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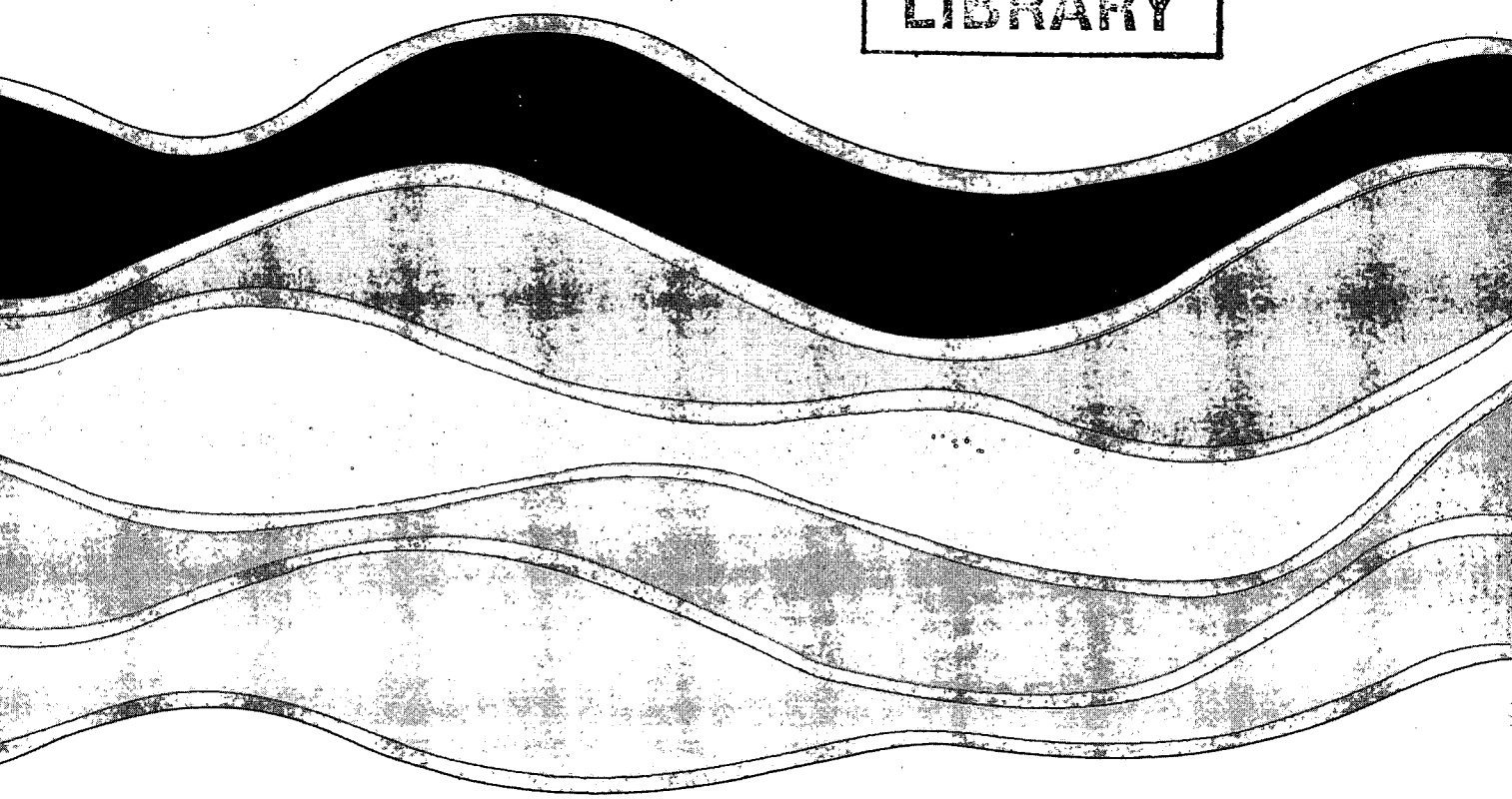

INTERLABORATORY STUDY NO. DF-1 FOR THE DETERMINATION OF DIBENZO-p-DIOXIN AND DIBENZOFURAN IN DEFOAMERS

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NWRI CONTRIBUTION 91-01

MANAGEMENT PERSPECTIVE

The presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) in the final bleached pulp was attributed to the use of defoamers contaminated with non-chlorinated dibenzo-p-dioxin (DBD) and dibenzofuran (DBF) in chlorine bleaching pulp mills. Under the auspices of Canadian Environmental Protection Act (CEPA), Environment Canada is developing regulations that would set the maximum concentrations of DBD and DBF in defoamers for these applications. An analytical method for the determination of DBD and DBF in defoamers to be referenced in the regulations has been jointly developed by the Pulp and Paper Research Institute of Canada and Research and Applications Branch at the National Water Research Institute.

An interlaboratory study was designed and conducted to validate the above-mentioned method. This interlaboratory study provides precision and accuracy statements for the proposed reference method for the determination of DBD and DBF in defoamers.

Dr. J. Lawrence
Director
Research and Applications Branch

PERSPECTIVE-GESTION

La présence de 2,3,7,8-tétrachlorodibenzo-p-dioxine (TCDD) et de 2,3,7,8-tétrachlorodibenzofurane (TCDF) dans la pâte blanchie finale a été attribuée à l'utilisation d'agents de démoussage contaminés par de la dibenzo-p-dioxine non chlorée (DBD) et du dibenzofurane non chloré (DBF) dans les usines où la pâte est blanchie au chlore. Conformément à la Loi canadienne sur la protection de l'environnement (LCPE), Environnement Canada élabore des règlements qui prévoiraient des concentrations maximales de DBD et de DBF dans les agents de démoussage utilisés à cette fin. Une méthode permettant de doser la DBD et le DBF dans les agents de démoussage, qui sera mentionnée dans les règlements, a été élaborée conjointement par l'Institut canadien de recherches sur les pâtes et papiers et par la Direction de la recherche et des applications de l'Institut national de la recherche sur les eaux.

Une étude interlaboratoire a été conçue et réalisée en vue de valider la méthode mentionnée ci-dessus. Cette étude interlaboratoire fournit des données sur la précision et l'exactitude de la méthode de référence proposée pour le dosage de la DBD et du DBF dans les agents de démoussage.

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ABSTRACT

An interlaboratory study in which eight laboratories participated was conducted for the determination of non-chlorinated dibenzo-p-dioxin (DBD) and dibenzofuran (DBF) in defoamers. Participants were requested to analyze DBD and DBF in two standard solutions and five fortified defoamers using a proposed reference method. The objectives of this study were to characterize the performance of the proposed method in terms of accuracy, interlaboratory (overall) precision, intralaboratory (within-lab) precision for the determination of DBD and DBF in defoamers as well as to assess the performance of participating laboratories.

In this study, most participants have demonstrated the capability of generating satisfactory results using the proposed reference method. Based on these interlaboratory results, it is concluded that the proposed reference method is shown to be a reliable analytical method for measuring low levels (from 1 to 100 ng/g) of DBD and DBF in defoamers.

RÉSUMÉ

Nous avons effectué une étude interlaboratoire sur le dosage de la dibenzo-p-dioxine non chlorée (DBD) et du dibenzofurane non chloré (DBF) dans les agents de démoussage, à laquelle huit laboratoires ont participé. Nous avons demandé aux participants de doser la DBD et le DBF dans deux solutions étalons et dans cinq agents de démoussage fortifiés, en utilisant une méthode de référence qui était proposée. Les objectifs de cette étude étaient de caractériser la performance de la méthode proposée, en termes d'exactitude, de précision interlaboratoire (précision globale) et de précision intralaboratoire (précision dans les laboratoires individuels), au cours du dosage de la DBD et du DBF dans les agents de démoussage, ainsi que d'évaluer la performance des laboratoires participants.

