Introduction

In paragraph 6 of the final report of the interlaboratory study iis04A02 for AZO dyes in textile and leather it was concluded from the reported results of sample 0419 that ”None of the participants detected 5-Chloro-o-toluidine, which could be very easily misidentified as 4-Chloro-o-toluidine. There is hardly any difference in UV, Retention time and Mass Spectra."

In response to this remark several participants contacted us with the question if it is possible to detect whether 4-Chloro-o-toluidine or 5-Chloro-o-toluidine is present in the sample. Also two critical remarks were made that caused us to do some further investigations into this matter:

Error in Mass Data?

The first remark was about the discrepancy between the spectrum and the list of intensities in appendix 4 of the report (see also the appendix of this addendum).
In the data of 5-chloro-o-toluidine the relative intensity of mass 140.0 is 49%. And in the data of 4-chloro-o-toluidine the relative intensity of mass 140.5 is only 32% (read from the mass spectrum). However in the table the relative intensity of mass 140.0 is 0%! Obviously this must be an error as intensities of 0% are usually not listed in these tables. But this error is of course rather confusing. Unfortunately this confusing error was not observed by us when we copied these data from http://www.aist.go.jp/RIODB/SDBS/sdbs/owa/sdbs_sea.cre_frame_disp?sdbsno=3565

The small intensity difference in mass intensities of the two isomers as observed above can be caused by the fact that the test conditions for recording of these two spectra were not identical. And thus must be concluded that this difference is probably not significant.

4-chloro-o-toluidine or 5-chloro-o-toluidine?

The second remark was about our conclusion that none of the participants reported the presence of 5-chloro-o-toluidine because the difference between 4-chloro-o-toluidine and 5-chloro-o-toluidine would be too small.
Our subcontractor that did prepare the textile materials by dying informed us that the brown cotton of sample 0419 was dyed in such a way that the allowed amine 5-chloro-o-toluidine would be found and not the forbidden 4-chloro-o-toluidine.
The results of the proficiency study were clear: not less than 39 laboratories reported the presence of 4-chloro-o-toluidine and none the presence of the expected 5-chloro-o-toluidine. However as the chromatographic and spectrometric properties of 4-chloro-o-toluidine and 5-chloro-o-toluidine are very similar and as we did not know of any analytical method that could differentiate between these isomers we did not find this result suspect and we draw the conclusion that all participating laboratories must have misidentified this aromatic amine.

But after receipt of the final report, one German participant in this study did send us a German publication [1] in which an analytical method was presented to differentiate between 4-chloro-o-toluidine and 5-chloro-o-toluidine. Although the difference between these two isomers indeed are too small to differentiate using the usual HPLC and GC/MS methods, the difference between

TFA-derivatives of these isomers is large enough to differentiate! The conclusions of the article are in short:

1 - Free amines cannot be separated by GC and the MS spectra are rather alike;
2 - Free amines cannot be separated under the usual HPLC conditions, but by using a special gradient (a 75 minute run!) a separation can be reached. The UV spectra are slightly different;
3 - The TFA derivatives of the amines can easily be separated by GC and the MS-spectra are also different.

And furthermore this participant informed us that he had used this analytical method and that he had concluded that the aromatic amine present in sample 0419 was 4-chloro-o-toluidine without doubt! Of course we immediately contacted our subcontractor that had dyed the sample material and it appeared that indeed some confusion about the identity of the aromatic amine was present. It was thought that the use of the dye Fast Red TR Base (azoic diazo component 11) [2,4,5,6,7] would result in the detection of 5-chloro-o-toluidine and use of the dye Fast Red KB Base (azoic diazo component 32) [3] would result in the detection of 4-chloro-o-toluidine, while it is just the other way around! This error was caused by an error in the literature [8] used as in table II on page 660 the amines listed for Fast Red TR and Fast Red KB are mixed up.

Thus we must conclude that the brown cotton of sample 0419 was erroneously dyed with a dye (namely Fast Red TR Base) that releases 4-chloro-o-toluidine rather than 5-chloro-o-toluidine and that our conclusions about the misidentification of 4-chloro-o-toluidine in paragraphs 4.1 and 6 are wrong. The corrected pages are attached to this addendum.

