

Figure 4—Deviations between dissolution profiles for an ideal (monodisperse) powder and sieve cuts of $R = 0, 0.5, 1.0, 2.0,$ or 1000 as a percent of powder undissolved plotted against percent undissolved for sieve cuts having $s = 0.91$.

sible sieve cut ($s = 0.91$) might contain up to 3.0% error if the treatment of results included the assumption that sieve cuts are equivalent to monosized powders. If, however, sufficient particle-size data are presented with the dissolution data to show that R is close to or equal to 1.0, then the errors could be considered much less than 1.0%. For the next widest sieve cut ($s = 0.83$), the errors in a cube root law slope could be as great as 6.0%. Errors of 7–10%

would be possible if wider sieve cuts ($s = 0.75$) were treated according to the cube root law. If, on the other hand, the particle sizes within a sieve cut could be described, then an exact equation like Eq. 8 might be useful.

It is concluded that when dissolution data are based on sieve cuts with no further definition of powder sizes and the assumption of a monosized powder is used in the data treatment, then the results may be in error by as much as 3% even for the narrowest sieve cuts. For wider sieve cuts, errors of 6 or even 10% should not be ruled out. These errors would occur solely from the assumption that sieve cuts behave like monosized powders.

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Comparison of Analytical Methods for Residual Ethylene Oxide Analysis

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Abstract □ A round-robin study compared four methods of residual ethylene oxide analysis. Results from the six participating laboratories, along with a statistical treatment of the data, are presented.

Keyphrases □ Ethylene oxide—residual analysis, comparison of four analytical methods by six laboratories □ Sterilants—residual-ethylene oxide analysis, comparison of four analytical methods by six laboratories □ Plastics—analysis of residual ethylene oxide, comparison of four analytical methods by six laboratories

Ethylene oxide gas has had wide use as a sterilant in the health field. Since many sterilized items are used in such a way that they contact sensitive human tissue, the residual ethylene oxide should be at a safe

level since ethylene oxide is in itself toxic. The Z79 Subcommittee on Ethylene Oxide Sterilization (an Association for the Advancement of Medical Instrumentation group) is concerned with the safe use of

Table I—Comparison of Methods by Lab-1

Sample	Ethylene Oxide, ppm			
	Head Space	Vacuum	Acetone	Gravimetric
1a	6310	7410	7900	7520
b	6980	5780	8860	8220
c	7330	10900	7450	8870
d	7290	12700	7560	8270
e	6280	7940	7300	7620
f	7230	8740	7140	8490
2a	1240	1930	1640	1800
b	495	818	486	1000
c	520	632	828	570
d	491	601	644	600
e	1860	2280	2000	1700
f	623	703	571	570
3a	488	537	650	530
b	202	217	271	290
c	171	234	382	310
d	472	622	701	210
e	69.1	75.9	140	180
f	728	759	800	1200
4a	40.0	51.5	62.1	ND ^a
b	55.6	63.2	89.1	ND
c	37.1	49.3	64.1	ND
d	81.3	139	137	ND
e	87.0	120	120	ND
f	63.7	76.4	77.9	ND
5a	6.5	7.12	13.0	ND
b	3.61	5.28	8.4	ND
c	5.23	6.45	10.0	ND
d	5.55	8.22	13.5	ND
e	4.41	5.40	8.8	ND
f	4.57	6.09	10.0	ND

^aND = not detected.

ethylene oxide. Late in 1972, an ad hoc committee was formed within Z79 made up of industrial, regulatory, and academic representatives. One charge to this committee was to establish uniform analytical test procedures for ethylene oxide, ethylene glycol, and 2-chloroethanol in plastic materials. The present report deals with the first part of this objective: the evaluation of methods for residual ethylene oxide analysis.

Methods for residual ethylene oxide analysis fall into four categories: colorimetric (1), titrimetric (2), gravimetric (3), and GC (4–12). A round-robin study was designed by the ad hoc committee to evaluate four of these methods. The major objective of this round robin was not to point out a single method to be used by the entire industry but rather to point out which method or methods could be used to check the validity of an in-house method. Six laboratories participated¹.