Au cours de cette étude, la plupart des participants ont montré qu'ils pouvaient obtenir des résultats satisfaisants à l'aide de la méthode proposée. Nous avons conclu, à partir des résultats interlaboratoires, que la méthode de référence proposée constitue une méthode fiable pour doser la DBD et le DBF présents en faibles concentrations (1 à 100 ng/g) dans les agents de démoussage.

1.0 INTRODUCTION

The Canadian Environmental Protection Act (CEPA) which became law in June 1988 is now the primary act for the national management of toxic substances. There is a concern about defoamers contaminated with non-chlorinated dibenzo-p-dioxin (DBD) and dibenzofuran (DBF) used in chlorine bleaching pulp mills as it has been reported (1) that a significant increase in the levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) was observed in the final bleached pulp. TCDD and TCDF are discharged into receiving waters as effluents after the pulp is washed, therefore there is a need to reduce the amounts of these toxic contaminants in pulp mill discharges. Environment Canada is developing regulations that would set the maximum concentrations of DBD and DBF in defoamers applied in chlorobleaching mills. In response to a recent request by the Industrial Programs Branch of Environment Canada, an analytical method for the determination of DBD and DBF in defoamers, to be referenced in the regulations, has been jointly developed by the Pulp and Paper Research Institute of Canada (PAPRICAN) and the Research and Applications Branch, National Water Research Institute (2).

As part of the validation of this proposed reference method, an interlaboratory study was designed and conducted for the determination of DBD and DBF in defoamers using the proposed

reference method. The objectives of this study were to characterize the performance of the proposed reference method in terms of accuracy, interlaboratory (overall) precision, intralaboratory (within-lab) precision for the determination of DBD and DBF in defoamers as well as the performance of participating laboratories.

2.0 STUDY DESIGN

The study consisted of 7 test samples for the determination of non-chlorinated dibenzo-p-dioxin and dibenzofuran. The detailed description of samples as well as their design values are given in Table 1. Briefly, Samples #1 and #2 in sealed glass ampules were standard solutions of DBD and DBF in iso-octane at various concentrations. These standard solutions were used to evaluate the performance of calibration standards and instrumentation of participants. Samples #3 to #7 were defoamers fortified with various concentrations of DBD and DBF designed for the evaluation of accuracy and precision of the proposed reference method performed by all participants. Samples #4 and #7 were blind duplicate which were used to evaluate the intralaboratory precision for the determination of DBD and DBF in defoamers by individual participating laboratory.

The defoamer reference samples for this interlaboratory study were prepared as follows: Weigh a 300 g subsample of

defoamer in a 500 mL Erlenmeyer flask containing a teflon magnetic bar. Spike content with appropriate amounts of DBD and DBF stock solutions in iso-octane at 10 μg/mL concentration. Agitate the mixture continuously with a magnetic stirrer for about 8 h. Store the sample in a refrigerator at 4°C in the dark overnight. Repeat the stirring procedure the next day. Store the resulting homogeneous bulk defoamer sample in a refrigerator until ready for subsampling. Before subsampling, mix the content in Erlenmeyer flask thoroughly again for 2 h. Transfer about 12 g subsample into a 25 mL amber glass vial. Seal the glass vial with a teflon-faced cap and store in a refrigerator. For each 300 g bulk sample, 25 subsamples can be prepared. Each subsample will be sufficient for at least two analyses using the proposed reference method.

Samples #3 to #7 were prepared according to the procedure described above. The homogeneity and integrity of subsamples as well as the design values of DBD and DBF in the reference samples were confirmed in advance by in-house analysis using the proposed reference method.

3.0 EXPERIMENTAL METHOD

A proposed reference method (2) that employed steam distillation using iso-octane as the extraction solvent was developed for the determination of DBD and DBF in defoamers. The extract was cleaned up on a basic alumina column. Final analysis

was performed by GC/MS in selected ion monitoring mode and the extraction recoveries of native DBD and DBF were monitored by using DBD-d_8 and DBF-d_8 as surrogates. Confirmation of DBD and DBF was done by comparing the ratio of peak areas for the quantitation and confirmation ions of each compound in the standard and the sample. Based on a 5 g sample and a concentration factor of 10, the method detection limits were 1 ng/g for both DBD and DBF. The detailed analytical procedures of the proposed reference method is given in Appendix A.

4.0 RESULTS AND DISCUSSION

In June 1990, 15 governmental, industrial and private laboratories in Canada and United States of America were invited to participate in this study. By August 1990, 10 sets of test samples were sent to those who had indicated an interest in participating. The participants were requested to analyze all 7 test samples for DBD and DBF using the proposed reference method enclosed (Appendix A). However, some laboratories chose to modify the method or use their own in-house method. If the in-house procedure was modified significantly, the participants were requested to submit the detailed deviations from the proposed reference method together with their results.

Laboratory DF009 had phoned that they would not participate in this study because they could not acquire all the

apparatus and chemicals on time to meet the deadline of September 28, 1990. Five out of the nine participating laboratories had submitted their results on schedule. Laboratory codes for these five participants are DF001, DF002, DF004, DF005 and DF010. On October 3, 1990, a letter was sent out to remind those participants who have not yet submitted their results. By October 10,1990, three more participants had submitted their results. Only one participant did not submit results when the study was closed. See the list of participants at the end of this report.

All participants had the capability of analyzing both DBD and DBF in defoamers. Most participants followed the proposed reference method for the determination of DBD and DBF in defoamers and experienced no difficulties. However, a few participants chose to modify some parts of the procedure or used their own method. Details of the modifications are summarized in Appendix B. One laboratory (namely, DF006) submitted two sets of results - one by the proposed reference method (assigned as DF006) and the other using their own method (assigned as DF006A). The detection limits of DBD and DBF in the proposed reference method were both 1 ng/g. In general, most participants have met or exceeded this requirement for both parameters with the exception of laboratory DF002, which had a high detection limits for both DBD and DBF and laboratory DF005, which had a high detection limit for DBD. It should be noted that laboratory DF002 concentrated the final extract to 100 μL instead of 500 μL and the sample size used was 1 g instead of 5 g.

In this case, the concentration factor (10 times) of final extract was the same as that in the proposed reference method. Some laboratories could not recover both surrogate standards of DBD and DBF in the cleanup step as specified in the proposed reference method. The low recoveries were perhaps due to the use of alumina of different activity.

The data submitted by all participants for DBD and DBF in standard solutions are summarized in Tables 2-1 and 3-1, respectively, while the data for DBD and DBF in defoamers are summarized in Tables 2-2 and 3-2, respectively. Mean and standard deviation of these samples for overall interlaboratory results were calculated after outliers (marked with a *) were removed by using Grubbs' test (3). Overall, results submitted by all participants are excellent with only 4 out of 117 (3.41%) results identified as outliers.

In this study, two standard solutions was used to evaluate the performance of the accuracy of participants' calibration standards as well as their instrumentation. Although no outliers were identified for those results as shown in Tables 2-1 and 3-1, laboratories DF002 and DF010 had a small systematic error in their in-house standards for both DBD and DBF. Also the results reported by laboratory DF006 had relatively low recoveries (about 33% and 50% for DBD and DBF, respectively) as compared with other participants.

