Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1967, 36, 963-974

The Second International Standard for Oxytetracycline

J. W. LIGHTBOWN,[1] J. M. BOND[1] & B. GRAB[2]

The National Institute for Medical Research, London, was requested by the WHO Expert Committee on Biological Standardization to establish a second International Standard for Oxytetracycline, since stocks of the first International Standard were depleted. A batch of 500 g of oxytetracycline dihydrate obtained in 1963 was distributed into ampoules in 125-mg amounts; the ampoules were filled with dried nitrogen and sealed. The usual procedure of exhaustively drying the material was omitted since removal of water of hydration adversely affects stability of oxytetracycline dihydrate. The proposed replacement material has been assayed biologically in terms of the first International Standard in 9 laboratories in 7 countries by means of plate-diffusion and turbidimetric assays.

Significant heterogeneity of the potencies obtained by the different laboratories and within 3 laboratories was not attributable to any known differences in assay technique; the weighted mean potencies obtained by all laboratories were within a range of ±2% of the over-all mean potency.

The potency of the second International Standard is approximately 3% less than that of the first International Standard but no significant difference in composition or purity could be detected by a variety of chemical and physical analytical methods. The material has been established as the second International Standard for Oxytetracycline with a defined potency of 880 IU/mg. The International Unit of Oxytetracycline is defined as the activity of 0.0011364 mg of the second International Standard of Oxytetracycline.

Since in 1963 stocks of the first International Standard for Oxytetracycline were becoming depleted, material suitable to serve as a second international standard was obtained. The sample, consisting of approximately 500 g of oxytetracycline dihydrate, was donated by the Pfizer Group, United Kingdom, through the offices of Mr B. A. Forder. In October 1963 the WHO Expert Committee on Biological Standardization (1964) noted that a second international standard was necessary and authorized the National Institute for Medical Research, London, to arrange a collaborative assay of the material which it had obtained, against the first International Standard.

THE PROPOSED SECOND INTERNATIONAL STANDARD FOR OXYTETRACYCLINE

The sample of oxytetracycline dihydrate was received at the National Institute for Medical Research in a single container in July 1963. The following data were supplied by the manufacturer:

Potency (microbiological)	903 " micrograms "/mg (i.e., 903 IU/mg) [3]
Potency (chemical)	917 micrograms/mg
Specific rotation	−199°
pH	5.95
Volatile	6.01%
Ash	0.07%
Heavy metals	< 10 ppm
Iron	< 5 ppm
Transmission	94%
Chloride	0.1%
Quaternary compounds	Nil

The sample was kept in its original sealed container at −10°C until March 1964, when it was distributed into 3700 ampoules each containing approximately 125 mg. The relative humidity during this operation was 51%. Following the procedure used for the first International Standard, the material was not dried since it was considered to be more stable

[1] Division of Biological Standards, National Institute for Medical Research, London, England.

[2] Health Statistical Methodology, World Health Organization, Geneva, Switzerland.

[3] See Humphrey et al. (1955).

in the form of the hydrate. The ampoules were therefore immediately constricted, filled with pure dry nitrogen and sealed by fusion of the glass. After sealing, the ampoules were tested for leaks and stored at −10°C.

When exposed to an atmosphere of 55% relative humidity in an unstoppered weighing bottle, a sample of the International Standard increased in weight by approximately 0.06% w/w in 10 minutes and about 0.4% w/w in 60 minutes.

THE COLLABORATIVE ASSAY

Participating laboratories were asked to estimate the potency of the proposed second international standard for oxytetracycline in terms of the International Standard for Oxytetracycline. They were supplied with 6 ampoules of each of the two materials. As is customary in these collaborative assays, laboratories were asked to use assay methods such that each assay would contain sufficient information to provide from its own internal evidence an estimate of potency and fiducial limits to that estimate. The assays were to be constructed so that they provided evidence of linearity and parallelism and so that all dilutions of standard and unknown which formed the basis of the assay were tested, with or without replication in a single unit of the experiment.

Nine laboratories in 7 countries agreed to participate in the collaborative study. They are listed in the annex together with the names of the workers who participated. Throughout the text of this report the laboratories are arbitrarily referred to by a code number which is not necessarily related to the order of appearance in the annex.

STATISTICAL ANALYSIS

Data

A total of 109 assays was performed by 9 collaborating laboratories. The diffusion method was used by 6 laboratories, while the other 3 used the turbidimetric method in their assays. In each assay the proposed second international standard for oxytetracycline was compared with the International Standard, established in 1954 with a potency of 900 IU/mg (Humphrey et al., 1955). The number of assays performed and details of methods used in each laboratory are shown in Table 1.

Method of statistical analysis

The standard statistical method for parallel-line assays was used for the analysis of the experimental results. For each individual assay the potency of the proposed second international standard and its precision were estimated and significance of departure from linearity and parallelism of dose-response regression lines were statistically tested. For assays based on the diffusion method, the diameter of the zone of inhibition of growth was used as the response metameter and was analysed in relation to the logarithm of the dose. When the turbidimetric method was used, the opacity reading on the electrophotometer was taken as the response metameter and was analysed in relation to the logarithm of the dose.

