decreased by 2^n times, i.e., 2^1 (where $n=1$), or in other words, by half. Again, n for phenol is approximately 6 (Jordan and Jacobs[11]); a twofold increase in concentration results in 2^6, i.e., 64 times decrease in the disinfection time. A threefold increase in concentration would result in a 3^6, i.e., 729 times decrease in disinfection time, and so on. The germicidal ability of aqueous dilutions of substances like phenol, having high concentration exponents will increase at a rapid rate with increasing concentration, and decrease equally rapidly with increasing dilution. Substances possessing low exponents, like mercuric chloride, will decrease in activity more slowly with increasing dilution. From the list prepared by Chick[12], supplemented by Rahn[13], and from the observations of Tilley[14], Withell[15], Brownlee and Tonkin[16] and Hoffmann and Rahn[17], it is evident that a wide range of concentration exponents exists amongst disinfectant substances.

The effect of concentration of the bactericide on disinfection rate. Chick[18] put forward the following empirical relationship connecting concentration and the time for disinfection: $\frac{1}{c_o - c_n} \log \frac{c_n\ t_n}{c_o\ t_o} = a$ constant, where t_o and t_n were the times for disinfection corresponding to concentration c_o and c_n. Watson[9] modified Chick's formula and showed that the relation of the death time t to the concentration c, was more suitably expressed by the equation $c^n t = constant$; because of the logarithmic or exponential nature of this relationship, n is referred to as the "concentration exponent." It is also known as the "coefficient of dilution" of the disinfectant.

It must be realised that Watson's equation is arbitrary and cannot be absolutely accurate over all ranges of concentrations of the germicide. Every disinfectant possesses a threshold value below which no effect can be detected under the experimental conditions; hence c can have a finite value whilst t can be infinite. Jordan and Jacobs[19,20] from experiments on the disinfection action of phenol on *Bact. coli*, were able to secure evidence that n increased as c approached the threshold value. These same workers[21] constructed graphs of the virtual sterilisation times (*v.s.t.*) against phenol and showed that with the more concentrated solutions of disinfectant the curves became asymptotic to the abscissa at the higher temperatures used.

Watson's equation can also be put into the form $n \log c + \log t =$ constant, and then n can be calculated from the disinfection times t_1 and t_2 at two different concentrations c_1 and c_2, thus:

$$n = \frac{\log t_2 - \log t_1}{\log c_1 - \log c_2}$$

When log t is plotted against log c a straight line should result if the relationship be true within the range of concentrations tested; the magnitude of the slope of the regression gives the value of the concentration exponent n.

The use of death rates for the determination of n. The original relationship as postulated by Chick[18] was based on the times for complete disinfection as determined by end-point methods; the calculations

of Reichel[22,23], Gegenbauer and Reichel[24], Gregerson[10], Gegenbauer[25] and Tilley[26] were based on this technique. As experimental evidence accumulated it was believed that the velocity of the disinfection process was constant throughout its course, and hence the death rate k (as determined by counting methods) could be substituted for the death time; Watson's equation then became $\frac{c^n}{k}$ = constant, from which

$$n = \frac{\log k_1 - \log k_2}{\log c_1 - \log c_2}$$

Death rates were used by Ikeda[28], Watson[9], Paul, Birstein and Reuss[29,30], Hobbs and Wilson[31] and Withell[32] for determining n. This method is satisfactory so long as the death rate of the process is constant throughout its course. Counting methods, although somewhat more laborious than end-point methods, have the advantage that they yield several points on the death curve, thereby enabling the death time to be estimated more accurately; a death time can then be assigned to any desired level of mortality. When the death rate does not vary the velocity constant is indirectly proportional to the death time, and under certain circumstances, for example when disinfection is rapid, constant values of k are often obtained during intermediate intervals of time. However, when the process is retarded (for instance, by using less concentrated solutions) the death rate at intermediate stages in the process may be shown to vary. Such variations can only be detected by counting methods; end-point methods give only the overall reaction velocity. When the death rate varies it is difficult to decide which value to use in the equation for the determination of n and hence its employment does not give conclusive estimations.