For the defoamer samples as shown in Table 2-2 and 3-2, overall only 4 out of 80 results were identified as outliers. In general, most laboratories have provided good results. Results of these samples from laboratories DF002 and DF010 again had the same systematic error as observed in their results for standard solutions. Results of laboratory DF006 for DBD in defoamer samples were approximately 35 to 45% lower than and for DBF were quite close to the respective interlaboratory means although this laboratory produced 3 times lower values for DBD and 2 times lower values for DBF in standard solutions than the corresponding interlaboratory means. Since there is a 2 to 3 times negative bias in the results of laboratory DF006 for the standard solutions, one would expect that the results for spiked defoamer samples would also be low. But this is not the case. The results for DBF are apparently good (matching the mean) and those for DBD only deviated some 50% from the interlaboratory mean. This suggests that the apparently satisfactory results for the defoamer samples were the consequence of low bias of the standards used and high bias of sample results resulting in apparently satisfactory data caused by some averaging effect. Therefore, inconsistency in laboratory performance is evidenced for this laboratory and their internal QA/QC activities should be critically reviewed to isolate the problem areas for corrective action.

As shown in Tables 2-1 and 3-1, the means and medians of interlaboratory results agreed very well (within ±10%) for DBD and

DBF in the standard solutions. Similar results were observed for both DBD and DBF in defoamer samples as shown in Tables 2-2 and 3-2. In order to determine the accuracy of overall interlaboratory results, the sample median values were used to compare with the respective design values since the former was not strongly influenced by small sample set of results and interlaboratory precision. In this study , the degree of agreement between interlaboratory medians and the design values of DBD and DBF in all samples was evaluated by the percent recovery of interlaboratory medians. The percent recovery was calculated as interlaboratory median divided by the design value and multiplied by 100% as follow.

$$\% \text{ Recovery} = (\text{Interlab. Median} / \text{Design Value}) \times 100$$

The range and average values of recoveries of interlaboratory results for DBD and DBF in standard solutions and defoamers are summarized respectively in Tables 4-1 and 4-2. As shown in these table, the recoveries of DBD and DBF in standard solutions were within ± 5% of the design values while the recoveries of DBD and DBF in defoamer samples were within ± 10% of the design values. These results were considered excellent since the analytes were present at ng/g level and the analytical procedure employed tedious sample extraction, clean-up and various concentration and quantitation steps.

The precision of interlaboratory results for DBD and DBF, expressed as the relative standard deviations (RSD), is given in Table 5-1 for standard solutions and in Table 5-2 for defoamer samples. The results showed that the overall precision was larger than ±25% although the same proposed reference method was used by most participants. Perhaps these larger variations resulted from the variation in in-house standards, instrumentation and skill of personnel of the participating laboratories. However, the overall precision for standard solutions and defoamers were similar for both DBD and DBF. In some cases the precision for DBF in defoamer samples was better than that obtained in standard solution even though tedious sample preparation were employed during the analysis of defoamer samples.

The intralaboratory (within-lab) precision for defoamers obtained from duplicate samples (#4 and #7) by individual participant showed that most laboratories achieved an RSD of ±12% or better for both DBD and DBF (Table 6). In general, better results were obtained for intralaboratory precision than interlaboratory precision since the latter involved more non-systematic errors as described above. Only two laboratories (DF006A and DF008) had poorer intralaboratory precision than interlaboratory precision for both DBD and DBF. This exception is likely attributed to the participants' inexperience in this method rather than to the inhomogeneity of samples. Our preliminary in-house study as well as the overall interlaboratory results

indicated that the subsamples used for this study were homogeneous between subsamples.

5.0 CONCLUSIONS

Based on the results obtained from the use of the proposed reference method, it is concluded that the method is capable of generating reliable results for the determination of DBD and DBF in defoamers in the concentration range of 1 to 100 ng/g. The results of this interlaboratory study indicate that 8 laboratories had acceptable accuracy and precision for the determination of DBD and DBF in defoamers by using either the proposed reference method or a method similar to it. Our conclusions are summarized as follows:

(1) The percent recovery, as defined earlier, for DBD and DBF in different defoamers varied from 91.6 to 110% and from 101 to 112.7%, respectively.

(2) The precision of the interlaboratory results for DBD and DBF in defoamers, expressed as the relative standard deviation (RSD) varied from 5.33 to 38.93% and from 4.87 to 26.15%, respectively. The larger RSDs for both DBD and DBF in the interlaboratory results were mainly attributed to the variation of in-house calibration standards used by the participants.

(3) The intralaboratory (within-lab) precision for duplicate defoamer samples was satisfactory (ca. ±12%) over 70% of

the results, indicating good in-house precision for the analysis. This also verified the homogeneity and integrity of the subsamples.

(4) Since some participants experienced problems in the recoveries of surrogates in the clean-up step (see Appendix B), the activity of alumina used should be checked to ensure quantitative recoveries for those compounds.

ACKNOWLEDGEMENT

The authors are grateful to the participating laboratories for the time and effort devoted to analyze the defoamer samples, reporting the results and commenting on the method. This interlaboratory study would not be successful without their active participation and cooperation.

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Table 1. Samples distributed in study DF-1.

Sample No.	Description	Design value*	
		DBD	DBF
1	Mixed standard solution, DBDF-1S	10.0	10.0
2	Mixed standard solution, DBDF-2S	1.0	2.0
3	Fortified defoamer, DBDF-1	5.0	24.0
4	Fortified defoamer, DBDF-2	15.0	50.0
5	Fortified defoamer, DBDF-4	50.0	100.0
6	Fortified deformer, DBDF-3	25.0	77.0
7	Duplicate of sample #4	15.0	50.0

Note: * The design values for samples #1 and # 2 are in µg/mL and while for samples #3 to #7 are in ng/g.

Table 2-1. Results for Dibenzo-p-dioxin (DBD) in Standard Solutions.

Sample Results (µg/mL)		
Lab Code	**1**	**2**
DF001	10.6	1.05
DF002	16.3	1.31
DF004	9.91	1.04
DF005	10.5	0.99
DF006	3.03	0.34
DF007	9.1	1.1
DF008	9.3	1.4
DF010	7.9	0.74
Mean	9.58	0.996
S.D.	3.65	0.332
Median	9.61	1.045
Design	10.0	1.0

Table 2-2. Results for Dibenzo-p-dioxin (DBD) in Defoamers.

Sample Results (ng/g)						
Lab Code	**3**	**4**	**5**	**6**	**7**	**D.L.**
DF001	5.5	14.0	49	25	14	0.5
DF002	15.0*	20.7	46.1	30.2	24.4	6.9
DF004	5.1	13.5	44.2	22.6	13.5	1.0
DF005	5.5	17.3	48.5	22.9	14.9	5.0
DF006	3.1	8.5	-	15.7	9	1.0
DF006A	3.4	8.7	-	14.8	16.1	1.0
DF007	6.0	24	46	42	17	1.3
DF008	6.9	17.0	51.2	28.5	28.6	1.0
DF010	4.3	8.5	25.0*	17.0	10.0	0.02
Mean	4.98	14.69	47.5	24.3	16.39	-
S.D.	1.30	5.58	2.53	8.58	6.38	-
Median	5.5	14.0	46.1	22.9	14.9	-
Design	5.0	15.0	50.0	25.0	15.0	-

Table 3-1. Results for Dibenzofuran (DBF) in Standard Solutions.