The χ^2 test (Humphrey et al., 1953) was applied to study the homogeneity of the potency estimates obtained within the same laboratory.

For laboratories whose results did not show heterogeneity, the average logarithmic potency was computed by weighting each potency value with the reciprocal variance based on the internal evidence of the assay. The variance of the average potency was then simply the reciprocal of the total of individual weights.

For laboratories whose results were found heterogeneous, the between-assay variance was computed and the weight of each logarithmic potency value was redetermined by taking the reciprocal of the total variance, i.e., including both within- and between-assay variances according to the methods described by Bliss (1952). The new weights were applied to the individual potencies in the computation of the average logarithmic potency and its variance was taken as the reciprocal of the sum of the new individual weights.

The same method was used for the computation of the over-all combined weighted log potency estimate from the laboratory average log potencies. The corresponding unweighted average estimate was also established for the purpose of comparison.

Results

Of the 109 assays analysed, the highest potency (1039 IU/mg) and the lowest potency (734 IU/mg) were obtained by Laboratory 9. The remaining potencies fell within the range 786 IU/mg to 991 IU/mg.

Among the 3 laboratories which used the turbidimetric method, 2 (Laboratories 8 and 9) did not apply any conventional design for their assays, the

TABLE 1

DETAILS OF ASSAY METHODS PERFORMED IN DIFFERENT LABORATORIES

Lab. No.	Test organism	Type of assay	Dose levels of each preparation (IU/ml)	Structure of assay	No. of assays performed	Temperature of incubation (°C)	Assay medium
1	*Micrococcus pyogenes aureus* (209P)	Diffusion; circular plates (diam. 140 mm), cups	2, 4, 8	3+3. One of each concentration on a plate. 5 plates per assay.	36	37	Bacto-Penassay seed agar (Difco)
	Bacillus pumilus (NCTC 8241)	As above	1.5, 3, 6				
2	*Staphylococcus aureus* (ATCC 6538P)	Turbidimetric	0.192, 0.240, 0.300	3+3. 3 replicates for each assay.	9	37	Peptone broth
3	*Bacillus cereus* var. *mycoides* (ATCC 9634)	Diffusion; Petri dishes, cylinders	0.125, 0.250, 0.500	3+3. One of each concentration arranged at random on a plate. 9 plates per assay.	8	37	Seed agar No. 01-176 Base Agar with low pH No. 01-550
4	*Bacillus cereus* (NCIB 8145)	Diffusion; Petri dishes (10 cm diam.), cups	1.0, 4	2+2. One of each concentration on a plate. 16, 15 or 8 plates per assay.	12	23 for 3 hours then 37	Medium No. 8, Grove & Randall (1955)
	Bacillus subtilis (ATCC 6633)	As above	2.5-10	As above.		As above	Medium A, pH 6.6, *British Pharmacopoeia* (1963)
5	*Bacillus pumilis* (NCTC 8241)	Diffusion; square plates (10 in × 10 in; 25 cm × 25 cm), fish-spine beads	62.5, 250	2+2. 16 replicates of each concentration arranged in 8 × 8 Latin square design. One plate per assay.	16	37	Medium A, pH 6.6, *British Pharmacopoeia* (1963)
	Bacillus cereus (NCIB 8035)	As above	5.0, 20.0	As above.	8	37	Peptone meat extract glucose agar, pH 6.4
6	*Bacillus cereus* (NCIB 8035)	Diffusion; square plates (12 in × 12 in; 30 cm × 30 cm) holes	0.4, 0.6, 0.9	3+3. 6 replicates of each concentration arranged in 6 × 6 Latin square design. One plate per assay.	4		Nutrient agar medium
7	*Bacillus pumilus* (NCTC 8241)	Diffusion; Petri dishes, cylinders	5.0, 20.0	2+2. One of each concentration on a plate. 6 plates per assay.	6	2 hours ambient temp. then 16 hours at 37	Nutrient agar medium
8	*Klebsiella pneumoniae* (PCI 602 - ATCC 10031)	Turbidimetric	0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08	6 or 7 doses for the standard solution; 3 or 4 doses for the test solution. 3 replicates per assay.	4	37	Peptone broth similar to Medium 3 produced by Difco
9	*Staphylococcus aureus* (NCTC 6571)	Turbidimetric	0.25, 0,50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 3.00, 4.00, 5.00	11 doses. 3 replicates per assay.	6	37	Peptone broth

results of which are shown graphically in Fig. 1 and Fig. 2. In each case the plotted points have been simply connected. No attempt has been made to fit the corresponding regression lines.

Doses tested by Laboratory 8

International Standard: 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 (2 assays only), 0.08 IU/ml

Proposed international standard: 0.03, 0.04, 0.05, 0.06 IU/ml

The statistical analysis was performed once on the basis of doses 0.03 IU/ml, 0.04 IU/ml, 0.05 IU/ml and 0.06 IU/ml (4 × 4 assays) and once with doses 0.03 IU/ml, 0.04 IU/ml and 0.05 IU/ml (3 × 3 assays). The combined potencies did not differ practically for the two series of doses. However, the 4-dose assays showed highly significant departure from linearity while the 3-dose assays were satisfactory for both parallelism and linearity. Results of the 3 × 3 assays are therefore incorporated in this report.