EXPERIMENTAL

Methods—Four methods of analysis were studied: a gravimetric method (3) and three GC approaches (9–12). The main difference between the GC methods is sample workup. The vacuum extraction technique (10) vaporizes the ethylene oxide and traps it in a cold coil before injection into the gas chromatograph. The head-space method (11) extracts the ethylene oxide by vaporizing it into the head space of an enclosed vial. An aliquot of the head-space gas is injected into the gas chromatograph. The vial is purged with nitrogen, and the process is repeated until all of the ethylene oxide is extracted from the sample.

¹ The laboratories and investigators were: Castle Co., Mr. J. Whitbourne and Miss Carolyn Eastman; 3M Co., Mr. G. E. Schwagerl and Mr. W. Peterson; Johnson & Johnson, Mr. A. Heutteman, Dr. G. Kaplan, and Mr. J. Weinberger; Ethicon Inc., Dr. S. J. Romano, Mr. J. A. Renner, Mr. P. J. Rafferty, and Mr. R. McGunnigle; Huntingdon Research Center, Dr. W. F. Stephen, Mr. M. Malik, Mr. R. O'Melia, and Mr. J. Morris; and C. R. Bard Inc., Mr. W. Greif.

Table II—Comparison of Methods by Lab-2

Sample	Ethylene Oxide, ppm			
	Head Space	Vacuum	Acetone	Gravimetric
1a	2950	3500	2040	7890
b	2950	46	978	9530
c	3410	839	1154	8180
d	1470	1520	1890	9690
e	5270	1.5	4515	6210
f	2880	475	537	7470
2a	494	ND ^a	408	15900
b	282	4	238	5420
c	631	793	414	5900
d	552	ND	611	40600
e	580	368	442	5880
f	471	ND	658	4290
3a	528	ND	631	—
b	232	106	149	4820
c	313	56	439	2150
d	141	101	124	4040
e	258	24	146	4610
f	105	77	126	2520
4a	40	32	31	1080
b	46	23	47	50700
c	44	40	27	—
d	19	11	30	470
e	22	22	57	1560
f	39	25	34	—
5a	4.5	1.6	3.3	1910
b	4.8	ND	4.0	4420
c	5.2	2.6	3.2	4840
d	4.0	1.2	2.7	3440
e	4.6	4.3	3.9	4200
f	4.6	0.5	4.2	3100

^aND = not detected.

Both the vacuum extraction and the head-space methods exhaustively extract the ethylene oxide. In the acetone extraction technique (9, 12), the ethylene oxide in the sample partitions between the sample and the acetone. An aliquot of the acetone solution is injected into the gas chromatograph. This extraction is not generally considered to be exhaustive unless the sample dissolves in the acetone.

Materials—In some cases, analytical results depend on the material being analyzed; *i.e.*, not all of the ethylene oxide may be extracted into acetone due to a highly polar sample. The ad hoc committee discussed this matter at length. The final decision was to use polyvinyl chloride tubing². Specifically, the material was 0.3 cm (0.125 in.) i.d. × 0.6 cm (0.25 in.) o.d. [0.15-cm (0.06-in.) wall]. The material was to contain approximately 5000, 1000, 500, 100, or 10 ppm of ethylene oxide.

The tubing was cut into 2-cm lengths and then into quarters along the length. These quarters weighed about 0.25 g. They were placed into a small glassine envelope and sterilized with pure ethylene oxide gas in a small laboratory sterilizer³. At predetermined times during degassing, samples were removed from the sterilizer and the contents of each envelope were placed into 7-ml vials with aluminum-lined septa caps. The vials were then stored in dry ice.

All samples were prepared at one location. Each laboratory received enough material to perform six replicate analyses at each level of ethylene oxide. The samples were shipped by air in insulated dry-ice chests and were received within 24 hr. Samples stored in this manner were shown to be stable for at least 18 days.

The laboratories were instructed to analyze the samples immediately upon opening a vial. All laboratories handled the samples in the same manner as follows. A vial was removed from the dry-ice chest, allowed to thaw for 5 min, and opened. One piece was weighed immediately for the gravimetric method. A second piece was placed in a tared vial of acetone for the acetone extraction method. Another was placed into a tared empty vial for head-space analysis. The final piece was weighed into the stoppered vacuum extraction flask.

The head-space and vacuum extraction methods were run concurrently, followed by the acetone extraction method. The gravimetric samples were stored in an open, dust-free area and weighed

² Tygon S-50-HL, Norton, Akron, Ohio.

³ A modified Amsco Cryotherm unit.

