

Minutes of the 5th Meeting of the Sub Group of CSC
to discuss the Analysis of Nitrosamines

The meeting was held on the 11th June, 1981 at the ITL Research Department, Bristol.

<u>Present</u>	Dr. A.S. Weaving	(ITL)
	Mr. P.D. Case	(BAT)
	Mr. G.K.E. Copeland	(LGC)
	Mr. E. Cumming	(CRL)
	Mr. H.F. Dymond	(BAT)
	Mr. B.E. Frost	(CRL)
	Mr. G. Haslett	(Gallaher)
	Mr. N. Hitchings	(ITL)
	Dr. R.A. Patrick	(Gallaher)
	Dr. D. Spincer	(ITL)
	Dr. J. Warren	(LGC)

Apologies for absence were received from Philip Morris.

1. Introduction

ASW welcomed the members to the 5th Meeting and in particular Mr. Case of BAT and Dr. J. Warren of LGC who were attending for the first time. Minutes of the meeting would be prepared by ITL members and draft copies would be circulated for comments before it was submitted to the CSC. He said that ITL members would open the meeting by reporting on methods they had developed and that this should stimulate discussion on the other items on the agenda. In conclusion he said that he hoped that the meeting would be interesting and informative with a free exchange of ideas.

2. ITL Presentation

DS outlined the work of ITL on volatile nitrosamines. He pointed out that ITL's safety regulations may be suggested as an industry standard at the next meeting of the industry with the factory inspectorate. ITL had been unable to work for 6 months due to the replacement of the detector housing. It has been shown that at least part of the nitrosamines found on a Cambridge filter placed between liquid traps and the smoking machine is due to artifacts.

Current ITL methodology for volatile nitrosamines was outlined. Smoke is collected in aqueous sulphamic acid and the nitrosamines are extracted into dichloromethane. After drying, this extract is passed down an alumina column and the nitrosamines eluted in dichloromethane. The eluate is concentrated on a Kuderna-Danish evaporator and injected into the G.C./TEA instrument. Separation is carried out on a 4 M x 10% Carbowax 20 M column at 180°C.

The overall recovery was found to be 75-80% for volatile nitrosamines. The recovery of N-nitrosodimethylamine was markedly improved by the addition of salt during the extraction of smoke traps and an alkali backwash of the dichloromethane extract from the traps.

The problems raised were:-

- 1) How can evaporation of the sample to less than 1 ml be achieved without greatly reducing the recovery of nitrosamines?
- 2) Why was the "solvent" peak for smoke samples so large and could its tailing be reduced?

ASW outlined the ITL work on non-volatile nitrosamines. He said that since the last meeting they had decided to develop a GC-TEA method for non-volatile nitrosamines. The best GC separation of NNN, NNK and NATB had been obtained on a 2 m x 3.5 mm glass column containing 3% OV-275 on Gas Chrom. Q (80-100 mesh) at 210°C. When this column was placed in the GC-TEA system there was a poor response for the nitrosamines and broad peaks were obtained. This was due to the fact that the GC-TEA link line was only insulated and not heated. When the line was heated to 350°C a good response was obtained and similar GC traces to those obtained with FID were obtained. A furnace temperature of 475°C was found to give maximum response for NNN. A method was developed for the determination of non-volatile nitrosamines in smoke in which 5 cigarettes were smoked onto each of two Cambridge filters and then ¹⁴C-labelled NNN added as tracer. The filters were macerated with dichloromethane and chromatographed on a column of alumina (neutral, activity 111). A nitrosamine fraction was eluted with dichloromethane/acetone (4:1) and this was extracted with 2N HCl. The acid extract was made alkaline and re-extracted with dichloromethane. After drying (Na₂ SO₄) the DCM extract was concentrated to 1 ml. Overall recovery of NNN from tracer measurements was 90%+. Separation of the smoke extract by GC-TEA showed only the presence of the three non-volatile nitrosamines. Levels of NATB, NNN and NNK for a UK filter cigarette were 75, 77 and 58 mg/cig. respectively. It was intended to investigate the effect of treating the filters with sulphamic acid since work on volatile nitrosamines had shown that there was artifactual formation on Cambridge filters. A comparison of results obtained by collection on a Cambridge filter with collection in liquid traps would be carried out.