FIG. 1. LOG-DOSE-RESPONSE LINES FOR TURBIDIMETRIC ASSAYS PERFORMED BY LABORATORY 8

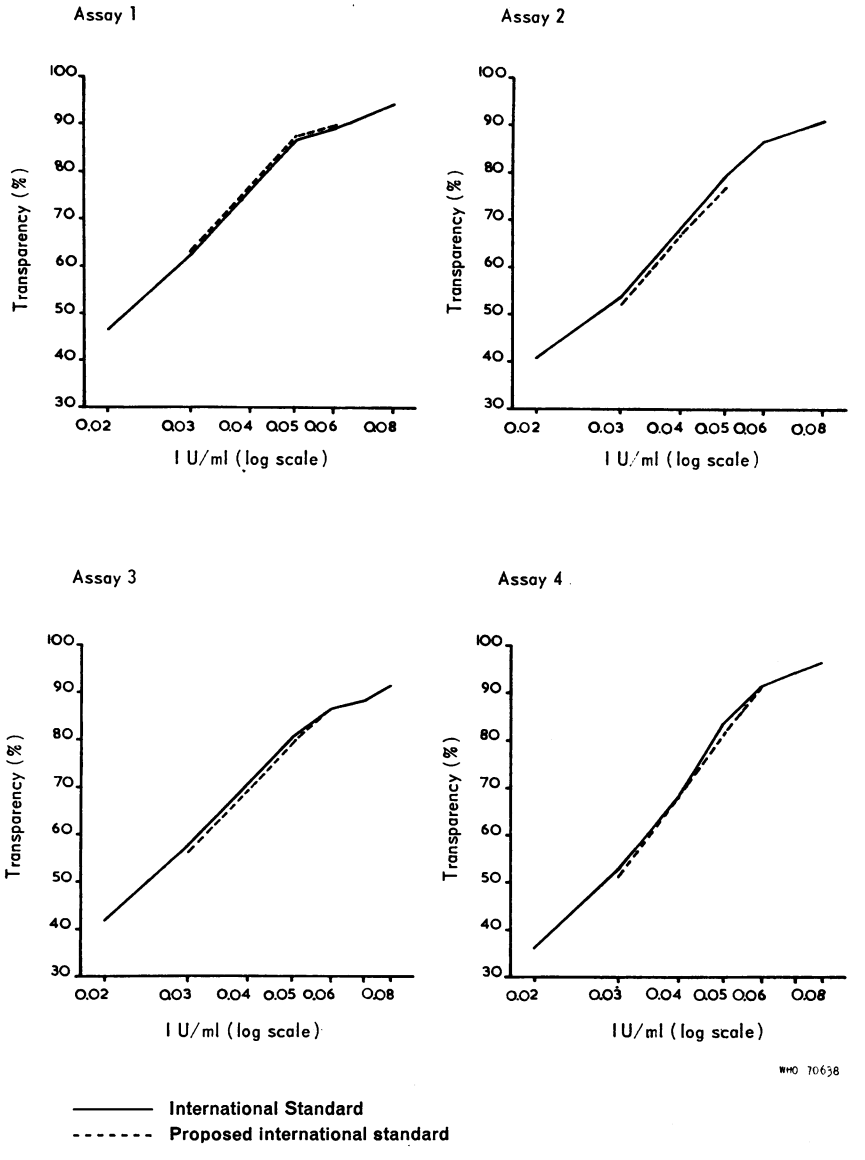


FIG. 2