Table III—Comparison of Methods by Lab-3

Sample	Ethylene Oxide, ppm			
	Head Space	Vacuum	Acetone	Gravimetric
1a	3732	7433	7801	10022
b	2451	3192	5003	7757
c	3593	5621	7904	12767
d	3570	—	10170	13341
e	2032	3352	5643	4464
f	3763	4604	9416	14895
2a	2421	1204	757	4236
b	2952	1204	1052	3840
c	454	1695	1225	2656
d	3476	1342	892	5219
e	460	1201	967	3328
3a	97	1011	513	2910
b	160	1053	356	2358
c	35	592	240	4459
4a	NR ^a	NR	789	3670
b	NR	NR	83	1702
c	143	349	127	NR
5a	NR	NR	58	3813
b	NR	NR	27	1835

^aNR = no response.

to constant weight. All of these procedures were required to ensure the four pieces in a vial would be at the same level of ethylene oxide when analyzed. In this way a comparison of the four methods was possible. It was assumed that the vial-to-vial variability would be large since, in effect, these samples are different although at the same nominal level of ethylene oxide. A total of 4 weeks was allowed for the analyses.

RESULTS AND DISCUSSION

Tables I-VI show the raw data reported by the six laboratories. The laboratories are only identified by number, and the order of numbering does not correlate with the order in Footnote 1. Lab-6 only performed the head-space and acetone methods, and each reported result is the average of two determinations. Lab-3 did not perform the required six replicates at the lower levels of ethylene oxide.

Upon examining the data, it is obvious that the original hypothesis of a large laboratory-to-laboratory variability was correct, although all laboratories found the five ethylene oxide levels to be in

Table IV—Comparison of Methods by Lab-4

Sample	Ethylene Oxide, ppm			
	Head Space	Vacuum	Acetone	Gravimetric
1a	3588	2285	5362	4455
b	3183	3459	5697	8876
c	4291	4525	5198	7789
d	2338	4469	2914	6760
e	1795	2887	3260	3572
f	2447	3238	4661	4930
2a	878	841	532	4756
b	606	1036	472	3594
c	388	558	346	1013
d	1663	1132	985	2823
e	520	446	382	1602
f	691	590	528	1402
3a	211	141	285	3355
b	115	214	114	2287
c	116	77	70	1410
d	147	187	140	1658
e	290	154	248	798
f	160	140	171	1368
4a	21	32	34	155
b	19	14	26	2205
c	15	18	18	1458
d	119	40	96	4301
e	57	24	50	544
f	22	6	255	2952
5a	16	4	81	1975
b	19	3	23	1251
c	9	3	44	1733
d	6	4	24	369
e	4	4	15	1664

Table V—Comparison of Methods by Lab-5

Sample	Ethylene Oxide, ppm			
	Head Space	Vacuum	Acetone	Gravimetric
1a	4431	5552	4661	6990
b	7273	7245	7144	9187
c	4499	5764	5246	6988
d	7052	7712	6945	9090
e	5978	7300	6330	6889
f	5521	6402	6098	2994
2a	709	810	886	969
b	1087	823	609	3479
c	1193	1342	1018	8155
d	859	998	1024	2977
e	1445	1426	1151	—
f	1083	1271	1109	7142
3a	354	125	156	7033
b	261	189	211	6298
c	574	441	583	8230
d	298	298	283	3659
e	323	339	387	1914
f	233	233	219	4762
4a	62	47	56	1875
b	124	97	102	1827
c	118	87	124	984
d	81	116	82	3157
e	77	52	45	—
f	106	94	100	—
5a	15.9	11.4	8.4	5976
b	14.6	7.3	10.8	—
c	13.3	7.7	10.8	1513
d	10.9	6.8	5.7	—
e	16.4	8.6	7.6	725
f	15.2	21	10.4	4483

the same order of magnitude for all methods. Another obvious fact is that the correlation between the GC methods and the gravimetric method breaks down below the highest levels of ethylene oxide studied.

Since most statistical treatments depend on equal replication and similar treatments, the data from Lab-6 could not be included in the statistical evaluations. For this same reason, only the two highest levels from Lab-3 were included. Since the noncorrelation of the gravimetric method is obvious, this method was not included in the statistical evaluation. These exclusions somewhat simplified the data, but a monumental statistical problem remained.