Sample Results (µg/mL)		
Lab Code	**1**	**2**
DF001	10.5	2.10
DF002	14.4	2.41
DF004	8.30	2.19
DF005	11.2	2.1
DF006	4.73	0.99
DF007	11.0	2.5
DF008	9.1	1.2
DF010	8.4	1.50
Mean	9.70	1.87
S.D.	2.81	0.57
Median	9.8	2.10
Design	10.0	2.0

Table 3-2. Results for Dibenzofuran (DBF) in Defoamers.

Sample Results (ng/g)						
Lab Code	**3**	**4**	**5**	**6**	**7**	**D.L.**
DF001	27	52	110	81	53	0.5
DF002	38.6	67.2*	89.4	78.1	64.4	4.9
DF004	27.1	47.2	90.3	77.3	48.9	1.0
DF005	25.6	50.0	104	83.0	51.8	1
DF006	-	50.7	-	76.8	49.4	1.0
DF006A	34.2	47.6	-	76.3	82.1	1.0
DF007	26	52	101	83	53	0.1
DF008	34.5	56.2	110	87.4	89.1	1.0
DF010	23	42	86	66*	46	0.31
Mean	29.5	49.71	98.67	80.36	59.74	-
S.D.	5.50	4.21	10.06	3.92	15.62	-
Median	27.05	50.7	101	78.1	53.0	-
Design	24.0	50.0	100.0	77.0	50.0	-

Table 4-1. **Range and average values of percent recoveries for the overall interlaboratory results of DBD and DBF in standard solutions.**

Parameter	Range	Average
DBD	96.1 - 104.5	100.3 ± 5.9 (2)
DBF	98.0 - 105	101.5 ± 4.95 (2)

Note: The numbers in parentheses are the numbers of samples.

Table 4-2. **Range and average values of percent recoveries for the overall interlaboratory results of DBD and DBF in defoamers.**

Parameter	Range	Average
DBD	91.6 - 110	97.3 ± 7.74 (5)
DBF	101 - 112.7	104.5 ± 5.02 (5)

Note: The numbers in parentheses are the numbers of samples.

Table 5-1. Range and average values of RSDs for the overall interlaboratory results of DBD and DBF in standard solutions.

Parameter	Range	Average
DBD	33.33 - 38.10	35.72 ± 3.37 (2)
DBF	28.97 -30.48	29.73 ± 1.07 (2)

Note: The numbers in parentheses are the numbers of samples.

Table 5-2. Range and average values of RSDs for the overall interlaboratory results of DBD and DBF in defoamers.

Parameter	Range	Average
DBD	5.33 - 38.93	28.73 ± 14.03 (5)
DBF	4.87 - 26.15	13.67 ± 8.62 (5)

Note: The numbers in parentheses are the numbers of samples.

Table 6. Intralaboratory Precision for DBD and DBF in QC sample DBDF-2 (Samples #4 and #7).

Intralaboaratoy Mean ± S.D. (%RSD) (ng/g)		
Lab Code	**DBD**	**DBF**
DF001	14.0 ± 0 (0)	52.5 ± 0.707 (1.35)
DF002	22.55 ± 2.62 (11.6)	65.8 ± 1.98 (3.00)
DF004	13.5 ± 0 (0)	48.05 ± 1.20 (2.50)
DF005	16.1 ± 1.70 (10.61)	50.9 ± 1.27 (2.50)
DF006	8.75 ± 0.35 (4.00)	50.05 ± 0.92 (1.83)
DF006A	12.4 ± 5.23 (42.18)	64.85 ± 24.40 (37.63)
DF007	20.5 ± 4.95 (24.15)	52.5 ± 0.71 (1.35)
DF008	22.8 ± 8.20 (35.96)	72.65 ± 23.26 (32.02)
DF010	9.25 ± 1.06 (11.45)	44 ± 2.83 (6.43)

APPENDIX A

The Proposed Reference Method

SECOND DRAFT (Revision 2.0)

A Proposed Reference Method for the Determination of Dibenzofuran and Dibenzo-p-dioxin in Defoamers

by

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Management Perspective

The use of defoamers contaminated with non-chlorinated dibenzofuran (DBF) and dibenzo-p-dioxin (DBD) in chlorine bleaching pulp mills was shown to cause a significant increase in the 2378-TCDF and 2378-TCDD levels of the final bleached pulp. In order to reduce the amount of these toxicants in pulp mill discharges, Environment Canada is developing regulations that would set the maximum concentrations of DBF and DBD in defoamers for those applications. An analytical method for the determination of DBF and DBD in defoamers to be referenced in the regulations has been jointly developed by the Pulp and Paper Research Institute of Canada (PAPRICAN) and Environment Canada.

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Abstract

Based on a method developed by the Pulp and Paper Research Institute of Canada, a procedure optimized for the determination of dibenzofuran (DBF) and dibenzo-p-dioxin (DBD) in defoamers at low ng/g levels is presented. The defoamer is steam extracted and the extract in iso-octane is cleaned up on a basic alumina column. Final analysis is performed by GC-MS in the selected ion monitoring mode and the extraction recoveries of native DBD and DBF are monitored by using DBF-d_8 and DBD-d_8 as surrogates. Confirmation of DBD and DBF was done by comparing the ratio of peak areas for the quantitation and confirmation ions of each compound in the standard and the sample. Based on a 5 g sample and a final volume of 0.5 mL, the method detection limit is 1 ng/g for both DBF and DBD.

NOTE: Mention of trade names or commercial products does not constitute endorsement for use by Environment Canada.

1. Scope and Application

1.1. This method applies to the determination of the non-chlorinated dibenzo-p-dioxin (DBD) and dibenzofuran (DBF) in defoamers at ng/g levels.

2. Principle and Theory

2.1. The defoamer is steam extracted into iso-octane and the extract is cleaned up by column chromatography. The concentrated extract is analyzed by gas chromatography-mass spectrometry (GC-MS) in the selected ion monitoring (SIM) mode.

3. Interferences

3.1. Other volatile and semi-volatile organics present in the sample not removed by the column cleanup step may interfere. Combination of a high resolution capillary column and a selective detector such as a GC-MS operating in SIM mode is usually sufficient to remove those interferences. Confirmation of compound identity is provided by comparing the ratio of peak areas of the quantitation ion and the confirmation ion in the sample to an authentic standard.

4. Sample Storage

4.1. No stability data for DBD and DBF in defoamers are available. Defoamers are currently stored at room temperature until analysis. To avoid contamination, glass bottles with foil- or Teflon-lined caps should be used for the storage of defoamers.

5. Apparatus

5.1. All glassware must be washed and dried by the same procedure used for trace

organic analysis.

5.2. Volumetric flasks, 10, 50 and 100 mL.

5.3. Steam distillation heads (see Figure 1 for construction and dimension, these may be purchased from Verrerie de Précision, Montreal, Quebec, tel: (514) 398-6217).

5.4. Round bottom flasks, 500 and 250 mL.

5.5. Graduated centrifuge tubes, 15 mL with standard tapered glass stoppers or Teflon-lined screw caps.

5.6. Chromatographic columns, 400 mm x 10 mm id with Teflon stopcocks.

5.7. Three-stage Snyder columns, ca. 30 cm in length.

5.8. Heating mantles with temperature controls.

5.9. Drying oven for glassware.

5.10. Oven for the activation of alumina at 130 ± 1 °C.

5.11. A GC-MS with good sensitivity operating in selected ion monitoring mode. As an example, a HP5880A GC interfaced to a HP5970B Mass Selective Detector (both available from Hewlett-Packard) and a data system were used. Other systems of equal or better sensitivity may be used.