The use of intermediate mortality levels for the determination of n. Counting methods enable the times for any level of mortality to be determined. Mention has been made in Part VIII[8] of this series of communications, of the times for different mortality levels used by research workers for the comparison of bactericidal activity. It has been argued (Withell[15]) that the value of t in Watson's equation need not necessarily be the extinction time, but that the times for other mortality levels more suitable for comparison purposes or more accurately determinable, might be substituted. However, Jordon and Jacobs[19] emphasised that the use of times for selected mortality levels must first be tried in Watson's formula to ascertain whether the equation is obeyed or not. A criterion is the relationship between the logarithms of the times for the fixed decrease in mortality (50 per cent., 99 per cent., etc.) and the logarithms of the concentrations of the disinfectant. If this is linear over the complete range of concentrations then the equation is obeyed. Nevertheless, even if Watson's equation is obeyed, the values of n may still differ when different mortality levels are used for the substitution in the equation.

Relationship between the log decrease in mortality time-log concentration regression, and the probit-log time regression. A rectilinear relationship between the logarithm of the decrease in mortality

time and the logarithm of the concentration of disinfectant is coupled with parallel probit-log time regressions; when this holds true, then any level of mortality may be chosen for the determination of n (over the range of parallelism established) because $log\ t_2 - log\ t_1 = constant$ (where t_2 and t_1 are the times for any mortality level in the range). Jordan and Jacobs[19] criticised Withell's[15] substitution of LT50 for t in Watson's equation, in that he did not prove parallelism of the probit-log time regressions over the range of concentrations of disinfectant considered. Jordan and Jacobs[19] also showed that the relationship of log LT50 to the logarithm of the concentration in some of Withell's experiments was curvilinear and could not therefore be used to give accurate and reliable values of n; a similar effect was observed when the log LT50's from their own results were plotted against the appropriate log concentrations. A rectilinear relationship, however, did exist over a smaller range embracing the lower concentrations of disinfectant, and here the probit-log time regressions were roughly parallel. Later results by these workers[20] have shown that this regression could be regarded no longer as bilinear, but that of a very asymmetrical sigmoid curve. The concentration exponent calculated from this portion of the curve did not differ significantly from that calculated when the *v.s.t.'s* were used. They further demonstrated that when the times for 99 per cent., 99·9 per cent. and 99·999999 per cent. mortality levels were used, the relationship became rectlinear over the whole range of concentrations of disinfectant used. The substitution of any mortality time down to 99 per cent. mortality for the extinction time in Watson's equation was therefore equally justified. These workers[21] also demonstrated that within the ranges of concentrations chosen, linear relationships existed between log *v.s.t.'s* and log phenol concentrations for experiments conducted at several temperatures.

Jordon and Jacobs[19] found that the value of n varied with the time for the degree of mortality (99 per cent., 99·9 per cent. or 99·999999 per cent.) chosen for the calculations. They preferred to use the value of the time from the highest mortality level because it was nearest to that which could be obtained from a technique based on extinction time—the complete sterility demanded in practice. Owing to the inaccuracies of end-point methods, n's calculated from extinction times themselves, cannot be considered reliable.

It is possible that inaccurate observations, due to the great speed of the reaction caused by the concentrated solutions employed, might have been responsible for the departure from linearity of the log LT50-log concentration of disinfectant relationship in Withell's[15] results. If this were so then it would not be possible to determine n with any considerable accuracy from such concentrated solutions. With his highest concentrations of phenol, Withell[31,32,33] obtained LT50's of less than one minute in many of his calculations. The error attached to the estimation of these times must have been large and consequently the divergence from linearity in log LT50-log concentration of disinfectant relationships must not be taken as final on the basis of these observations.

When disinfection is rapid the log percentage survivor-time curve is likely to be rectilinear, suggesting a constant death rate of the disinfection process. The average of the intermediate death rates may then be taken as equal to the overall death rate, and in these circumstances the time for any mortality level may be used in the equation $c^n t$ = constant for calculating n. Jordan and Jacobs[19] plotted some of Withell's[33] results for the disinfection of *Bact. coli* against *para*chlor*meta*cresol, as log LT50 against log concentration of disinfectant and found that a rectilinear relationship existed, although many of the LT50's were less than 1 minute and in some instances less than 30 seconds. To what extent a linear relationship really holds can only be determined by experiment.

