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The relation between the bactericidal activities and certain physico-chemical properties of some fluorophenols

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The antibacterial activities, as demonstrated by the Rideal-Walker coefficients and viable counts, of mono-, 2,4,5- tri-, 2,3,5,6- tetra- and pentafluorophenol increase with the number of substituent fluorine atoms. The thermodynamic activities (Ferguson values) range from 0·083 to 0·022, and indicate a non-specific physical mode of action. Linear correlations occur between the equitoxic concentrations of the compounds to *Escherichia coli* (99·9% mortality after 50 min contact) and their molecular weights, number of fluorine substituents and water solubilities. A similar relation exists between the molar concentrations at the Rideal-Walker end points and the oleyl alcohol-water partition coefficients; no correlation occurs between toxicity and the cyclohexane-water partition coefficients. The surface tensions of equitoxic solutions vary between 57·5 and 63·2 mNm^{-1} (dynes/cm).

The bactericidal activities of *o*-chloro-, 2,4-dichloro- and 2,4,6-trichlorophenol, increase with increasing halogen substitution (Klarmann, Shternov & von Wowern, 1929; Ordal, 1941; Sykes, 1965). The low solubilities of tetra- and pentachlorophenol may interfere with the demonstration of their intrinsic activities (Wolf & Westveer, 1952), but using an aqueous suspension of organisms, Allawala & Riegelman (1954) demonstrated increasing toxicity throughout the chlorophenol series. The antibacterial activities of the monofluorophenols have been reported by Pinney & Walters (1967). This paper describes the activities of some polyfluorinated phenols and the relations between their toxicities and certain physico-chemical properties.

EXPERIMENTAL

Materials

Phenol A.R. (B.D.H.) and *o*-, *m*- and *p*-fluorophenols (Koch Light Limited) as described by Pinney & Walters (1967). 2,4,5-trifluorophenol, 2,3,5,6-tetrafluorophenol and pentafluorophenol (Imperial Smelting Corporation Ltd.). Their purity was confirmed by gas chromatography using the system of Pinney & Walters (1967) with nitrogen as the carrier gas.

Methods

Organisms and bactericidal evaluations. *Salmonella typhi* (NCTC 786) for Rideal-Walker evaluations and *Escherichia coli* (NCTC 5933), in aqueous suspension, for viable counts as described by Pinney & Walters (1967).

Solubility. Excess of each phenol was added to separate 20 ml quantities of distilled water in 50 ml "Quickfit" conical flasks. The flasks were held at 40° for

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1 h and then shaken 100 times/min at 25° for 36 h. After standing at 25°, the supernatant aqueous phases were removed and further clarified by centrifugation. The solutions were suitably diluted for spectrophotometric assay. Calibration curves obeyed Beer's Law and the respective λ_{max} of the phenols in water are given in Table 2.

Phase distribution. (*a*) Cyclohexane–water. Amounts of 20 ml of approximately 10^{-3}M solutions of each phenol and 20 ml of cyclohexane in 50 ml "Quickfit" flasks were shaken 20 times/min at 25° for 24 h. The aqueous phases were separated after standing at 25° for 1 h and the concentrations of phenol in solution determined spectrophotometrically. Water similarly treated with cyclohexane, was used as the reference solvent. The apparent partition coefficients (Reese, Irwin & others, 1964) were calculated from the respective concentrations in the two phases. The results are the means of duplicate experiments.

(*b*) Oleyl alcohol–water. Amounts of 5×10^{-3}M solutions (5×10^{-2}M for pentafluorophenol) and oleyl alcohol were used as above.

Surface tension. Deionized water containing 0·1% potassium permanganate and 0·1% sodium hydroxide was double distilled in an all glass apparatus. Distillate having a surface tension greater than 71 mNm^{-1} (dynes/cm) was collected. Solutions of the phenols were prepared with this water using glass apparatus which had been degreased with 1·0% sodium nitrate in sulphuric acid, rinsed with double distilled water and dried at 105°.

Surface tensions were determined by the Wilhelmy plate method using an apparatus devised by Padday (1957) and modified by Warburton (1966).

RESULTS

The log survivor-time curves for *E. coli* in aqueous solutions of the polyfluorophenols are shown in Fig. 1. The times to reduce the viability by 99·9% were obtained from these curves, and plots of the logarithms of these times against the log molar concentration of bactericide were linear (Fig. 2). The slopes of the lines in Fig. 2 gave the concentration exponents for the respective compounds (Table 1). The concentrations of phenol and the fluorophenols which reduce the initial viable population by 99·9% in 50 min were calculated from the data in Fig. 2. Table 1 gives the ratios of phenol : fluorophenol for these concentrations. The Rideal-Walker coefficients of the compounds were also determined and are presented in Table 1.

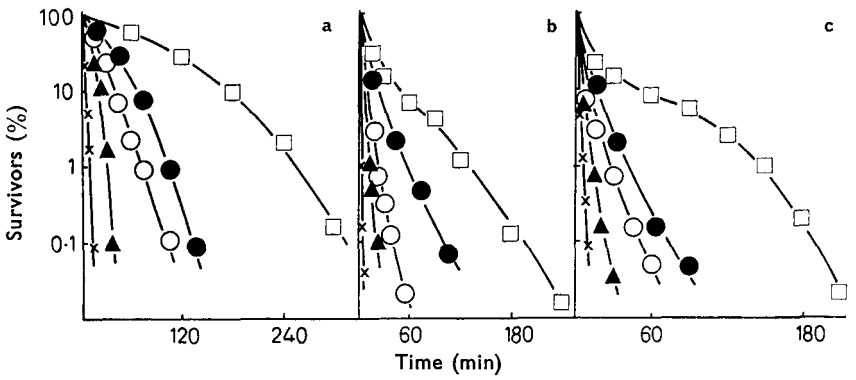

FIG. 1. Log survivor-time curves for *E. coli* exposed to fluorophenols.
a. Trifluorophenol (%): × = 0·4. ▲ = 0·3. ○ = 0·275. ● = 0·25. □ = 0·225. b. Tetrafluorophenol (%): × = 0·25. ▲ = 0·2. ○ = 0·175. ● = 0·15. □ = 0·125. c. Pentafluorophenol (%): × = 0·2. ▲ = 0·15. ○ = 0·125. ● = 0·1125. □ = 0·1.

