Table III. α-AOA-hydroxamic acid derivatives

X–NH–O–CH(R)–CO–NH–O–Y

No.	X	R	Y	in vitro inhibition mcg/ml								
				$H_{37}R_v$	INH res.	Strepto-mycin res.	PAS res.	*M. kansasii*	*Staph. aur.* haem. Wood	*Bact. subtilis*	*E. coli*	*Ps. pyocyanea*
1	H	H	Bzl(pCl)	0.1–1	1–5	1–5	0.1–1	–	>50	>50	>50	>50
19	H	H	Bzl	5–10	5–10	10–25	5–10	–	–	–	–	–
17	H	H	Bzl(pNO_2)	5–10	5–10	5–10	5–10	–	–	–	–	–
16	AO-acetyl	H	Bzl(pCl)	1–5	1–5	5–10	5–10	–	–	–	–	–
28	Gly	H	Bzl(pNO_2)	>50	>50	>50	>50	–	–	–	–	–
34	H	H	dodecyl	0.1–1	0.1–1	1–5	1–5	–	–	–	–	–
53	H	Bzl (L)	Et	5–10	1–5	10–25	–	1–5	>50	>50	>50	>50
54	H	Bzl (L)	dodecyl	10–25	5–10	5–10	–	1–5	>50	>50	>50	>50
59	H	CH_3 (DL)	Bzl(pCl)	5–10	10–25	10–25	–	10–25	>50	>50	>50	>50
68	H	C_6H_5 (DL)	Bzl(pCl)	>50	>50	>50	–	>50	>50	>50	>50	>50

The in vitro experiments were carried out in liquid culture-medium[2] with *M. tub.* $H_{37}R_v$ strain and its resistant variations resp. after inoculation with 0.1 mg and incubation for 3 weeks at 37 °C. The upper limit of the given figures resulted in complete inhibition. The investigation of other bacterium strains were carried out in bouillon culture-medium.

prepared with excellent yield for example by reacting protected α-AOA-OPCP esters with corresponding amines and hydrazides respectively. Some representatives of the 3 groups and the in vitro activities of the compounds are listed in the Tables.

The most potent compounds were investigated under in vivo conditions, too. It was found that 7.5 mg of the compound No. 5/A in Table II inhibited the generalization of *M. tuberculosis* infection in a 3-month-experiment on guinea-pigs. Similarly good results were obtained from experiments carried out on white mice. The compounds show fairly low toxicity in acute experiments.

Zusammenfassung. Es wurde festgestellt, dass einzelne α-Aminooxy-carbonsäure-Derivate, d.h. α-AOA-Amide, α-AOA-Hydrazide und α-AOA-Hydroxamsäure, in vitro und auch in vivo eine ausgeprägte Hemmung auf verschiedene *M. tuberculosis*-Stämme aufweisen.

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Censored Distribution Techniques in Analysis of Toxicological Data

Truncated and censored samples are both common in bioassays. Truncation occurs if the population assayed cannot have values beyond a given cut-off point or if the number of observations exceeding the cut-off point is unknown[1]. Censoring refers to situations where observations beyond the cut-off point are not measured, but the number of such observations is known.

Recently, two different approaches to analyzing censored data have been compared. We report here details of the statistical techniques involved. The large body of data available permitted assessment of the internal consistency of the results obtained by these techniques; such assessment is not possible when only a few data sets are to be analyzed. Thus, the validity of the assumptions made could be verified.

The data examined are part of a large series designed to evaluate the effects of 100 MDOP compounds and related open-ring analogs on the hepatic microsomal enzyme function in the mouse as measured by the prolongation of hexobarbital narcosis[2]. For each replicated experiment 16 control mice were given 75 mg/kg of hexobarbital which would induce sleep for about 30 min and 5 groups of 8 mice each were given in addition doses of 2.5, 10, 40, 160 and 640 mg/kg, respectively, of the MDOP compound. The mice were observed for 300 min and the duration of narcosis recorded. For the active compounds some mice in the higher dose groups slept for longer than 300 min but the duration was not known.

When deaths occurred after the cut-off point and no record was available of whether the mice regained consciousness before death, it could be argued that truncation occurred. However, the approach was taken that all sleeping mice that were alive at the cut-off point had extended narcosis and their later death did not affect the sample size, thus censoring prevailed. The sample size of 8 was reduced if the mice died before 300 min as these deaths could be attributed to causes other than drug effect.

Most of the 100 drugs were tested at least twice, and if the reproducibility of the results was not satisfactory when inspected visually, they were replicated again, resulting in a total of 240 replications. Of these, 114

had measurements at each of 5 doses, 50 had some deaths before the cut-off time but no prolonged narcosis and 76 had prolonged narcosis. Of these 76 replicates, censoring occurred only at the highest dose in 56, at 2 doses in 8 and at 3 doses in 4 experiments, resulting in 102 censored distributions (Table I).