LOG-DOSE-RESPONSE LINES FOR TURBIDIMETRIC ASSAYS PERFORMED BY LABORATORY 9

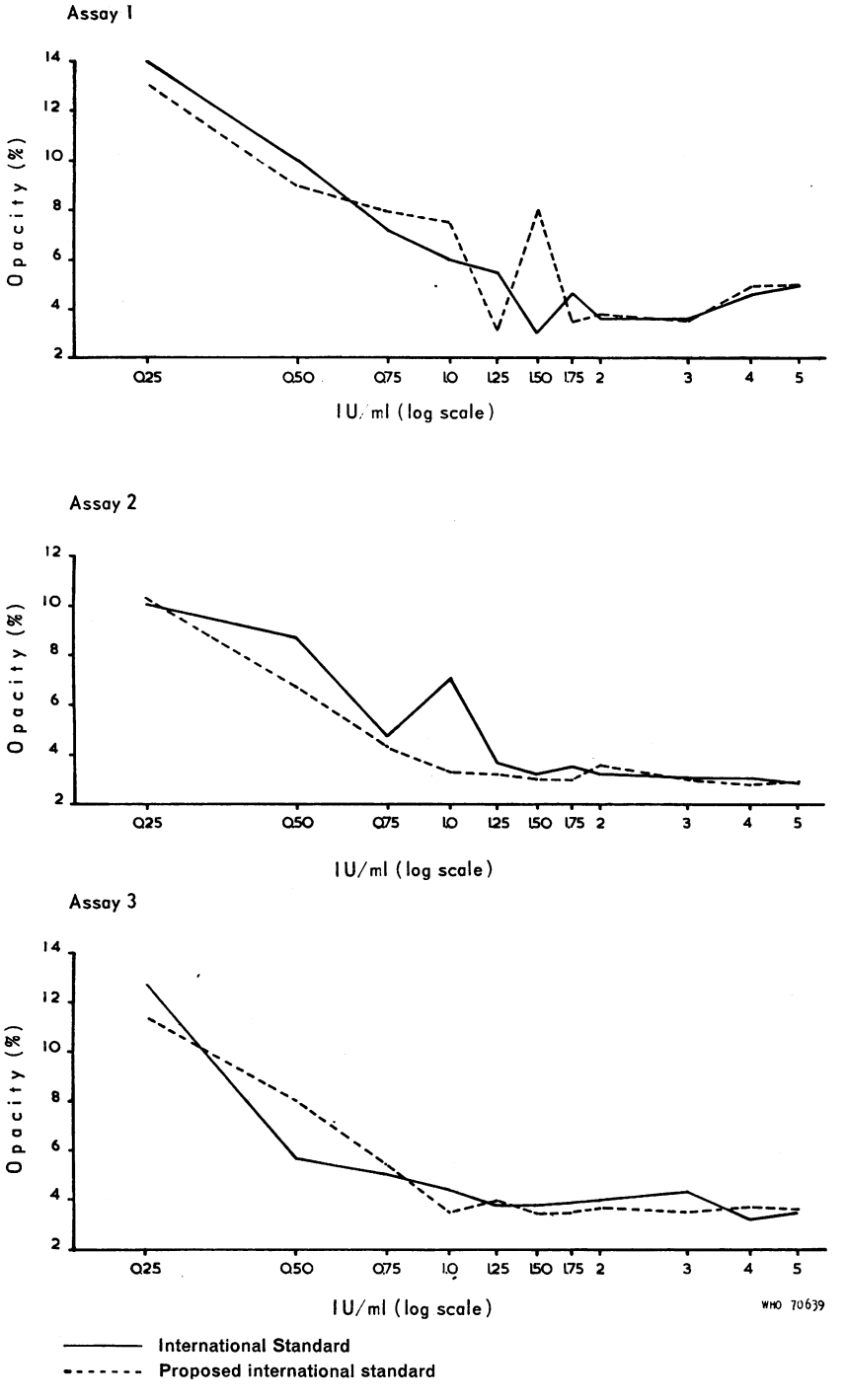
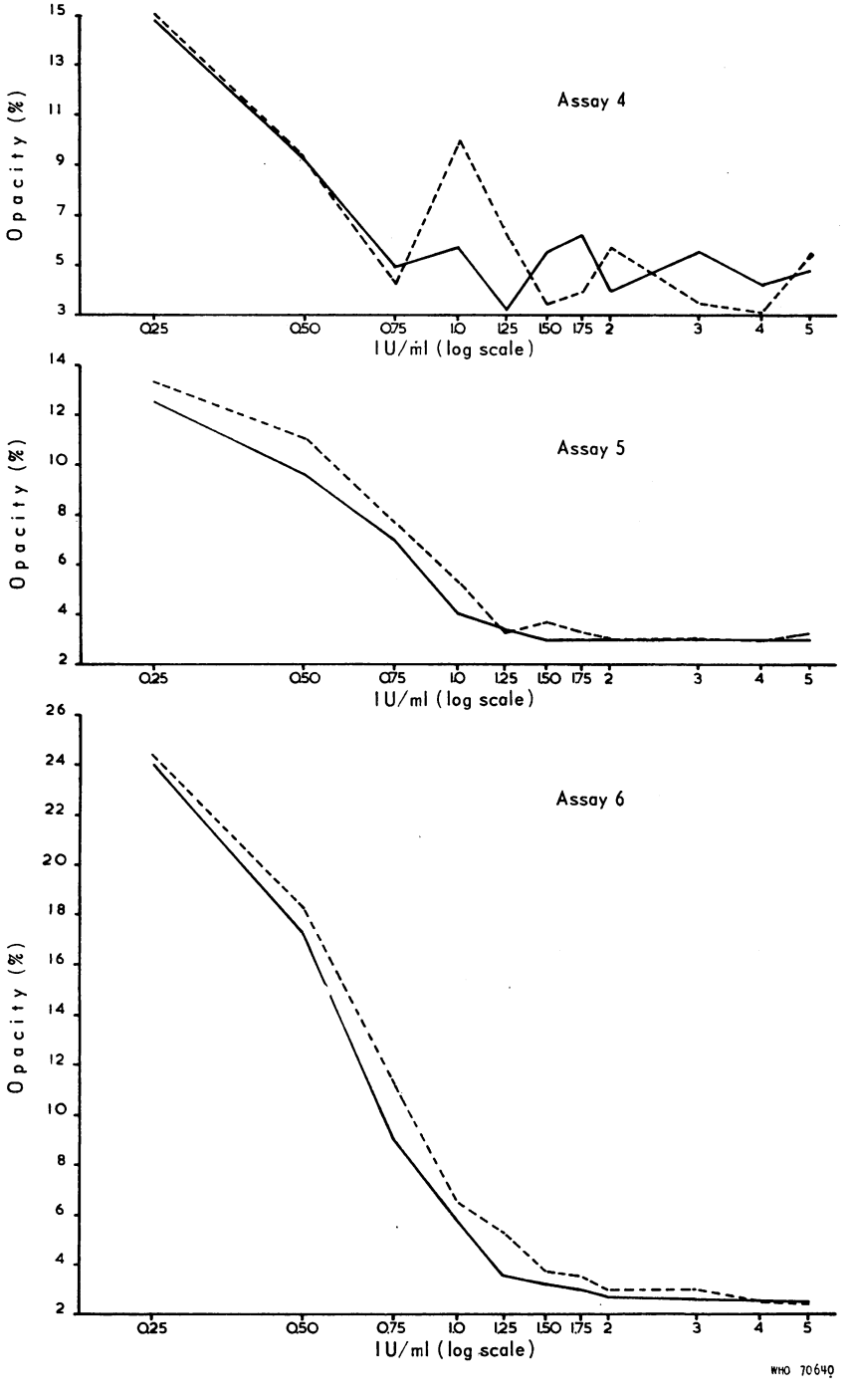