5.12. Fused silica capillary column, 30 m x 0.25 mm id coated with 5% diphenyl, 94% dimethyl, and 1% vinyl polysiloxane phases, 0.25 μm film thickness, such as DB-5, SPB-5 and SE-54 from various suppliers.

5.13. Hamilton syringes, 10, 50, 100, 250 and 500 μL.

6. Reagents

6.1. All organic solvents must be of distilled-in-glass grade with blanks suitable for residue analysis.

6.2. Iso-octane.

6.3. Petroleum ether (P.E., b.p. 30 - 60°C).

6.4. Dichloromethane.

6.5. Alumina, basic, 100 - 200 mesh, Brockman Activity I, activated at 130°C overnight and kept in a tightly sealed bottle placed inside a desiccator until use. Reactivate adsorbent once every two weeks.

6.6. Anhydrous sodium sulfate previously heated at 600°C overnight.

6.7. Reagent water - defined as water in which an interferent is not observed at the method detection limit of the parameters of interest. For example, a sample prepared by passing distilled water through a 4-cartridge Milli-Q purification unit was used.

6.8. Dibenzofuran, 99+%, Aldrich Chemicals.

6.9. Dibenzo-p-dioxin, 98+%, Ultra Scientific.

6.10. Dibenzofuran-d_8 and dibenzo-p-dioxin-d_8, 99.0 atom % D, MSD Isotopes (Division of Merck Frosst Canada Inc.). (See note 14.1)

6.11. Hexamethylbenzene, 99%, Aldrich Chemicals.

6.12. Boiling chips, anti-bumping granules of fused alumina from BDH Inc. Teflon boiling stones can also be used.

7. Extraction Procedure

7.1. Shake sample well just before a subsample is taken for analysis (See note 14.2).

7.2. To 5.00 g defoamer sample in a 500 mL round bottom flask, add 50 μL of a mixture of DBD-d_8 and DBF-d_8 internal standard surrogates of 5 ng/μL each in iso-octane, 200 mL of reagent water, and boiling chips (See note 14.3).

7.3. Place the flask into a heating mantle and attach a steam distillation condenser.

7.4. Add 3 mL of water and 2 mL of iso-octane inside the condenser.

7.5. After a steady flow of cooling water is passing through the condenser, adjust the heater control of the mantle to bring the suspension to a vigorous boiling without bumping for 3 hr.

7.6. At the end of the extraction, let the condenser cool down to room temperature. Carefully drain off as much water as possible before draining the organic extract from the condenser into a 15 mL centrifuge tube.

7.7. Using a Pasteur pipet, transfer the organic extract into a second centrifuge tube containing a small amount of anhydrous sodium sulfate while leaving the water behind in the first tube.

7.8. Rinse the condenser and the first tube twice with 2 mL aliquots of petroleum ether (P.E.) and transfer the rinsings to the second tube again.

7.9. Evaporate the combined extract down to 2 mL using a gentle stream of nitrogen and a water bath of 45°C.

8. Cleanup Procedure

8.1. Plug a 400 mm x 10 mm id glass column with a piece of glasswool. Add 1 cm of granular anhydrous sodium sulfate to the bottom.

8.2. Fill the column with 5.00 g of activated basic alumina and then with 1 cm of anhydrous sodium sulfate at the top.

8.3. Elute the column with 20 mL of P.E. and discard this fraction.

8.4. Quantitatively transfer the sample extract in step 7.9 to the column, elute the column with 50 mL of P.E. and also discard this fraction.

8.5. Continue the elution with 50 mL of 5 % (v/v) dichloromethane in P.E. and collect this fraction in a 250 mL round bottom flask as it contains all the native and deuterated DBD and DBF.

8.6. Evaporate the solvent down to ca. 5 mL with a three-stage Snyder column and a heating mantle (See note 14.4).

8.7. After cooling, transfer the extract to a 15 mL centrifuge tube and add 1 mL of iso-octane. Rinse the Snyder column and the flask with 2 x 2 mL of

P.E. and combine the rinses in the above tube.

8.8. Using a gentle stream of nitrogen and a 45°C water bath, evaporate the solvent down to just below 0.5 mL.

8.9. After cooling, add 10 μL of a 25 ng/μL solution of hexamethylbenzene recovery standard in iso-octane and adjust volume to 0.5 mL before GC-MS analysis.

9. GC-MS Analysis

9.1. An example of the GC-MS operating conditions for the analysis of DBD and DBF is given below.

Instrument:	HP5880A GC, HP5970B MSD and data system
Column:	30 m x 0.25 mm x 0.25 μm SPB-5 (Supelco)
Carrier gas:	Helium with a head pressure of 10 psi, linear velocity 32 cm/sec
Injection:	2 μL splitless (valve time 0.75 min)
Injector temp.:	250°C
Oven program:	70°C for 0.75 min then programmed to 140°C at 30°C/min, followed immediately by a 2°C/min temperature increase to 180°C. At the end of the run, bake the column at 280°C for 15 min. (See note 14.5).
Ionization:	Electron impact (70 eV)
Source temp.:	200°C
Dwell time:	100 msec
EM voltage:	200 V above autotune value
Ions monitored:	m/z 147[a] for hexamethylbenzene
	m/z 168[a] and 139[b] for DBF
	m/z 176[a] for DBF-d_8
	m/z 184[a] and 155[b] for DBD
	m/z 192[a] for DBD-d_8
where:	[a] = quantitation ion
	[b] = confirmation ion

9.2. Prepare a series of standards in iso-octane that cover the expected concentration range of DBD and DBF in the sample extracts. Each solution must also contain DBD-d_8, DBF-d_8 and hexamethylbenzene at a concentration of 500 pg/μL.

9.3. To maximize sensitivity, divide the ions into three groups or retention time windows. Monitor m/z 147 (hexamethylbenzene) in group 1, m/z 139, 168 (DBF) and 176 (DBF-d_8) in group 2 and m/z 155, 184 (DBD), and 192 (DBD-d_8) in group 3.

9.4. Inject 2 μL of the standard. Analyze the standard by GC-MS in the selected ion monitoring (SIM) mode using the above masses. A typical chromatogram is depicted in Figure 2 and the order of elution is: hexamethylbenzene, DBF-d_8, DBF, DBD-d_8 and DBD.

9.5. Analyze the samples in the same way as the standards (See note 14.6).

10. Calculations

10.1. From the integrated ion chromatograms for the calibration runs, calculate the relative response factors for the native DBD and DBF relative to the corresponding perdeuterated DBD and DBF internal standards (=RRF_x) according to the following equation:

$$RRF_x = A_{ix}/A_x * C_x/C_{ix}$$

where: A_{ix} = peak area of the quantitation ion for the appropriate labelled internal standard (m/z 176 for DBF-d_8 and m/z 192 for DBD-d_8)

A_x = peak area of the quantitation ion for the native analyte x (m/z 168 for DBF and m/z 184 for DBD)

C_x = concentration of native analyte x, pg/μL

C_{ix} = concentration of appropriate internal standard x, pg/μL

10.2. If the RRF for DBD and DBF are constant (<10% RSD) over the working range, then the RRF can be assumed to be invariant and the average RRF can be

used. Alternatively, the results can be used to plot a calibration curve of response ratios, A_{ix}/A_x vs. RRF.

