds in STP that contribute to bioactivity