Three approaches were taken in an attempt to make some statistically significant statements concerning the data. The data averages were graphed, subjected to analysis of variance, and subjected to a nonparametric ranking treatment.

Graphing of Averages—An accepted method of testing data interactions is the graphing of the averages. The following assumptions were made: (a) comparison between methods can only be

Table VI—Comparison of Methods by Lab-6

Sample	Ethylene Oxide, ppm	
	Head Space	Acetone
1a	3860	3150
b	4902	4057
c	4096	3865
d	3865	3380
2a	648	841
b	438	442
c	465	411
d	412	415
3a	95	86
b	245	237
c	171	222
d	182	238
4a	28	31
b	60	70
c	57	59
d	49	57
5a	8	4
b	13	6
c	5	2
d	5	4

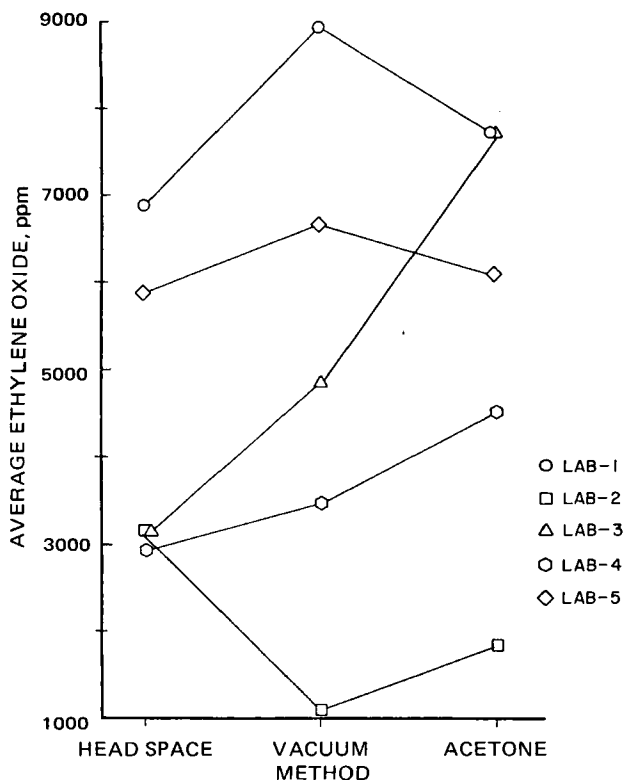


Figure 1—Level 1 interaction.

done within an ethylene oxide level, and (b) all samples within a level are identical or at least less variable than the variability between methods.

For each set of data, at each level, a method average was computed for each laboratory. These averages were then plotted versus the method on an arbitrary scale (Figs. 1-5). There were no obvious trends, i.e., one method was not always low or high. What is obvious is that some laboratories were able to get consistently good correlations between methods while others were not.

The next step was to take the method average for each level of ethylene oxide, regardless of the laboratories, and plot these values versus method (Fig. 6). These data show that the methods gave similar results; however, the acetone extraction technique usually gave higher results than the other two methods. This result could be due to the loss of ethylene oxide in both the vacuum and head-space methods. Conversely, acetone impurities or other factors may cause high ethylene oxide results in the acetone method. The important point to note is that sample-to-sample and laboratory-to-laboratory variability was high, so only very general trends can be noted.

Analysis of Variance—Analysis of variance allows one to test statistically the significance of differences between sets of data by computing ratios of variance (*F* ratio test) and comparing these to critical values found in tables. The following qualifications were made in testing the data: (a) levels were tested individually, (b) all results reported as ND were assumed to be zero, and (c) where a data point was missing (only in a few cases), the method average at that level was substituted. With these qualifications, the data were coded and fed into a computer for processing. Table VII gives the results.

A significant difference among methods was found at the very high and very low levels of ethylene oxide. In the area where the industry generally works (1000-50 ppm), no significant difference was found among methods.

A significant difference was found among all laboratories at all levels. This result was expected since samples were "different" and the time of analysis was not the same. However, the possibility that the laboratories made errors in standardization cannot be ruled out. In this case, all results would be internally consistent but a significant difference in the results from the laboratories would occur.