Several methods of analysis were considered and rejected as being unsuitable for these data. A large number of drugs were being screened and in order to assess their relative effects it was necessary that each be administered at the same dose levels and analyzed in comparable manner.

A measure of central tendency commonly used for a censored distribution is the median, but this requires that measurements exist for more than half the animals. Thus with 8 mice and no deaths measurements would be required on at least 5 mice, but these were not always available (Table I).

In some situations it is possible to circumvent the problem by basing the analysis only on those dose levels for which all measurements were available. In the dose range chosen as being suitable for most drugs, occasionally censored distributions occurred at 3 of the 5 dose levels for the more potent drugs, so this would not have been a feasible solution.

A simple approach which we tried and will refer to as the trend-test, degrades the measurements to simple counts. A specific time, x, is selected and the number of mice who sleep for longer than x minutes are counted. Standard techniques[3] are then used to test whether the proportion of prolonged-sleepers increases with increasing dose. This test is most powerful when x is the median for each set of data, but for these data it was necessary to use the same values for every drug. The values chosen here were 30 min and 40 min, the approximate medians for the aggregated control animals and the experimental animals, respectively.

The preferred method, which we will refer to as the GUPTA method, is again a standard technique. There are several methods of estimating the mean of a censored sample, and each makes assumptions about the underlying distributions. An estimate can be simply obtained when the observations are exponentially distributed[4]. The maximum likelihood estimate consists of assigning the cut-off time to all mice with prolonged narcosis, then adding all times, both observed and assigned, and dividing this sum by the number of observed times. That the bias is positive can be readily seen; whenever less than half the times are recorded the estimated mean is greater than the cut-off point. An unbiased method was essential because a regression line was to be fitted to the resulting means. Two methods have been given by GUPTA[5]; a maximum likelihood method for normal distributions which is biased and an unbiased linear method; the latter was selected for these data.

In GUPTA's linear method, for each sample of size n the k known times are ordered, $x_1, x_2, \ldots x_k$, and the estimate of the mean is

$$\mu = \sum_{i=1}^{k} \beta_i x_i$$

where the values of the β_i are obtained from his table for samples up to size 10. This limitation exists because the variance-covariance matrix for order statistics was only available for samples up to this size. Methods for larger samples[5], have been further developed[6], but these were not applied as none of the samples studied here exceed 8.

Inspection of the sleeping times from the combined controls showed that after transformation to the log-scale (base e) the distribution was fairly symmetrical and could be regarded as normal with a mean of 3.28 log-min and standard deviation of 0.22. The clumping of the data was of some concern; the mice had a tendency to wake up in groups and although the GUPTA method also yields variances these were not used in fitting the weighted linear dose-response to the GUPTA means.

When using the trend-method for a large comparative series it was necessary to choose a critical level, x, and use this same level in every replicate. When the level was set at 30 min, in some replicates all mice exceeded this time and thus no estimate of slope could be obtained (Table II). This occurred in fewer replicates when the level was set at 40 min, but the opposite phenomenon of very few measurements exceeding this level occurred. This dependence of the method on the critical level chosen leads to different estimates of the significance of the slope; replicate 2, Table II, shows different t-values for each method.

All replicates in which no deaths occurred before the cut-off time were examined by the trend-test using a 40 min critical level. Each replicate was classified as exhibiting a dose-response slope if the t-value was greater than required for significance at the 5% level. These replicates were then compared with the GUPTA method (Table III) using the same criteria for slope. In the absence of censoring the GUPTA estimates are the usual arithmetic means, thus for complete replicates linear regression is compared with the trend test and of the 111 replicates evaluable by both methods the proportion of slopes detected is similar but agreement is poor. Examination of the censored replicates, for which some method different from linear regression is needed, showed an increased proportion of non-evaluable replicates.

As the assumptions underlying the GUPTA method were not precisely attained by these data, the matter of first interest was whether the estimates obtained were

Table I. Distribution of 102 individual dose samples in which one or more mice slept for more than 300 min

No. mice living to cut-off	Number of sleeping times observed								Total
	0	1	2	3	4	5	6	7	
8	9	4	5	11	10	8	7	20	74
7	0	1	2	0	3	2	1		9
6	3	0	3	0	1	1			8
5	0	2	1	2	0				5
4	0	0	0	1					1
3	0	1	0						1
2	1	2							3
1	1								1
Total	14	10	11	14	14	11	8	20	102

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Table II. Example of raw data and 3 analyses for dose-response