Calculation of the Concentration Exponents of Ethylene Glycol and its Monoalkyl Ethers at 20°C. and 30°C.

For all the compounds investigated, the mean log LT50's at each concentration (collected from Tables 2 and 4, Part VIII[8]) were plotted against log concentration. Every regression simulated rectilinearity and in some instances the fit was remarkably good. The magnitude of the slope of the regression gives the value for the concentration exponent n. A more accurate value of n is obtained by calculation of the regression coefficient; this method also affords a means of estimating the standard error of the slope and is therefore to be preferred. Table 1 sets out the relevant data for all the compounds. The slopes of the regressions have been calculated in the usual manner by the method of least squares. The error mean squares of these regressions have been computed and used to estimate the standard errors of each slope.

Discussion

Justification of the use of LT50 as the basis for the determination of n. Over the ranges of concentrations of disinfectants investigated, the standard errors of the log percentage concentration-log LT50 regressions are satisfactorily small, indicating that a rectilinear relationship may be assumed to exist. These results diverge to a certain extent from the relationship found by Jordan and Jacobs[19], who showed that taken over a wide range of concentrations of phenol, the regression of log percentage concentration-log LT50 was curvilinear. Perhaps if the present investigations had been conducted over a wider range of concentrations a similar result would have been observed. Nevertheless, the utilisation of LT50 in Watson's[9] formula, $c^n t$ = constant, is quite justified in the present series of experiments since the probit-log time regressions for the different concentrations of the same substance have previously been shown to be parallel (Parts VI[6] and VII[7]). Furthermore, concentrations which would give very small values of LT50 (and possibly subject to large experimental error), were avoided; from Table I it is seen that the values of LT50 rarely fell below 10 minutes, and the small standard errors indicate that these points have been satisfactorily estimated.

The magnitude of the concentration exponents. The values of n for ethylene glycol (15·8654 at 20°C. and 18·4582 at 30°C.) are extremely high; in fact they are the highest ever recorded for a disinfectant sub-

TABLE I

CALCULATION OF THE CONCENTRATION EXPONENTS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS

(*a*) FOR EXPERIMENTS AT 20°C.

Ethylene glycol		Monomethyl ether		Monoethyl ether		Monopropyl ether		Monobutyl ether		Mo
log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cen conc.
860	2·237	1·628	2·734	1·398	2·493	0·892	2·101	0·544	2·123	$\bar{1}$·602
875	2·108	1·653	2·357	1·439	2·025	0·954	1·738	0·574	1·847	$\bar{1}$·628
889	1·811	1·677	2·008	1·477	1·739	1·000	1·432	0·602	1·484	$\bar{1}$·658
903	1·546	1·699	1·786	1·512	1·218	1·079	0·884	0·628	1·384	$\bar{1}$·677
917	0·982			1·544	1·977			0·653	0·989	$\bar{1}$·699
929	1·374									
954	0·793									
− 15·865418 ± 1·256		− 13·187371 ± 1·882		− 10·533373 ± 0·863		− 6·449768 ± 0·680		− 10·036182 ± 1·158		− ±

(*b*) FOR EXPERIMENTS AT 30°C.

Ethylene glycol		Monomethyl ether		Monoethyl ether		Monopropyl ether		Monobutyl ether		Mo
log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cent. conc.	log LT50	log per cen conc.
796	2·006	1·544	1·954	1·097	2·198	0·477	2·200	0·176	2·162	$\bar{1}$·512
813	2·053	1·574	1·736	1·176	1·987	0·602	1·959	0·301	1·699	$\bar{1}$·544
829	1·453	1·602	1·413	1·243	1·563	0·699	1·740	0·398	1·345	$\bar{1}$·574
845	1·198	1·628	1·104	1·301	0·885	0·788	1·431	0·477	0·915	$\bar{1}$·602
										$\bar{1}$·627
− 18·458239 ± 2·896		− 10·227064 ± 1·685		− 6·289254 ± 0·693		− 2·484941 ± 0·469		− 4·061068 ± 0·469		− ±

(In this table the magnitude of the slope of the regression, *b*, equals the concentration exponent *n*.)

extent of the range of concentrations over which it is estimated, particularly so when this range borders on the threshold value.