10.3. Calculate the concentration of the native DBD and DBF in the sample, C_{sx}, as follows:

$$C_{sx} = RRF_x * A_x/A_{ix} * Q_{ix}/W$$

where: C_{sx} = concentration of native parameter x in the sample, ng/g

RRF_x = response factor of native parameter x relative to its perdeuterated internal standard

A_x = peak area of the quantitation ion for native parameter x in the sample (m/z 168 for DBF and m/z 184 for DBD)

A_{ix} = peak area of the quantitation ion for appropriate labelled internal standard x in the sample (m/z 176 for DBF-d_8 and m/z 192 for DBD-d_8)

Q_{ix} = amount in ng, i.e. 250 ng, of the appropriate labelled internal standard x added to the sample before extraction

W = weight of defoamer sample in grams

10.4. Calculate the percent recovery of the perdeuterated internal standards, % R_{ix}, measured in the sample extract using the formula:

$$\% R_{ix} = (A_{ix}/A_r)Spl * (A_r/A_{ix})Std * 100$$

where: A_r = peak area of the quantitation ion (m/z 147) for hexamethylbenzene recovery standard

A_{ix} = peak area of the quantitation ion for the appropriate labelled internal standard x (m/z 176 for DBF-d_8 and m/z 192 for DBD-d_8)

Spl = measurement made for sample

Std = measurement made for calibration standard

11. Confirmation of Identity

11.1. Integrate the reconstructed ion chromatograms for the quantitation ions

(m/z 168 for DBF and m/z 184 for DBD) and confirmation ions (m/z 139 for DBF and m/z 155 for DBD) in the sample. If the ratio of peak areas for the quantitation and confirmation ions at the expected retention time in the sample is within ±20% of that of an authentic standard, then the presence of the parameter is confirmed.

11.2. For the confirmation of DBD and DBF in samples of ≤ 10 ng/g, further evaporation of the final extract to 100 μL or less may be necessary.

12. Quality Control

12.1. The acceptable range of surrogate recovery is from 50 to 120% for surrogate level of 50 ng/g. If the recovery of the surrogates is outside this range, the sample should be repeated and or the entire analytical technique should be reviewed.

12.2. Method blanks should be run frequently to correct for background contamination.

13. Method Performance

13.1. Based on a 5 g sample and a final volume of 0.5 mL, the method detection limit (MDL) for both DBD and DBF is 1 ng/g. It was obtained by replicate analysis of spiked defoamer samples in a single laboratory. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and sample matrix effects.

13.2. The method has been tested for linearity of spike recovery from defoamers and has been demonstrated to be applicable over the concentration range from 1 to 100 ng/g.

13.3. In a single laboratory, six replicate determinations of DBD and DBF in a defoamer sample spiked at 10 ng/g level gave a mean recovery and a coefficient of variation of 103% and 8.9%, respectively for DBF, and 106% and 5.9%, respectively for DBD.

14. Notes

14.1. ^{13}C-labelled DBF and DBD surrogates, when available, can be used in place of the deuterated surrogates.

14.2. Since some defoamers are supplied in the form of a suspension, the sample should be homogenized by shaking to ensure a representative subsample is taken for analysis. A larger sample, i.e. 5 g instead of 1 g or less, also helps to minimize this potential inhomogeneity problem.

14.3. In order to obtain quantitative recovery of DBD and DBF by steam distillation, about 100 (0.6 to 0.7 g) boiling chips were added to the water/defoamer sample to achieve vigorous boiling without bumping.

14.4. Other techniques can be used for the evaporation of solutions containing DBD and DBF. However, the analyst must demonstrate that losses of these compounds and their surrogates are negligible in the evaporative steps.

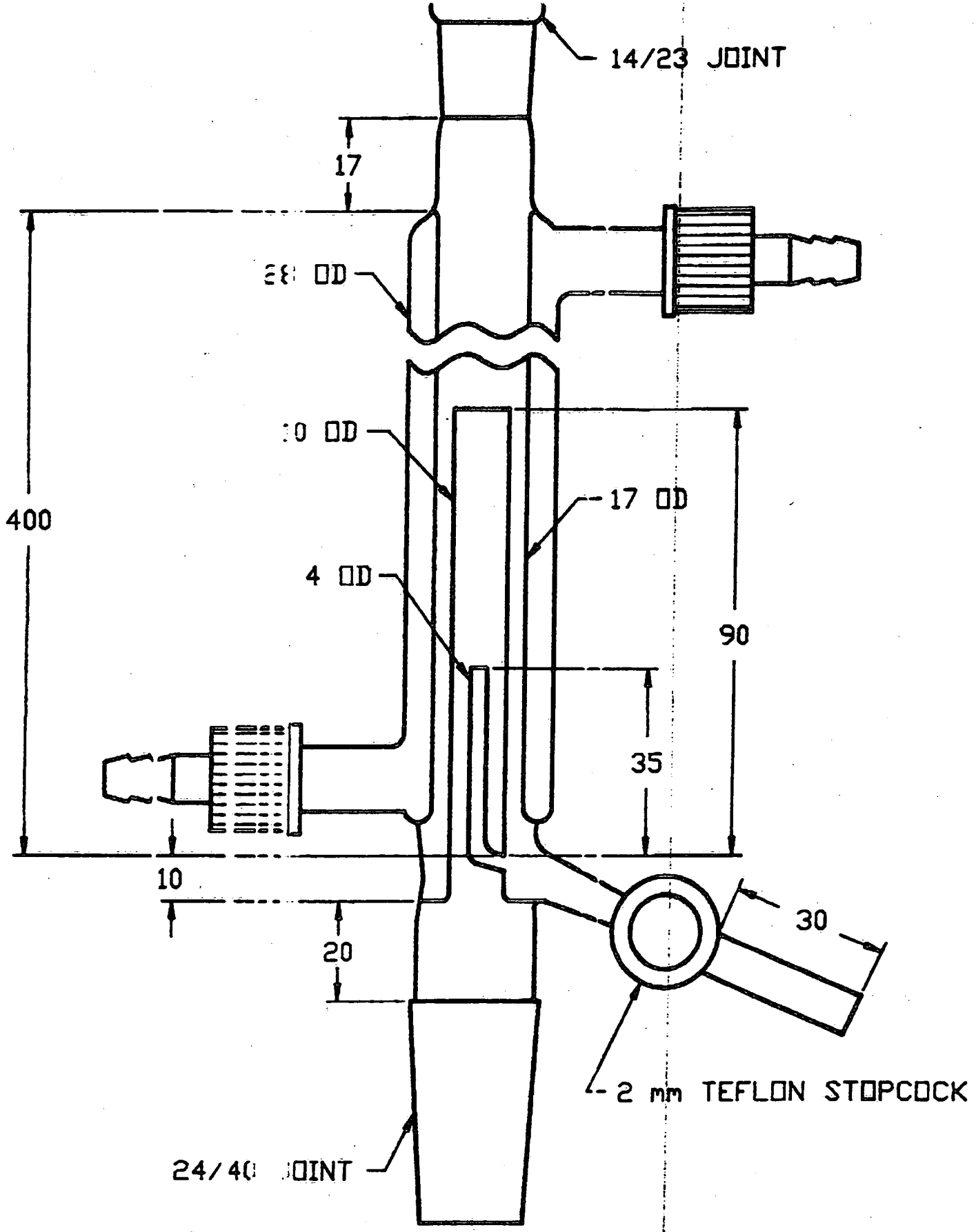
14.5. To avoid interference by the high boiling co-extractives in the GC-MS analysis, it is necessary to bake the capillary column at 280°C for 15 min before the next defoamer extract is injected.