	Dose mg/kg					t-value
	2.5	10	40	160	640	
Replicate 1						
	36	31	33	36	45	
	36	31	34	37	45	
	36	32	44	37	45	
	41	33	45	41	46	
	41	37	45	42	97	
	41	38	45	46	118	
	49	50	52	47	215	
	64	50	52	78	> 300	
No. sleeping times > 30 min	8	8	8	8	8	no value
No. sleeping times > 40 min	5	2	6	5	8	2.11
GUPTA mean	3.74	3.61	3.76	3.78	4.47	1.94
Replicate 2						
	18	24	37	41	45	
	19	24	37	41	51	
	27	24	37	50	52	
	27	30	38	50	62	
	31	30	38	50	77	
	31	30	38	70	139	
	31	30	45	90	260	
	32	35	45	100	> 300	
No. sleeping times > 30 min	4	1	8	8	8	3.76
No. sleeping times > 40 min	0	0	2	8	8	5.39
GUPTA mean	3.27	3.33	3.67	4.06	4.58	6.73
Replicate 3						
	48	50	53	55	57	
	48	50	53	56	78	
	48	50	53	56	195	
	48	50	54	75	214	
	69	51	54	76	276	
	69	51	54	99	> 300	
	69	51	63	193	> 300	
	69	60	64	212	> 300	
No. sleeping times > 30 min	8	8	8	8	8	no value
No. sleeping times > 40 min	8	8	8	8	8	no value
GUPTA mean	4.05	3.94	4.02	4.49	5.49	2.35

Each replicate shows sleeping-time in min for 8 mice at each dose level after administration of Tropital. Control data not shown.

Table III. Comparison of 2 methods of analysis for detecting dose-response. For each method replicates are classified as having slope if test statistic was significant at 5% level

		Trend method			Total
		No slope	Slope	Not evaluable	
Replicates complete					
GUPTA method	No slope	22	12	0	34
	Slope	16	61	3	80
	Total	38	73	3	114
Censored replicates					
GUPTA method	No slope	1	3	0	4
	Slope	2	26	8	36
	Total	3	29	8	40

credible. To examine this the censored distributions in which no deaths occurred but at least 2 measured times were available, were selected. These 61 samples, all of size 8, afford a mean of comparing the estimates obtained with varying numbers of observations (Figure 1). When 7 observations fell below 5.7 – the natural logarithm of the 300 min cut-off point – the mean would be expected to fall below this point for most samples; but when 4 out of 8 were observed, the mean would be expected to fall above the cut-off in half the samples. That this occurred confirms to some extent the assumptions made, as the GUPTA method does not utilize knowledge of the cut-off point but is computed entirely from the observed times.

Further confirmation of the suitability of the method is obtained from those experiments where more than one censored sample occurred. In all but one censored replicate the estimated means were greater for the larger dose.

Estimation of the means thus seemed satisfactory, but estimates of the standard deviations of the means were occasionally very small for heavily censored samples. Whereas for 7 observations the means and standard errors (S.E.) were unrelated and the S.E.'s fell between 0.01 and 0.43 which is comparable to the standard deviation of 0.22 for controls, for 3 out of 8 there seemed to be a correlation (Figure 2). This could be explained by a tendency for the observations to be clumped, a group of mice being observed to have recovered at the same time, possibly because of the technique used to assess recovery. Use of these variances when fitting the dose-response regressions would have unduly weighted low estimates of the means, so the regression was fitted using the number of observations as weights. Estimates based on only 2 responses were also excluded whenever estimates were available for the other doses.

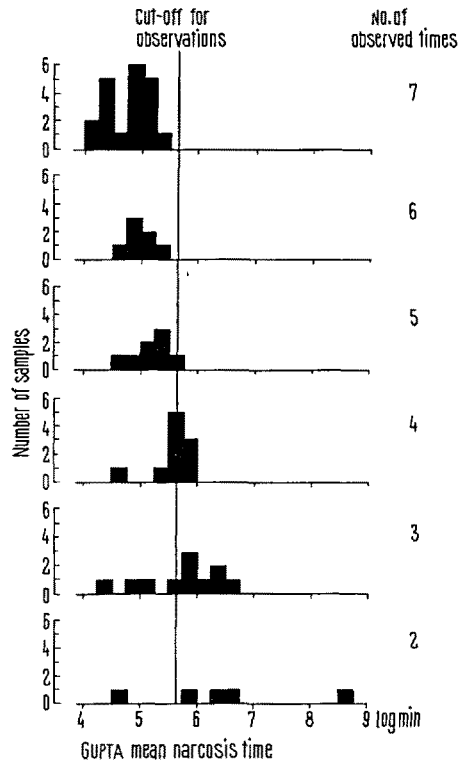

Fig. 1. Distribution of mean narcosis times estimated by GUPTA method for censored samples of size 8.

