nicotine aza-arenes

Objective: to determine whether the production of aza-arenes from nicotine constitutes a significant contribution to Salmonella/microsome activity of CSC.

Proposal: to add nicotine citrate to LTF-5E at a level equivalent to 5% nicotine, prepare sheets, cut, and make cigarettes. Prepare control LTF-5E by the same procedure. Smoke, collect IT-CSC, and fractionate into acids, bases, neutrals. Prepare a pot residue from each base fraction. Test CSC's, acids, neutrals & PR's in S/M assay.

Timetable of activities:

activity	Time Span	Personnel
Prepare nicotine citrate	2 days	A. Warfield
Prepare LTF + nicotine citrate	~~Prepare~~ 3 days	D. Petri, [redacted]
Prepare LTF-5E (200g)	~~Prepare~~ 3 days	"
Cut + make cigarettes	3-4 days	D. Petri
Smoke	2 days	N. McGee or D.P.
Sample CSC + Fractionate	4 days	S.T., R.L.
Prepare P.R.	4 days	S.T.
Prepare Samples for testing	2 days	S.T.
Test in S/M assay		6906
Evaluate Results + write report		S.T., A.W., K.R.S.
analyze level of nicotine (gc) in CSC, Bases, PR	2 days	A.H.W. or D.P.

An initial experiment should be carried out where LTF-5E is smoked, IT-CSC trapped, and a base fract. prepared and tested in S/M assay. The % bases should be recorded to ascertain the feasibility of detecting any S/M activity due to nicotine azaarenes.

the nicotine citrate could be added to a small amount of LTF to determine whether there is any chance of finding activity. This may or not mean anything, especially if negative results are obtained.

For the above reason, I believe it would be better to calculate what the minimum # of cigarettes is, with the highest reasonable level of nicotine, that would be capable of yielding positive results if the activity due to nicotine azaarenes is significant.

Facts:

1. 98% of nicotine survives unchanged. 2% is pyrolyzed.

2. 0.03–0.006% of ^{14}C nicotine in ~~MS~~ filler is transferred to the aza-arene fraction. (Monthly Sum., 2525, July 1982). of MS-CSC
   0.3–1.5 mg of aza-arenes would be effected from 100 cigarettes @ 5% nicotine in LTF.

3. The 0.3 – 1.5 mg would be diluted by some factor, which could be determined by smoking the LTF-5E control and fractionating as described above. The wt of P.R. would indicate a dilution factor.

4. CSC from LTF-5E is ca 23 mg/cig.

5. if base fr. = 5% of CSC, then 1–2 mg base fr/cig.

6. calc. of amt. of cmpds (aza arenes) likely in PR.

   0.003–0.015 mg aza arenes/cig

   $\frac{0.003\text{ mg}}{2\text{ mg}}$ (worst case) = 0.15% active cmpds.

   $\frac{0.015\text{ mg}}{1\text{ mg}}$ (best case) = 1.5% active cmpds.

   10,000 rev/mg × .15% = 1.5 rev/mg
   * 1,000,000 rev/mg × .15% = 1500 rev/mg
   } 0.15% active cmpds.

   10,000 rev/mg × 1.5% = 15 rev/mg
   * 100,000 rev/mg × 1.5% = 1500 rev/mg
   } 1.5% active cmpds.

7. conclusions: the aza-arenes collectively would have to have a S/M activity of 100,000 to 1,000,000 rev/mg. to exhibit an overall PR S/M activity of 1500 rev/mg at 5% nicotine in LTF, assuming 5% base fr. from LTF-5E.

at 10% nicotine, chances are doubled for detecting activity, or if the base fr. from LTF-5E is only 2.5% of CSC, the same would be true. the # of cigarettes to be smoked would depend on the yield of base fraction from LTF-5E. Probably would smoke at least 60 if LTF + nicotine citrate is prepared, since it would likely be prepared in 100 g batches.