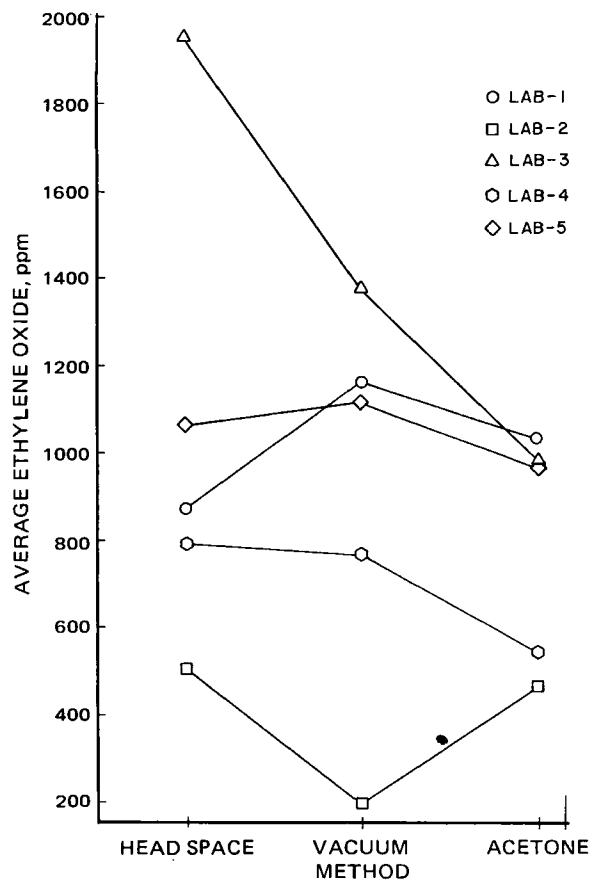


Figure 2—Level 2 interaction.

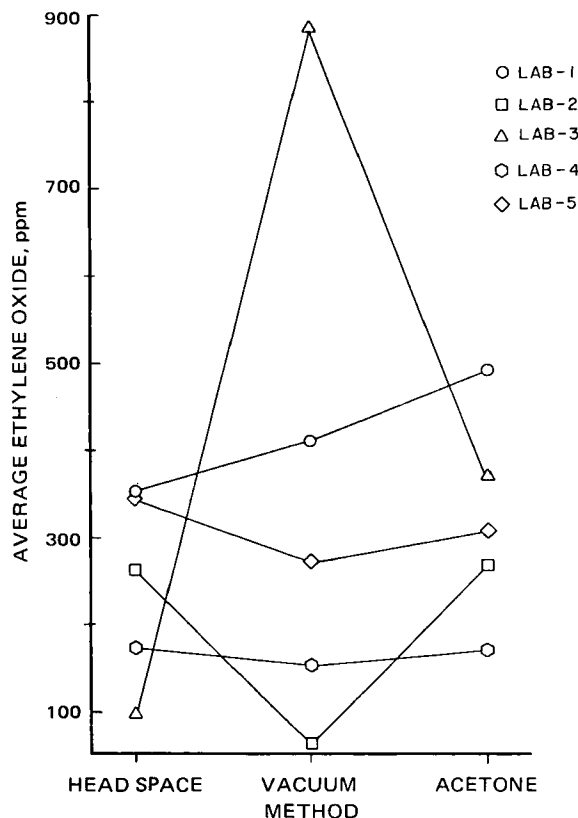


Figure 3—Level 3 interaction.

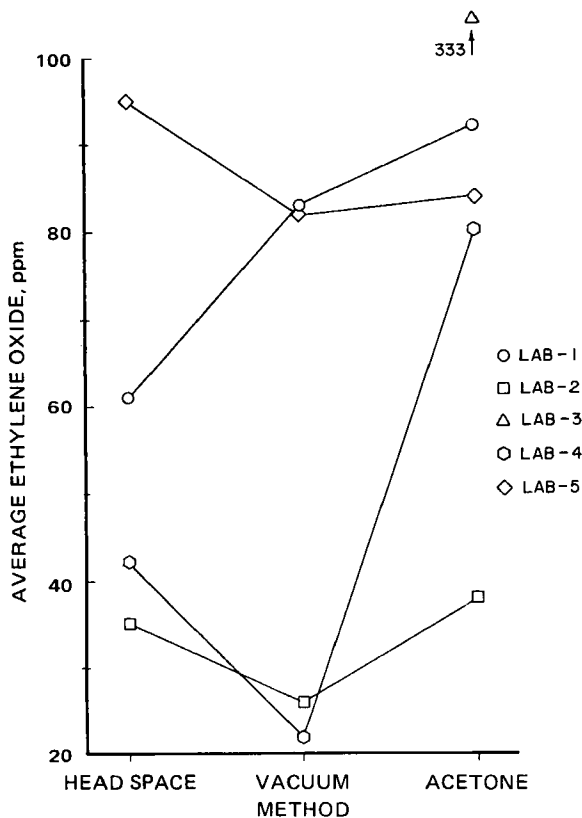


Figure 4—Level 4 interaction.