FIG. 2. LOG-DOSE-RESPONSE LINES FOR TURBIDIMETRIC ASSAYS PERFORMED BY LABORATORY 9 (*continued*)



Doses tested by Laboratory 9

International Standard and proposed international standard: 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 3.00, 4.00 and 5.00 IU/ml.

The graphical presentation of the 6 assays carried out by this laboratory showed that doses over 1.25 IU/ml did not affect the response for practical purposes.

The statistical analysis was performed separately for the following sets of doses:

(*a*) 0.25, 0.50, 0.75, 1.00, 1.25 IU/ml (5 × 5 assays)

(*b*) 0.25, 0.50, 0.75, 1.00 IU/ml (4 × 4 assays)

(*c*) 0.25, 0.50, 0.75 IU/ml (3 × 3 assays)

(*d*) 0.25, 0.50, 1.00 IU/ml (3 × 3 assays, doses on log scale).

In all the 4 analyses a large degree of heterogeneity was observed between the individual potencies and in each case at least 3 assays showed significant departure from linearity. The most reasonable results seem to have been obtained with the doses of series (*c*); the departure from linearity was significant in 3 assays only and the total statistical weight was relatively high. Results incorporated in this report refer to series (*c*).

Considering all laboratories, only a few assays showed significant departure from parallelism. Their distribution according to laboratory and level of statistical significance was as follows:

Lab. No.	*No. of non-parallel assays*	*Level of statistical significance* 5 %	1 %
1	2	2	—
3	1	1	—
5	4	3	1
9	1	1	—
Total	8	7	1

Deviation from linearity was observed in 11 assays. In 10 cases the sum of squares for " quadratics " was statistically significant; the sum for quadratics was negative in 8 cases and positive in 2 cases, but the excess of negatives is not statistically significant at the 5% level of probability when the sign test is applied. For 2 out of these 10 assays the sum of squares for " difference of quadratics " was also statistically significant; in one additional assay (Laboratory 2) only the latter sum of squares was statistically significant.

The findings can be summarized as follows:

Lab. No.	*No. of assays with significant SS quadratics*	*Level of significance* 5%	1%	*Sign of sum for quadratics* − (convex)	+ (concave)
1	6	3	3	6	—
2	1	1	—	—	1
9	3	1	2	2	1
Total	10	5	5	8	2

Lab. No.	*No. of assays with significant SS for difference in quadratics*	*Level of significance* 5%	1%
2	1	1	—
9	2	—	2
Total	3	1	2

A closer examination of all these cases revealed that the statistical significance of departure from parallelism or from linearity had been brought out frequently by the fact that in these assays the error variance was smaller than in the other assays which showed no significant departure. Furthermore, the corresponding potencies did not deviate substantially from those observed in the other assays (except from Laboratory 9). Since a slight degree of curvature and non-parallelism is often observed in bio-assays, the results of all the assays were used in the final statistical analysis, but an over-all combined potency was also computed after elimination of the non-valid assays.

The results from different assays performed in the same laboratory were homogeneous in Laboratories 3, 4, 5, 6 and 7. For these laboratories the average log potency shown in Table 2 was computed by weighting each potency value with the reciprocal variance based on the internal evidence of the assay.

Among the laboratories using the diffusion method, only one (Laboratory 1) got heterogeneous results (significant χ^2 at 5% probability level); while within-laboratory heterogeneity was observed in all the 3 laboratories which applied the turbidimetric method (significant χ^2 at 5% probability level for Laboratory 2 and at 1% probability level for Laboratories 8 and 9).

The break-down of the χ^2 for Laboratory 1 showed that the source of heterogeneity was neither the difference between " organisms " nor the difference between " day/ampoules ".[1]

Source of variation	*Degrees of freedom*	χ^2	
Between organisms	1	0.59	
Within first organism:			
Between day/ampoules	5	10.56	
Within day/ampoules	12	23.81*	(critical 5% value: 21.0)
Within second organism:			
Between day/ampoules	5	0.70	
Within day/ampoules	12	16.89	
Total:	35	52.55*	(critical 5% value: app. 50)

[1] In the following tabulation and those overleaf, * indicates a value significant at the 5% probability level and ** a value significant at the 1% level.