3. Storage, Handling and Disposal of Nitrosamines

DS circulated the ITL regulations for volatile nitrosamines and also an IARC monograph on the handling of chemical carcinogens in the laboratory. The biggest problem was stated as being the destruction of solutions (particularly old concentrated solutions) prior to disposal. The LCC treat contaminated solid materials with glacial acetic acid/HBr mixture and send liquids for burning. BAT send for Rechem. International to dispose of their wastes. Gallahers and CRL burn their waste materials. DS pointed out that this problem will reduce now that ITL are using ISOPAC standards. ASW circulated the latest Sigma information on these reagents.

JW pointed out that Castegnaro at IARC is organising a study on the methods of destroying nitrosamines and anyone wishing to take part should contact Castegnaro.

NRH stated that ITL's monitoring programme showed that nitrosamine contamination was only found in the freezer used for storing concentrated solutions. It was agreed that the handling of non-volatile compounds was far less of a problem.

4. Collection of Cigarette Smoke

ITL are using an eight channel "simultaneous" smoking machine (Filtrona 302) and have found the collection efficiency for volatile nitrosamines in liquid traps to be greater than 96%. The LGC are using a similar approach but are considering the use of a Borgwaldt rotary smoker to speed up smoke collection. ITL are collecting non-volatile nitrosamines on Cambridge filters.

BAT are using a Borgwaldt RM20CS smoking machine and collecting volatile nitrosamines in 20 ml ascorbic acid as outlined at the last meeting. Their preliminary work on non-volatile nitrosamines has used Cambridge filter collection.

Gallaher, working only on non-volatile nitrosamines, have not changed their procedures from the last meeting. They are, however, purchasing a Borgwaldt smoking machine to reduce sample preparation time.

CRL are using a Mason smoking machine as described at the last meeting.

5) Isolation of Nitrosamines

Apart from ITL all other laboratories continue to use the procedures previously described. BAT are attempting to analyse non-volatile nitrosamines using a nitrogen specific detector and examining what clean up is necessary. The object of this approach is to be able to analyse volatile and non-volatile nitrosamines at the same time, which cannot be done if the TEA is used for both analyses. They believe that TAC non-volatile nitrosamine samples are of high purity.

The LGC are using liquid chromatography to isolate the non-volatile nitrosamines prior to detection on the TEA which has been modified for this purpose.

ITL asked if anyone knew how to concentrate the final solution for the analysis of volatile nitrosamines to below 1 ml without seriously reducing the recovery. LGC suggested using 0.5 ml of hexane as "keeper".

6. Separation and Determination of Nitrosamines

Laboratories were using the same separation conditions as at the last meeting although CRL were considering a change to TPA terminated Carbowax for the volatile compounds. BAT were considering the use of capillary columns and a nitrogen detector for the analysis of non-volatile nitrosamines. Gallaher doubted whether this approach would be successful as they had not been able to produce results by these techniques.

ITL commented that the simplest and most successful trapping system for the TEA in their experience was one made of Teflon placed in liquid nitrogen. They did not recommend the expensive Thermo Electron CTR trap. BAT had found that only one in 4 CTR traps had improved instrument performance. They had had some problems when using a Teflon trap in a liquid nitrogen/isopentane slush. These appeared to be due to temperature variations with depth of coolant.

The LGC commented that they used a stainless steel trap immersed in a liquid nitrogen/n pentane slush as sensitivity was reduced if the depth of liquid nitrogen was too great. CRL had found that a small bore Teflon trap in liquid nitrogen became blocked and was not useful. Gallaher still do not use a trap in their system.