References

[1] K. Friedrichs, H-D. Winkeler, G. Prior, Herkunft, textile Verwendung und Identifizierung von 4-Chlor- und 5-Chlor-o-toluidin, GIT Labor-Fachzeitschrift 5/97, p 488-494
[2] http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr165.html
[3] http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr187.html
[4] http://www.cdc.gov/niosh/rtecs/xu501bd0.html
[5] http://www.sigmaaldrich.com
[6] http://www.serva.de/products/data/21317.01.html
[7] http://bgchemie.de/webcom/show_article.php/_c-85/_nr-25/_p-1/i.html
[8] K. Venkatraman, Chemistry of Synthetic Dyes, Academic Press, New York, 1952

CORRECTED PARTS (*corrections are given in italic*)

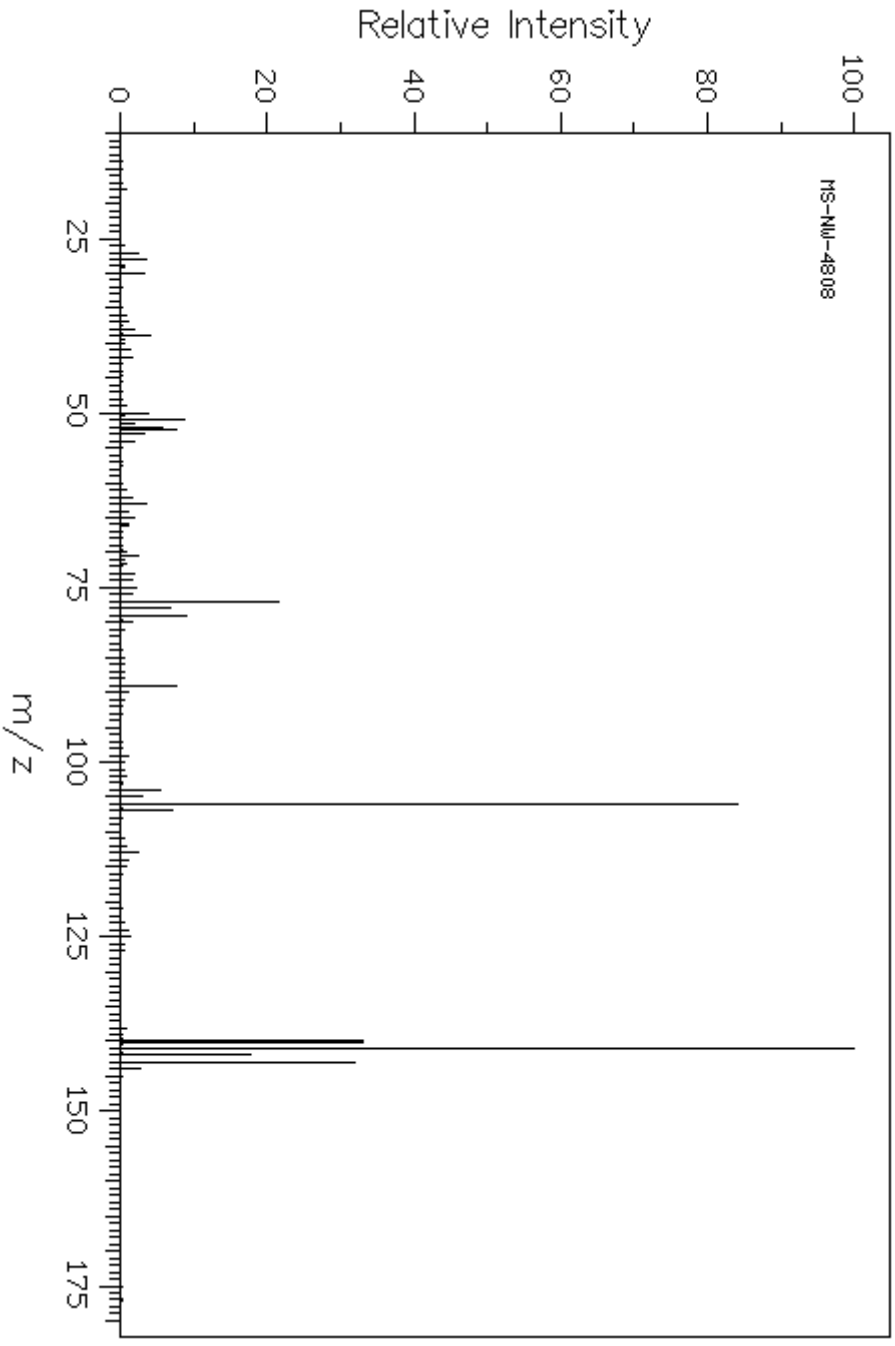
4.1 EVALUATION PER SAMPLE

Textile 0419: Sample 0419 (brown fabric) was treated by the third party laboratory with dyes in order to find the following aromatic amines: Benzidine, 3.3-Dimethoxybenzidine, *4*-Chloro-o-toluidine and 3-Chloroaniline. Most of the 43 participants, that did analyse this sample, detected besides *three* of the four mentioned aromatic amines also o-Toluidine. Only six (!) participants reported the presence of the expected 3-Chloroaniline. Almost all reporting participants reported the presence of *the banned* p-Chloroaniline instead of the *allowed* isomer *3-Chloroaniline* (see also paragraph 6: discussion)