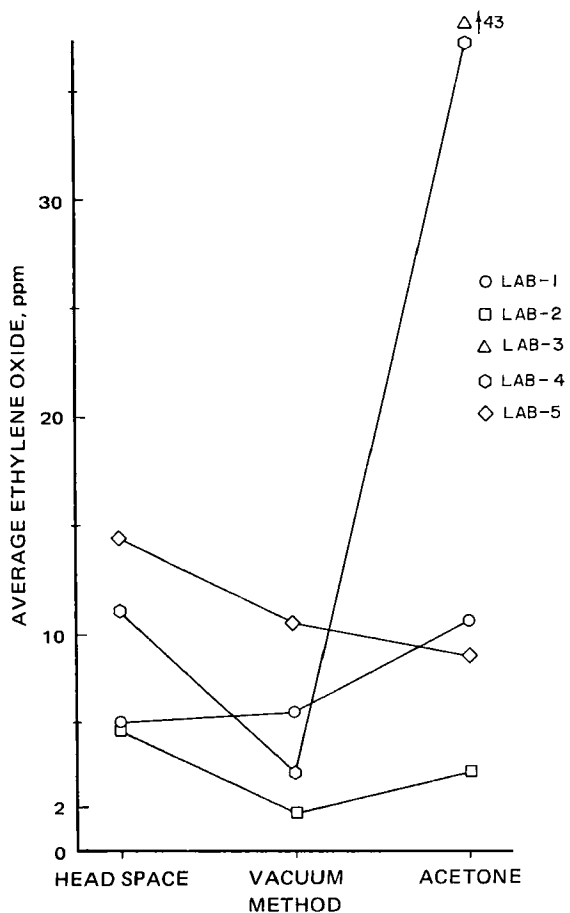


Figure 5—Level 5 interaction.

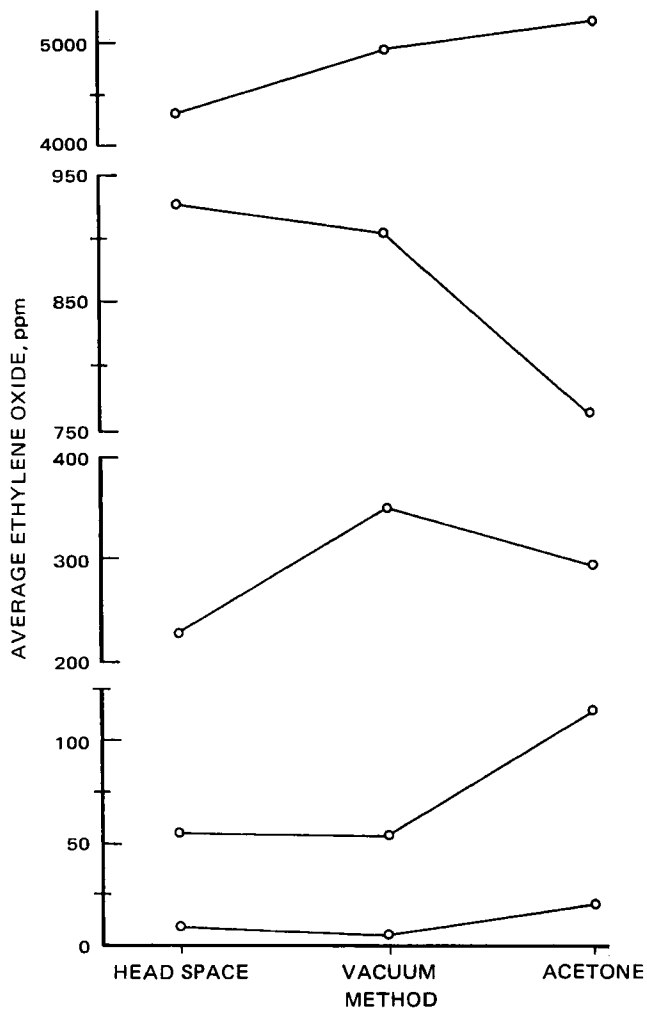


Figure 6—Method average.