When the regression lines had been fitted to each replicate the position of the GUPTA estimate relative to the fitted line was examined (Table IV). In most instances the GUPTA estimate fell above the line and, as these were the higher doses, the effect of using these estimates was thus generally to increase the slope of the line compared with the value that would have been obtained if the doses at which censoring occurred had been omitted. When censoring occurred at more than one dose, it was essential to estimate the response at these doses and the results show that omission of the highest dose, when it alone was associated with prolonged narcosis, would generally have led to reduced estimates of potency. When regression lines had been obtained for each replicate, they were compared before a combined line, corrected for individual control values, was computed for each drug. In only one drug was incompatibility of replicates detected and in all drugs where censoring without death occurred the combined estimate indicated that the drug was active.

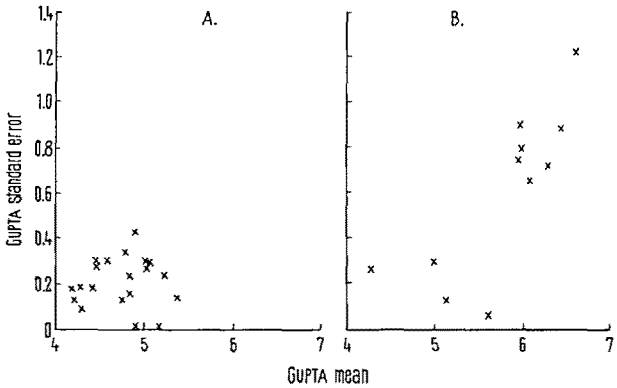

Fig. 2. Relationship between means and standard errors computed by GUPTA method. a) 7 observations from sample size 8. b) 3 observations from sample size 8.

Table IV. Distribution of 61 GUPTA means in relation to fitted linear regression line

	Number of sleeping times observed						
	2	3	4	5	6	7	Total
Dose 5							
Above line	2	7	6	4	3	7	29
Total	3	9	6	5	3	10	36
Doses 3 and 4							
Above line	2	1	4	2	3	5	17
Total	2	2	4	3	4	10	25

In general, the replicated results obtained using the GUPTA method for censored samples were comparable, even when censoring only occurred in some replicates. In some drugs where censoring did not occur over the selected dose range but individual replicates showed a consistent slight trend with dose, repeated replication was necessary before the slope could be established as significant at the 5% level. This suggests that in experiments of this type the experimenter can obtain unequivocable results with fewer replications by using a dose-range that produces some censored samples.

It may thus be concluded that the GUPTA method of computing means for censored samples was satisfactory, leading to estimates that were reasonably distributed about the cut-off point and plausibly related to adjacent dose estimates. The effect was in general to increase the slope of the fitted linear regression which may have indicated that the log response-log dose relationship was in some instances non-linear at these doses. The purpose of the experiments was, however, to relate the potency of a series of drugs over the same dose-range and for these purposes significant potency could be more rapidly determined when the upper doses of the range lead to censored samples in some or all replicates.

Zusammenfassung. Es werden Methoden der Dosis-Effekt-Analyse verglichen, die es erlauben, nicht mehr gemessene Werte in höheren Dosisbereichen zu schätzen. Obwohl die Daten eng beieinander liegen, ergab die von GUPTA[5] 1952 entwickelte Methode zur Bestimmung von Mittelwerten befriedigende Schätzungen, verifiziert durch die Übereinstimmung innerhalb der Gruppen.

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Ethyl-α-*p*-Chlorophenoxyisobutyrate Induced Hepatic Microbody Proliferation in Rat Liver and Ubiquinone Concentration

Male rats fed ethyl-α-*p*-chlorophenoxyisobutyrate (CPIB), a hypolipidemic drug, show a significant increase in the number of hepatic microbodies (peroxisomes) together with a two-fold increase in the content and activity of catalase protein, one of the principal enzymes of microbodies[1–4]. The precise mechanism by which CPIB elicits the hypolipidemic effect and microbody proliferative response is not understood. Accumulated experimental evidence suggests that the hypolipidemic property and the microbody proliferating effect are possibly two independent actions of CPIB and may not interrelate with one another[5,6].

The work of RAMASARMA et al.[7–10] demonstrated 1. that CPIB and ubiquinone (co-enzyme Q) have remarkably similar effects on inhibition of hepatic synthesis of cholesterol and lowering of serum sterol concentration, 2. that CPIB increased ubiquinone concentration in the liver to the same extent as did feeding with exogenous ubiquinone and 3. that the catabolism of ubiquinone was lowered with CPIB administration. From these results it