TABLE 2

SUMMARY RESULTS OF ASSAYS OF PROPOSED SECOND INTERNATIONAL STANDARD FOR OXYTETRACYCLINE

Lab. No. (1)	No. of assays (2)	Homogeneity within laboratory (3)	Weighted geometric average potency (IU/mg) (4)	Weighted average log potency (5)	Within-laboratory variance of average log potency (6)	Total weight of each laboratory (7)	Average weight per assay (8)
1	36	Heterogeneous	870.3	2.93966	0.00000660	151454	4207
2 (turbidimetric)	9	Heterogeneous	861.9	2.93543	0.00001611	62060	6896
3	8	Homogeneous	889.7	2.94923	0.00000575	173851	21731
4	12	Homogeneous	867.9	2.93847	0.00000840	119091	9924
5	24	Homogeneous	884.8	2.94686	0.00000160	624699	26029
6	4	Homogeneous	897.1	2.95282	0.00001013	98700	24675
7	6	Homogeneous	879.9	2.94445	0.00021164	4725	788
8 (turbidimetric)	4	Heterogeneous	888.6	2.94870	0.00001453	68826	17207
9 (turbidimetric)	6	Heterogeneous	878.5	2.94376	0.00050787	1969	328
Total	109					1305375	

For Laboratory 2, the source of heterogeneity was found to be the difference between " days ":

Source of variatio	*Degrees of freedom*	χ^2
Between days	4	12.78* (critical 5% value: 9.49)
Within days	4	6.69
Total:	8	19.47* (critical 5% value: 15.51)

The experimental design used by Laboratory 8 did not allow the source of heterogeneity to be isolated.

In Laboratory 9 the between-assay heterogeneity was due to the difference between " ampoules ":

Source of variation	*Degrees of freedom*	χ^2
Between ampoules	2	46.98** (critical 1% value: 9.21)
Within ampoules	3	2.69
Total:	5	49.67** (critical 1% value: 15.09)

For Laboratories 1, 2, 8 and 9 with heterogeneous results, the average log potency was obtained by using a series of weights based on both within- and between-assay variances and calculated by the method described by Bliss (1952).

There were small variations in the average potency value from one laboratory to another (see Table 2). However, owing to the relatively high weight (25% of the over-all weight) of the 3 laboratories with the lower average potencies, the χ^2 for heterogeneity turned out to be 25.61, exceeding the critical value (20.09) at the 1% probability level for 8 degrees of freedom.

Grouping Laboratories 1, 2 and 4 with lower average potencies on the one hand and the remaining laboratories on the other hand, the χ^2 for heterogeneity would be split up as follows:

Source of variation	*Degrees of freedom*	χ^2
Between groups	1	22.63** (critical 1% value: 6.63)
Within groups	7	2.98
Total:	8	25.61** (critical 1% value: 20.09)

It is seen that the difference between such groups of laboratories could be considered as a significant source of heterogeneity.

Nevertheless, the combined weighted log potency estimate should be established on the results of all laboratories. In order to take into account the additional variance component originating from the between-laboratory variability an attempt was made to estimate the between-laboratory variance component with the formula given by Bliss (1952). However, this attempt was not successful as the between-laboratory component turned out to be negative. This is due to the extremely large variance for Laboratory 9 which is mainly responsible for making the average within-laboratory variance larger than the between-laboratory variance. Therefore, owing to the absence of the additional variance component originating from the between-laboratory variability, we may accept that the average potency values obtained from different laboratories are reasonably homogeneous.

Consequently, a combined weighted log potency estimate was computed by applying to each laboratory log potency given in column (5) of Table 2, the corresponding weight shown in column (7). The variance of this general average log potency was estimated simply by computing the reciprocal of the sum of weights (1/1305375 = 0.00000076606).

The resulting combined potency of the proposed second international standard for oxytetracycline was 882.2 IU/mg with the 95% confidence interval of 878.1-886.3 IU/mg.

For purposes of comparison a general unweighted geometric mean potency of all the 109 assays was calculated as being 877.4 IU/mg with the 95% confidence interval of 870.4-884.5 IU/mg.

When the 18 invalid assays (all assays with statistically significant departure from parallelism and/or from linearity) were excluded, the corresponding unweighted geometric mean potency based on the remaining 91 assays was 878.9 IU/mg with the 95% confidence interval of 872.2-885.6 IU/mg.

The distribution of the log potencies of the 109 individuals assays performed in all laboratories is shown in Fig. 3.

DISCUSSION

Heterogeneity of potency estimates, both within and between laboratories, was observed in this collaborative study. Within the results of Laboratory 9 there was a suggestion that heterogeneity resulted from a variation between ampoules. This laboratory performed assays on the basis of replicate weighings from single ampoules but since the weighings were made on different days the importance of the variation is not clear. Laboratory 1 also performed its assays in such a way that variation between ampoules could be examined, but in this case replicate weighings were also made from the same ampoules within one day. The variation within ampoules within days was significant, whereas between days it was not. This variation within ampoules includes variation resulting from the operation of weighing and dilution; the quantity of material weighed for each assay (2 mg to 4 mg) was small and although a " semi-micro " balance was used it seems that " weighing variation " was the most likely cause of the heterogeneity. None of the 7 other laboratories produced evidence of heterogeneity between ampoules.