The results reported by the participants varied strongly (Benzidine: 2.35 – 34.7 mg/kg, *4-Chloro-o-toluidine* 5 - 753.7 mg/kg and 3.3-Dimethylbenzidine 13 – 165.9 mg/kg). All laboratories, except one (3122), that reported results for sample 0419, would reject this textile sample for containing too much forbidden aromatic amines (>30 mg/kg).
Finally, the spreads were very large and not in agreement with the requirements of the respective standard or Horwitz equation. These large spreads may partly be explained by the observed differences in the analysis methods as applied (see appendix 3). See paragraph 6 for the discussion.
Thirteen participants reported also other amines (see appendix 2).

6 DISCUSSION

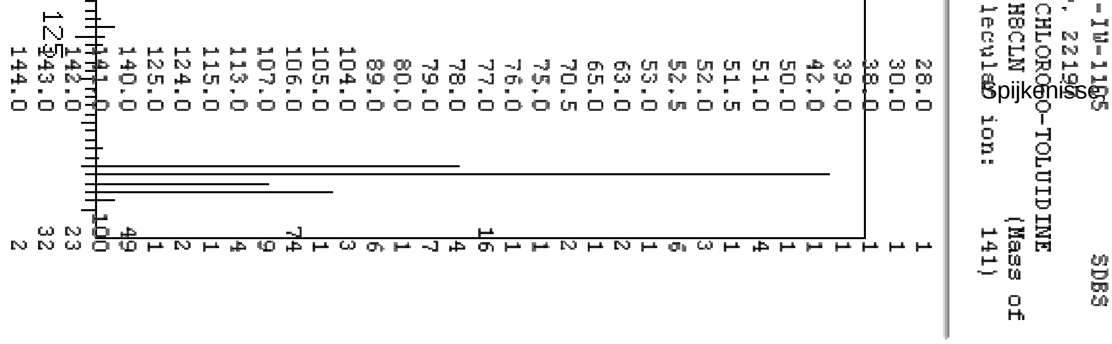
Identification and confirmation seems also problematic for *many* participants. A mismatch is easily made as confirmed in the present round robin by sample 0419. *A non-banned* isomer of p-Chloroaniline should be found in this sample. However only six (!) participants detected *correctly* the *non-banned* isomer 3-Chloroaniline instead of p-Chloroaniline.

APPENDIX 4



MS-NW-4808 SDBS NO.
3565
4-CHLORO-O-TOLUIDINE
C_7H_8ClN (Mass of molecular ion: 141)

27.0	2
28.0	3
30.0	3
37.0	1
38.0	1
39.0	4
41.0	1
42.0	1
50.0	3
51.0	8
51.5	1
52.0	5
52.5	7
53.0	3
54.0	1
62.0	1
63.0	3
64.0	1
65.0	1
68.0	1
70.5	2
73.0	1
74.0	1
75.0	2
76.0	1
77.0	21
78.0	6
79.0	9
80.0	1
89.0	7
90.0	1
99.0	1
104.0	5
105.0	2
106.0	84
107.0	7
113.0	2
114.0	1
124.0	1
125.0	1
140.5	0
141.0	100
142.0	17
143.0	31
144.0	2



Spijkenisse, August 2004