14.6. If excessive interference is experienced in the analysis of sample extracts, the use of a GC-MS system operating at a resolution of 5000 or higher is recommended. In this case, the ions monitored are: 147.1174 for HMB, 168.0575 and 139.0548 for DBF, 176.1077 for DBF-d_8, 184.0524 and 155.0497 for DBD, and 192.1026 for DBD-d_8.

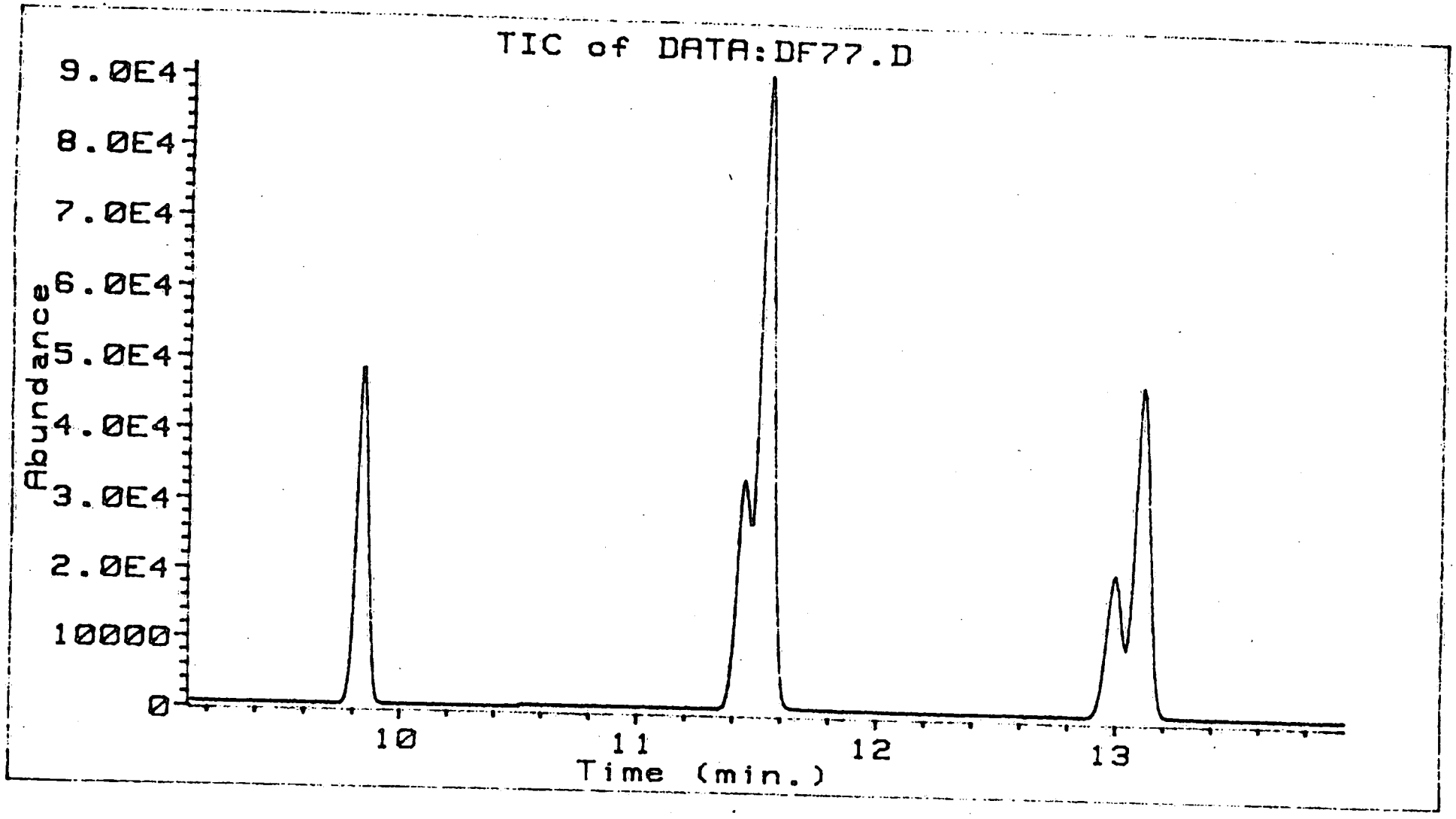
15. References

15.1. R.H. Voss, C.E. Luthe, B.I. Fleming, R.M. Berry and L.H. Allen, 1988, "Some New Insights into the Origins of Dioxins Formed During Chemical Pulp Bleaching", Pulp Pap. Can. 89(12):151-162 (1988).

15.2. D.W. Kuehl and R.C. Dougherty, Environ. Sci. Technol., 14(4), 447-449 (1980).



NOTE: ALL DIMENSIONS ARE IN MILLIMETERS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2.0E4
10000
0
Abundance
10
11
12
13
Time (min.)

FIGURE 2

APPENDIX B

Analytical Methodology

Laboratory DF002

The method is slightly different from the proposed reference method as follows.

A mixture of defoamer (typically 1 g) , water, and deuterated dibenzofuran solution D_8-DBF (50ng) is refluxed for 45 minutes with water and iso-octane (2 mls) in the extractor. The iso-octane extract and hexane rinses are transferred into disposable centrifuge tube and allowed to stand for 24 hours. The extract is then transferred with rinsings into another tube containing a small amount of sodium sulphate.

An alumina column (0.5 gm Al_2O_3 topped with a small amount of Na_2SO_4 in a disposable pipette) is pre-eluted with hexane (2 mls). The extract is added to the top of the column and the column is eluted with hexane (5mls), followed by 15% dichloromethane in hexane (4 mls), which removes the DBF and DBD. The DBF/DBD fraction is evaporated under nitrogen to almost dryness.

The extract is reconstituted with 100 μl of iso-octane immediately. The performance standard hexamethylbenzene (50 ng) is added and the sample is analyzed by GC/MS, using the selected ion monitoring mode.

Laboratory DF005

The proposed reference method was strictly followed without any difficulties. Recoveries of spiked surrogates (DBD-d_8 and DBF-d_8 for the 5 defoamer samples are within a range of 63 to 100%.

LABORATORY DF006

The defoamer samples were analyzed using both the Environment Canada Reference Method and the method normally used by us. The method that we normally use involves dissovling a portion of sample in hexane and cleaning the extract up using a macro-alumina packed column. The detection system for both methods was GC/MS/MS. It is interesting to not that two methods produce comparable results and surrogate recoveries. In fact, our method is both simpler and more cost effective.

The surrogate recoveries for defoamer samples ranged from 11 to 66% for $^{13}C_{12}$-DBD and from 0 to 73% for $^{13}C_{12}$-DBF using reference method. While the surrogate recoveries for defoamer sample ranged from 27 to 67% for $^{13}C_{12}$-DBD and from 32 to 73% for $^{13}C_{12}$-DBF suing our own method.

LABOARATORY DF008

The methodology followed was as given in the appendix A of the proposed reference method. A rotary evaporator was used in place of a 3-stage Snyder column for extract concentration.

The amounts of DBD and DBF givenare corrected for surrogate recoveries. The surrogate recoveries for defoamer sample are ranged from 70 to 81 % for DBD-d_8 and 35 to 75% for DBF-d_8.

LABORATORY DF010

$^{13}C_{12}$-DBD and $^{13}C_{12}$-DBF were used as the internal standard surrogates in place of the DBD-d_8 and DBF-d_8 reference in the method. The concentration of the 5% $MeCl_2$/pet ether wluate was accomplished by the use of ratory evaporator instead of the described three-stage Snyder method.

