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Summary: The probable reason for some of the differences in the levels of perfluorooctanoic acid (PFOA) in blood from 2000 to 2001 is the use of a new and improved analytical method for the 2001 samples.

Dr. Daryl Cobranchi, DuPont CR&D CCAS, and I visited NMS to determine why some blood PFOA levels from 2000 and 2001 did not agree, although the same laboratory ran them. The samples were taken from individuals at the DuPont Washington Works facility in West Virginia. The data below show that the results from 2000 for some of the same individuals are generally significantly lower than the results from 2000.

Job Description	2001		2000	
Granular Polykettle Operator	3.7	776395	8.5	495972
	3.7	776394	9.0	495964
	0.76	776413	1.7	495935
	2.9	776419	6.2	495955
	1.4	776388		
	0.55	776412		
	0.91	776415	0.80	495917
Fine Powder/Dispersion Autoclave Operator	2.9	776408	4.7	495925
	1.2	776406		
	1.9	776405		
	2.6	776397		
	1.9	776398	4.3	495946
	0.79	776399		
	1.9	776407	4.9	495916
	1.1	776411	2.4	495947
	0.49	776409		
Other DuPont Employees	1.1	776403	2.8	495941
	0.22	776396		
	0.39	776410	0.33	495906
	0.14	776401	0.22	495950
	0.33	776404		
	0.07	776414		
	0.45	776400		
	0.76	776413		
	0.60	776423		
	0.12	776416		
	0.23	776417		
	1.5	776421		
	1.3	776420		

	1.6	776422
ONYX Employees	2.2	776392
(* = PSA)	1.3	776393
	0.48	776386
	2.5	776391
	0.66	776387
	0.26	776389
	0.71	776385
	1.2*	776402

The most probable reason for the difference in the reported levels in the two sample sets is that NMS introduced a new method of sample preparation for our 2001 samples. The two methods (old and new) are described below.

Old Method
Perfluorooctanoic Acid (Freeze-drying)
Method#3425
Effective date: 12/92

New Method
Perfluorooctanoic Acid (w/out Freeze drying)
Method#3425
Effective date: November 1, 2001

The method was changed to reduce the interferences from the acetyl chloride peak impurity from the kit used in the old analysis. The interfering peak came immediately before the PFOA (methyl ester) peak, sometimes making the peak integration so difficult that the analyst had to manually integrate the PFOA peak. Because of the interference, the laboratory often could not reproducibly detect PFOA at the 10 ppb (10 ng/mL) reporting limit. In the new method the analyst makes up methylation reagent from high-performance liquid chromatographic grade methanol and concentrated (37%) hydrochloric acid. The data show that there is no interfering peak. The new method does a better job homogenizing the blood so that one can more easily extract a representative sample for gas chromatographic analysis with an electron-capture detector. The new method also uses less extraction solvent, improving the dilution factor fourfold.

A comparison of the two methods is given below:

New Method	Old Method
whole blood	whole blood
GC/ECD	GC/ECD
PFDA* internal standard	PFDA internal standard
5890 GC	6890 GC
HPLC-grade methanol/37% HCl	Alltech instant methanolic kit
0.5 mL hexane	2.0 mL hexane
no freeze drying	freeze drying
no NaOH	NaOH
no sonication	sonicate for 30 minutes
no water added	1 mL of water added
65-75% completeness of methylation (estimate)	100% completeness (estimate)

*perflurodecanoic acid (PFDA)

Overall, the new method seems to be more precise with better recoveries of spikes, especially at the lower levels.

A characteristic of the new method is lower % methylation reaction completion, although this is somewhat compensated for with the PFDA internal standard. Both the old and the new method use PFDA, which has measurable amounts of PFOA as an impurity. Retention of the PFOA methyl ester peak could be improved in both methods, perhaps by using a pefluorinated stationary phase. Both methods use the electron capture detector, which is not specific for PFOA (other compounds could co-elute causing an enhanced peak and higher reporting levels from what is actually present in the sample). This is especially important since the retention time is so short. Analytes with short retention times have increased probability of coelution with another material.

Conclusion: The NMS new method is an improvement over the old method. The 2001 results are more reliable than the 2000 results for our two sample sets. The determination could be improved by increasing both the sensitivity (10 ng/mL reporting level) and specificity.