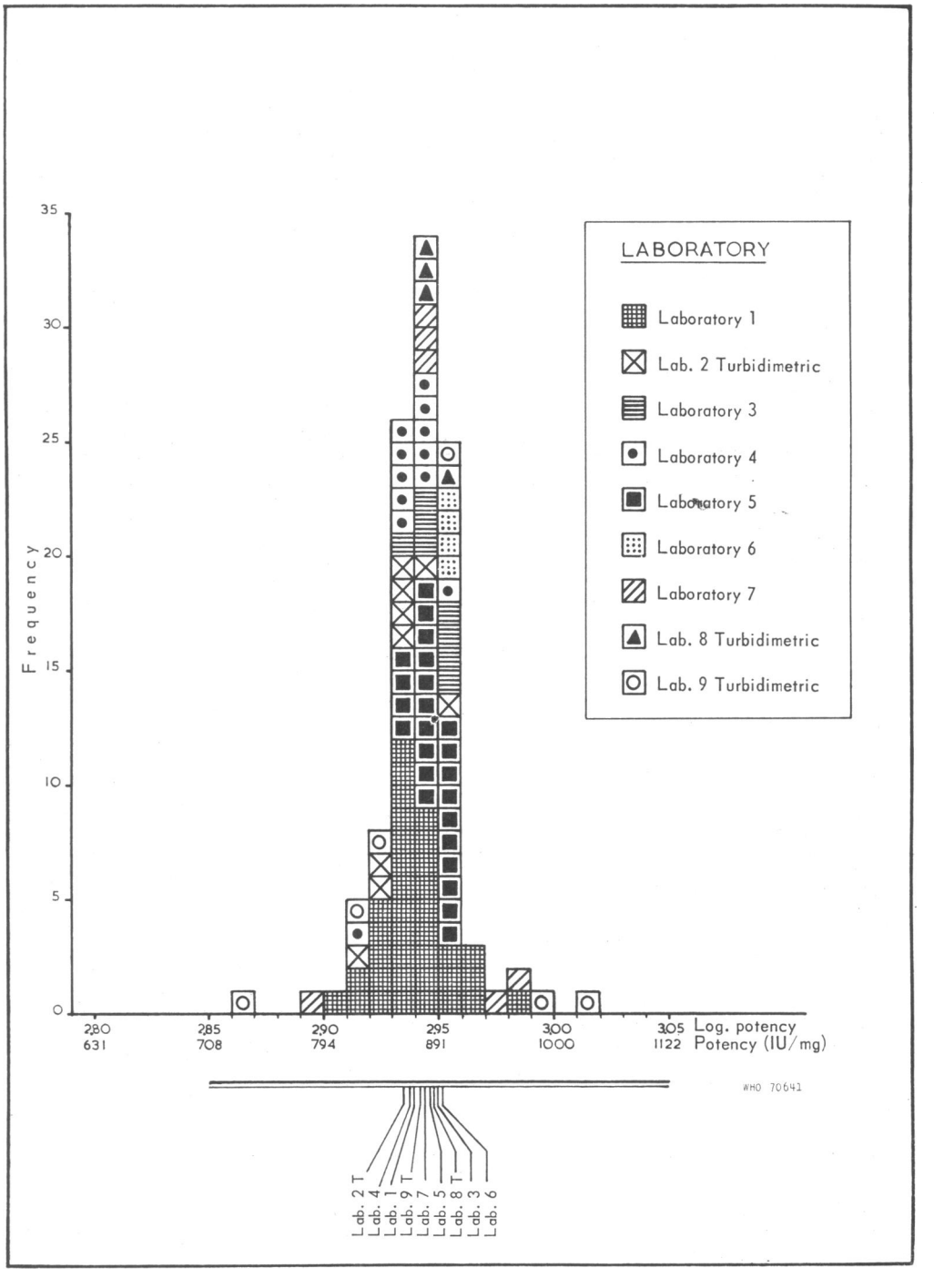
Although the mean potencies obtained by the 9 laboratories fell within the narrow range of 862 IU/mg to 897 IU/mg, these results were heterogeneous mainly as a result of relatively low values from Laboratories 1, 2 and 4. This variation could not be attributed to any obvious difference in technique.

The potency of the proposed second international standard is obviously significantly less than that of the first International Standard. The mean loss in weight of the second standard when dried *in vacuo* at 100°C over P_2O_5 was found to be 6.24% w/w compared with the value of 7.28% w/w previously found for the first International Standard under the same conditions of drying. It thus appears that the proposed second international standard is approximately 3% less potent on a dry weight basis. The two samples were compared by ultra-violet absorption, the ferric chloride assay, and infra-red spectrophotometry; no significant differences were observed between the two samples.

Two possible explanations for the difference in potency were considered, either that the first International Standard contained a small percentage of chlortetracycline which was not present in the proposed second international standard or that the difference was caused by a trace of penicillin in the first International Standard. Examination by thin-layer chromatography showed that neither sample contained a detectable level of chlortetracycline, i.e., <0.1% w/w; electrophoretic studies demonstrated that any contamination by penicillin was at a level less than 1 IU in 10 g. Examination of the two standards chromatographically (Addison & Clark, 1963) showed no significant difference in content of epimers or anhydro derivatives.