ITL asked if anyone had experienced problems with inefficient decomposition of nitrosamines during analysis. LGC have found occasional peak broadening during the analysis of a large number of "dirty" samples. This was overcome by burning off material deposited on the catalyst in oxygen. This was unlikely to occur if only smoke samples were analysed.

ITL reported a peak in the analysis of volatile nitrosamines which is eluted before N-nitrosodimethylamine. Other laboratories also observed this peak. ITL are attempting to identify the compound responsible for this by M.S. This would thought to be useful.

There was some discussion on whether capillary columns rather than packed ones should be used in conjunction with the TEA. The general view was that they could be used but that any advantage in improved separation may well be counteracted by a loss in sensitivity due to dilution with the necessary make up gas.

ITL pointed out that integration of nitrosamine peaks was not always simple or accurate, due to noise and peak shape, and that in the case of N-nitrosopyrrolidine the area was measured within a specific time "window". In the case of non-volatile compounds ITL preferred to use peak height measurement. Gallaher stated that they use peak height measurement for non-volatile nitrosamines as their "old" integrator was not good enough.

It was generally agreed that for non-volatile nitrosamines no internal standard could be used and that radio traces were unnecessary due to good recoveries.

7. Any Other Business

1) ITL had indicated that deliveries of N-nitrosodimethylamine were of the order of:- 50-80 ng per cigarette for Gauloises Plain, less than 1 ng/cigarette for a typical U.K. filter cigarette and less than 3 ng per cigarette for a typical U.K. plain cigarette. The work at the LGC indicated that U.K. filter cigarettes deliver less than 1 ng per cigarette and less than 6 ng per cigarette for U.K. plain cigarettes.

2) ITL results for non-volatile nitrosamines were of the order of 50-80 ng per cigarette for U.K. filter products. CRL reported that Gauloises cigarettes delivered 200 ng per cigarette of N-nitrosocotine whereas the delivery from U.K. filter cigarettes was less than 5 ng per cigarette. Gallaher stated that their results were similar to those of ITL.

3) It was agreed that it was too early for a collaborative exercise as not all laboratories had finalised their methods.

4) Gallaher reported that their examination of the non-volatile nitrosamine content of tobacco suggested that this was the major source of these compounds in smoke.

5) G.K.E.C. raised the question as to why there had been no discussion on sidestream smoke at this meeting. The general reply was that the analysis of sidestream smoke was unrealistic as the level of nitrosamines varied with the method of production. It was, therefore, more realistic to measure nitrosamines in ambient atmospheres - a task which none of the other members present were in a position to do as they were having enough problems analysing mainstream smoke. G.K.E.C. then suggested that the TAC approach to sidestream and mainstream smoke nitrosamines was slightly illogical and ambivalent. Much effort was being spent on minimising artifact formation during the analysis of mainstream smoke whereas this formation was permitted in the case of ambient atmospheres. He suggested that the best measure of "total insult" to the smoker would be to allow artifact formation to be completed prior to the analysis of mainstream smoke. This would represent a true measure of the nitrosamine level received by a smoker by inhalation and formation within the human system. ITL and BAT pointed out that what reactions occur in smoke once it enters the human system are completely unknown. Such a measure could be a gross overestimate of the total insult. (The same arguments could of course be applied to the analysis of ambient atmospheres). TAC, therefore, have decided that, since the changes occur within the human systems are unknown, all analyses should measure the level of compounds that emerge from a cigarette. Whatever reactions occur within the human system, these analyses can at least rank the exposure from cigarettes based on the assumptions and restrictions imposed by the standard smoking parameters. Using this approach, is was likely that the analysis of compounds like acrolein and nitric oxide produce overestimates of what the smoker actually receives. Gallaher and CRL supported these views.

There being no other further business ASW thanked the members for coming and for their contributions.

Basic Research & Specialist Services Division
17th July, 1981
DS/KT