No significant difference was found among the six replicates within a method in each individual laboratory. This finding is surprising, since there appears to be large differences among replicates. They are, after all, different samples taken from different parts of the sterilizer. This point was conceded at the start of the study, since the only samples thought to be the same are the four samples in the same vial used for the four methods. This data analysis, however, indicates that the differences among samples at a particular level are not significant. The overall interaction showed no significant differences among laboratories, replicates, and methods.

This data analysis indicates the three methods give comparable results and supports the claim that the methods are equivalent in the hands of an experienced operator.

Nonparametric Ranking—Ranking is a rapid statistical procedure based on the assignment of serial numbers, or ranks, to the experimental observations arranged in order of their magnitude. Conclusions are drawn about the significance of differences between categories under study from the sums of the assigned ranks. Such methods are called nonparametric or distribution free. They are simple to compute and require few assumptions about the nature of the sampled population (13).

Results of the ranking of data indicated there was no significant difference among the methods overall. However, some slight differences were detected within each laboratory. For example, Lab-1 found the head-space method to be lower in general than either the vacuum or acetone extraction method (possibly the vials used leaked during heat up), and Lab-2 found the vacuum method gave generally lower results than either of the other two methods (ethylene oxide may have been lost through a warm trapping coil).

These three data treatments are by no means the only statistical

Table VII—Analysis of Variance

Source of Variance	Level of Ethylene Oxide ^a				
	1	2	3	4	5
Methods	+	—	—	—	+
Laboratories	+	+	+	+	+
Methods and laboratories	+	—	—	—	+
Replicates	—	—	—	—	—
Methods and replicates	—	—	—	—	—
Laboratories and replicates	+—	—	+	—	—
Methods, laboratories, and replicates	—	—	—	—	—
Grand means	4980	918	272	61.7	9.79

^a+ indicates a significant difference; — indicates no significant difference.

treatments which could be applied to the data, but other treatments probably would draw the same conclusions.

Regardless of the results of this study, one must deal with realistic samples on a daily schedule, and each method has its strong and weak points. The following is a subjective evaluation of each technique studied in the round robin.

The gravimetric method presents the ultimate in simplicity, since it does not use complex apparatus or equipment. However, it is a nonspecific method. Therefore, it also measures other volatile residues such as carbon dioxide or dichlorodifluoromethane⁴ from the sterilizing gas and absorbed water as ethylene oxide. The tendency is toward positive errors.

Acetone extraction GC is a relatively simple procedure with which most laboratories have little trouble. However, acetone has impurities which could interfere in the analysis, particularly at low levels. The solvent and plasticizers extracted reduce the life of GC columns. Unless the sample is swollen or dissolves in acetone, one cannot be certain that all of the ethylene oxide has been extracted. Therefore, acetone extraction is not a universal method.

The head-space method is rather simple to perform, and it is very fast and sensitive. The GC columns last indefinitely since no solvents are injected. It is a universal method. Nevertheless, errors may occur due to leaky vials or syringes. Also reabsorption of ethylene oxide on the sample may occur if the sample is allowed to cool.

The vacuum extraction method is a universally applicable method and gives long GC column life and extreme sensitivity since sample size is unlimited. It has the disadvantage of using complex equipment, which could be subject to leaks and, therefore, low results. It also requires more operator time than the other methods.

CONCLUSIONS

In general, it may be concluded from this work that the three

⁴ Freon-12.

GC methods give comparable results in the hands of experienced operators. The correlation between the gravimetric and the GC methods breaks down below the highest levels of ethylene oxide studied. At the lower levels of ethylene oxide, the gravimetric method seems to give very high results overall. At higher ethylene oxide levels than were studied here, *i.e.*, above 0.5% ethylene oxide, the correlation possibly could be better. One should be cautious in its use, however, due to the large errors possible at low levels.

In general, for polyvinyl chloride, the use of whichever GC method feels the most comfortable is recommended. If GC is not available, then the method of choice should be checked against any or all of the GC methods used in this study. Also, since this study pointed out the large variability that could be expected between laboratories and samples, care must be exercised in choosing a statistically significant number of samples when validating an in-house method.

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