FIG. 3

FREQUENCY DISTRIBUTION OF THE LOG POTENCIES OF THE PROPOSED SECOND INTERNATIONAL STANDARD FOR OXYTETRACYCLINE OBTAINED IN DIFFERENT LABORATORIES



The slightly lower potency of the material used for the second International Standard therefore remains unexplained but this does not detract from its value in continuing the definition of the International Unit.

In the collaborative assay of the first International Standard for Oxytetracycline it was reported that the potency obtained under certain assay conditions varied with the duration of incubation of the assay plates, probably as a result of differences in stability of the two preparations being compared. The assay of the proposed second international standard against the first International Standard was performed under the same conditions which had demonstrated this phenomenon, but the potency obtained for the proposed second international standard was found to be independent of the duration of incubation.

ESTABLISHMENT OF THE INTERNATIONAL STANDARD AND DEFINITION OF THE INTERNATIONAL UNIT

In accordance with the instructions of the Expert Committee on Biological Standardization (1964), the above material was established as the second International Standard for Oxytetracycline and with the agreement of the participants in the collaborative assay the potency defined as 880 IU/mg. The International Unit for Oxytetracycline is defined as the activity contained in 0.0011364 mg of the second International Standard for Oxytetracycline.

RÉSUMÉ

Le Comité OMS d'experts de la Standardisation biologique avait chargé le National Institute for Medical Research de Londres d'établir un second étalon international d'oxytétracycline, le premier étant épuisé. Un lot de 500 g d'oxytétracycline dihydratée, obtenu en 1963, a été réparti en ampoules de 125 mg qui ont été remplies d'azote sec et scellées. Le matériel n'a pas été soumis à l'habituelle dessiccation complète, car ce traitement nuit à la stabilité de l'oxytétracycline dihydratée. Le matériel de remplacement a ensuite été soumis à des essais biologiques par comparaison avec le premier étalon international dans neuf laboratoires de sept pays par des méthodes de diffusion sur plaque et de turbidimétrie.

Les différences significatives d'activité qui ont été enregistrées entre les laboratoires et entre épreuves successives à l'intérieur de trois d'entre eux ne sont pas attribuables à des différences de technique. Les moyennes pondérées des activités se situent toutes dans un intervalle de $\pm$ 2% autour de la moyenne générale.

L'activité du second étalon international est inférieure d'environ 3% à celle du premier, mais diverses méthodes d'analyse chimique et physique n'ont permis de constater aucune différence sensible de composition ou de pureté. Le matériel a donc été constitué en deuxième étalon international d'oxytétracycline et sa valeur fixée à 880 UI/mg. L'unité internationale d'oxytétracycline est définie comme l'activité de 0,0011364 mg du deuxième étalon international d'oxytétracycline.

REFERENCES

Addison, E. & Clark, R. G. (1963) *J. Pharm. Pharmacol.*, **15**, 268

Bliss, C. I. (1952) *The statistics of bioassay with special reference to vitamins*, New York, Academic Press, pp. 580-582

Grove, D. C. & Randall, W. A. (1955) *Assay methods of antibiotics*, New York, Medical Encyclopedia, Inc. (Antibiotics Monograph No. 2)

Humphrey, J. H., Lightbown, J. W., Mussett, M. V. & Perry, W. L. M. (1955) *Bull. Wld Hlth Org.*, **13**, 903-915

Humphrey, J. H., Mussett, M. V. & Perry, W. L. M. (1953) *Bull. Wld Hlth Org.*, **9**, 15

WHO Expert Committee on Biological Standardization (1963) *Wld Hlth Org. techn. Rep. Ser.*, **274**

WHO Expert Committee on Biological Standardization (1964) *Wld Hlth Org. techn. Rep. Ser.*, **293**

Annex

PARTICIPANTS IN THE COLLABORATIVE ASSAY

FRANCE

Dr J. Desbordes and Mlle Simonne Chaniot
Laboratoire National de la Santé Publique
Ministère de la Santé Publique
Paris

GERMANY, FEDERAL REPUBLIC

Professor G. Heymann and Dr G. Siefert
Paul-Ehrlich Institut
Frankfurt-am-Main

POLAND

Professor P. Kubikowski, Mr I. Dzierzynska
and Mr M. J. Zawadzka
Instytut Lekow
Warsaw

PORTUGAL

Dr J. do Souto Teixeira
Serviços de Comprovação de Medicamentos
Direcção-Geral de Saude
Lisbon

SWITZERLAND

Dr J. S. Pitton
Laboratoire des Antibiotiques
Institut Universitaire de Microbiologie Médicale
Geneva

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

Mr J. W. Lightbown and Dr J. M. Bond
Division of Biological Standards
National Institute for Medical Research
Mill Hill
London

Mr O. Hughes
The Pfizer Group
Sandwich
Kent

UNITED STATES OF AMERICA

Mr R. C. Kersey
Chas. Pfizer and Co. Inc.
Brooklyn, N.Y.

Mr. A. Kirshbaum
Division of Antibiotics and Insulin Certification
Food and Drug Administration
US Department of Health, Education, and Welfare
Washington, D.C.