The qualitative and quantitative determinations were performed by HRGC/HRMS using a VG-70S at 10,000 resolution. Additional confirmation ions were also monitored. A copy of experimental page showing exact masses monitored and conditions is attached.

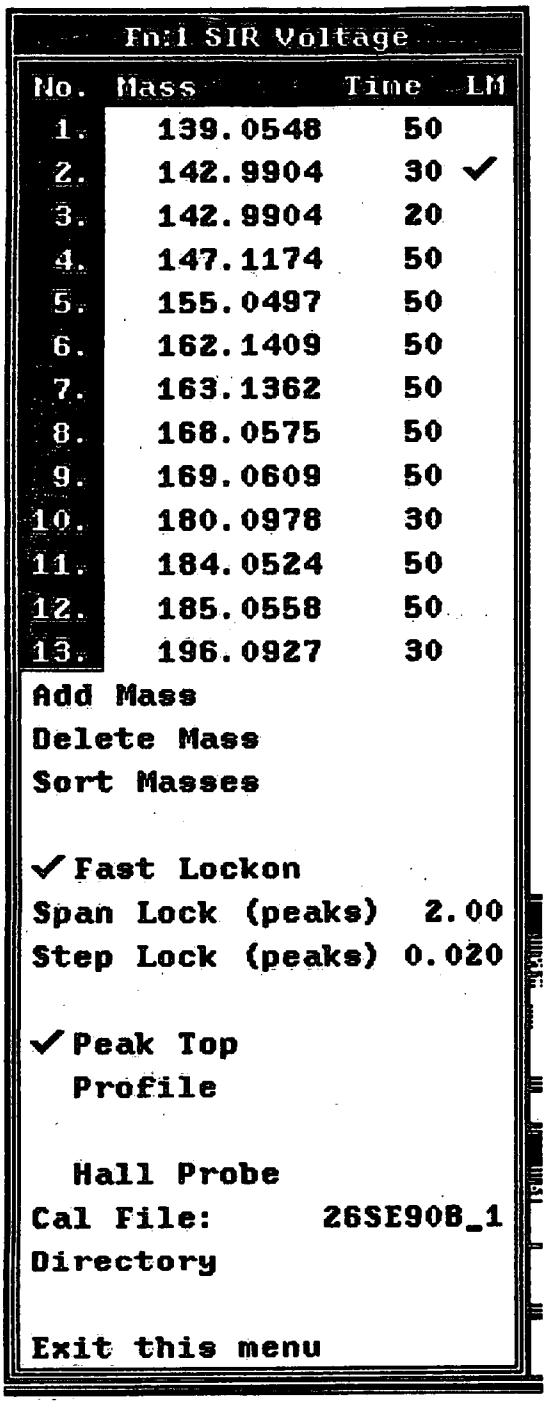
Also enclosed are the results from our in-house MDL study that was initiate prior to the analysis of defoamer samples in study DF-1.

METHOD DETECTION LIMITS[1] FOR DBD AND DBF

COMPOUND	SPIKE LEVEL (PPB)[2]	AVERAGE	STANDARD DEVIATION	MDL
DIBENZOFURAN	1	1.09	0.071	0.22
DIBENZODIOXIN	1	0.74	0.11	0.11

[1]"Method 1.11", 40 CFR 136 (49 Federal Register 43430) October 26, 1984, Appendix B to Part 136.

[2]Assuming a 5 gram sample size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5. 155.0497 50
6. 162.1409 50
7. 163.1362 50
8. 168.0575 50
9. 169.0609 50
10. 180.0978 30
11. 184.0524 50
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Sort Masses
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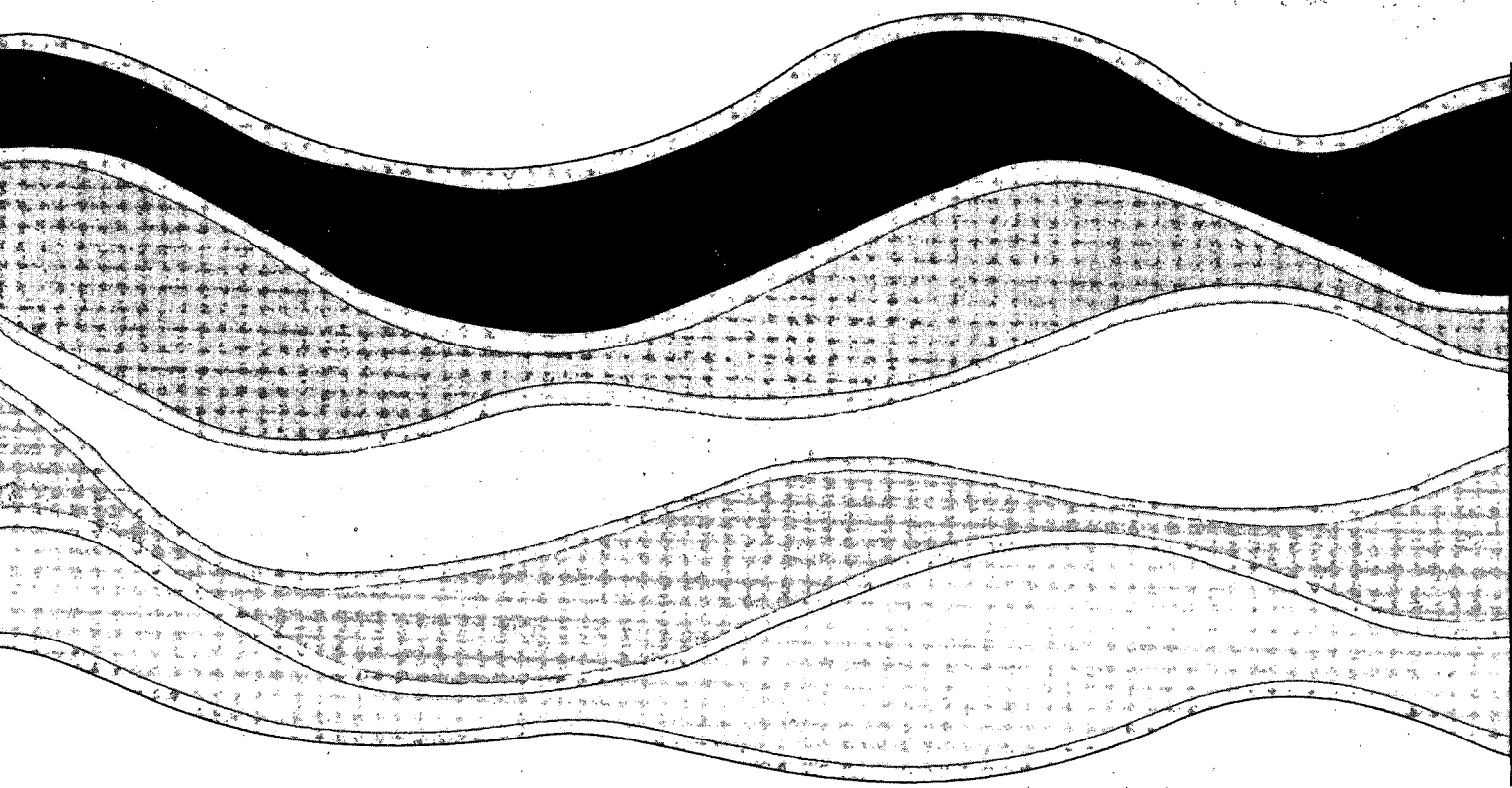

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