A more satisfactory concentration exponent. Jordan and Jacobs[21] asserted that in the light of this evidence, Watson's[9] formula could not be

TABLE II

TEST OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN n'S OF THE SAME COMPOUND AT 20°C. AND 30°C.

(a) SUMMARY OF TOTALS FROM CALCULATIONS OF LOG CONCENTRATION-LOG LT50 REGRESSIONS AT 20°C. AND 30°C. (ABSTRACTED FROM TABLE I.)

Item	Ethylene glycol			Monomethyl ether		
	20°C.	30°C.	Total	20°C.	30°C.	Total
$S[(x-\bar{x})(y-\bar{y})]$ ...	−0·099969	−0·024531	−0·124499	−0·037913	−0·040181	−0·078044
$S(x-\bar{x})^2$	0·006301	0·001329	0·007630	0·002811	0·003924	0·006735
$S(y-\bar{y})^2$	1·779256	0·529233	2·308489	0·515185	0·415485	0·930670
N	5	2	7	2	2	4
SS for individual regressions ...	1·586034	0·452799	2·038833	0·511347	0·410422	0·921769
b	−15·8654	−18·4583	−16·3170	−13·4874	−10·2271	−11·5877
SS pool	—	—	2·031481	—	—	0·904683

Item	Monoethyl ether			Monopropyl ether		
	20°C.	30°C.	Total	20°C.	30°C.	Total
$S[(x-\bar{x})(y-\bar{y})]$ ...	−0·140452	−0·145678	−0·286130	0·140483	−0·125574	−0·266057
$S(x-\bar{x})^2$	0·013334	0·023163	0·036497	0·021471	0·050534	0·072005
$S(y-y)^2$	1·490587	1·006395	2·496982	0·920581	0·320812	1·241393
N	3	2	5	3	2	5
SS for individual regressions ...	1·479433	0·916206	2·395639	0·919169	0·312044	1·231044
b	−10·5334	−6·2893	−7·7789	−6·4498	−2·4849	−3·7024
SS pool	—	—	2·237723	—	—	0·983447

Item	Monobutyl ether			Monohexyl ether		
	20°C.	30°C.	Total	20°C.	30°C.	Total
$S[(x-\bar{x})(y-y)]$ ...	−0·074338	−0·205222	−0·279560	−0·058343	−0·075445	−0·133785
$S(x-\bar{x})^2$	0·007409	0·050534	0·057943	0·005943	0·008313	0·014256
$\bar{S}(y-y)^2$	0·761985	0·840435	1·602420	0·605356	0·708874	1·314230
N	3	2	5	3	3	6
SS for individual regressions ...	0·745868	0·833420	1·579288	0·572759	0·684704	1·257463
b	−10·0362	−4·0611	−4·8247	−9·8171	−9·0755	−9·3847
SS pool	—	—	1·349189	—	—	1·255748

2. The conditions for the legitimate substitution of an intermediate mortality level for the extinction time in Watson's[9] equation have been elucidated.

3. The concentration exponents of ethylene glycol and its monoalkyl ethers at 20°C. and 30°C. have been calculated from the log LT50-log concentration regressions. The standard errors of these regressions were all satisfactorily small.

4. The values of *n* for ethylene glycol (15·8654 at 20°C. and 18·4582 at 30°C.) are believed to be the highest ever recorded for a disinfectant substance.

5. At both temperatures of the experiments the value of *n* decreased as the homologous series of the monoalkyl ethers was ascended. The minimum value was reached in the monopropyl ether after which an increase was observed.

6. For ethylene glycol, the monomethyl ether and the monohexyl ether, no significant difference between the values of *n* at the 2 temperatures could be detected. The values of *n* at 30°C. for the monoethyl, monopropyl and monobutyl ethers were significantly lower than the corresponding values at 20°C.

7. Reference has been made to the proposal of Jordan and Jacobs[21] to establish a more satisfactory concentration exponent which was constant over a wide range of concentrations and for all temperatures.

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