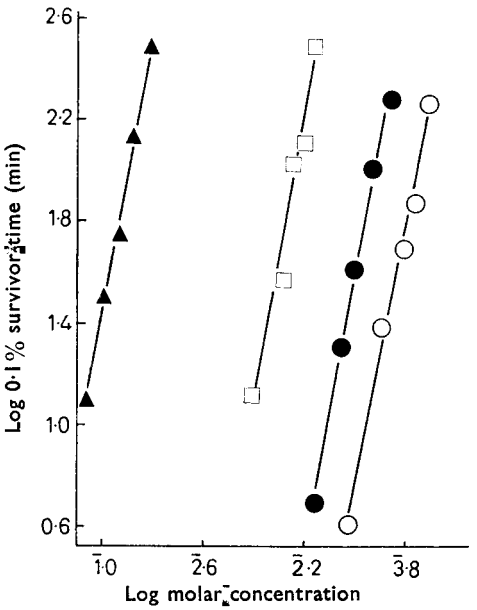

FIG. 2. Concentration exponent plots for phenol and fluorophenols against *E. coli*. ▲ = phenol. □ = trifluorophenol. ● = tetrafluorophenol. ○ = pentafluorophenol.

Table 1. *Comparison of the relative bactericidal activities of phenol and fluorophenols by viable counting and Rideal-Walker techniques*

Compound	Concentration exponent	Concn for 99·9% mortality of *E. coli* in 50 min at 25° %	Ratio: concn phenol / concn fluorophenol	Rideal-Walker Coefficient
Phenol	6·2	0·83	1·0	1·0
Trifluorophenol	5·5	0·30	2·8	2·5
Tetrafluorophenol	5·4	0·16	5·2	3·4
Pentafluorophenol	5·1	0·12	6·9	4·3

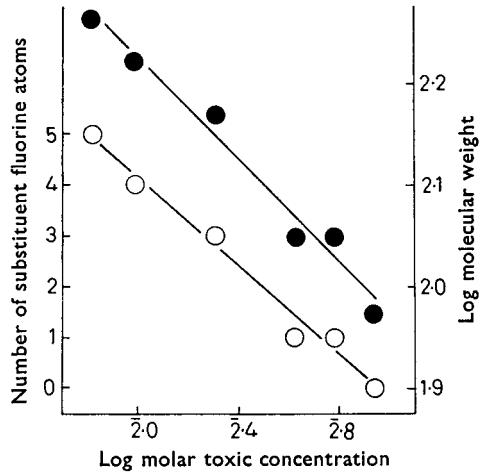

FIG. 3. Correlation of log molar concentrations of fluorophenols which produce 99·9% mortality in 50 min with their log molecular weights (●) and with the number of fluorine atoms substituted in the phenol molecule (○).

The correlation coefficients relating the log molar toxic concentration (99·9% mortality in 50 min) with the number of substituent fluorine atoms and with the log molecular weights (Fig. 3) are 0·991 and 0·987 respectively (d.f. = 6) The regressions are linear since the calculated coefficients correspond to P' values $< 0{\cdot}001$.

Table 2. *Solubilities and thermodynamic activities of toxic solutions of phenol and fluorophenols. (Equitoxic concentration is that which produces 99·9% mortality of* E. coli *in 50 min at 25°)*

Compound	λ_{max} nm	Solubility mol/litre at 25° (So)	Equitoxic concn mol/litre (St)	Thermodynamic activity of toxic solution St/So
Phenol	270	0·93	0·088	0·095
o-Fluorophenol	267	0·72	0·060	0·083
m-Fluorophenol	267·5	0·69	0·042	0·061
p-Fluorophenol	277	0·72	0·060	0·083
Trifluorophenol	274	0·42	0·020	0·048
Tetrafluorophenol	226·5	0·37	0·0096	0·026
Pentafluorophenol	265	0·30	0·0066	0·022

The log molar solubility (from Table 2) plotted against log molar toxic concentration is linear (Fig. 4) with a correlation coefficient of 0·985 (d.f. = 6; $P' < 0{\cdot}001$). Ferguson (1939) cites such linear correlations as being indicative of a physical mode of action. The relatively high thermodynamic activities of the toxic solutions (Table 2), calculated according to the method of Ferguson (1939), confirm physical toxicity.

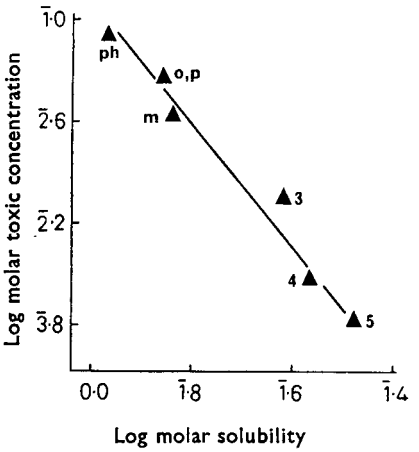

FIG. 4. Relation between log molar solubility of fluorophenols and their log molar toxic concentration (99·9% mortality in 50 min). Phenol = ph; monofluorophenols: *ortho* = o, *meta* = m, *para* = p; trifluorophenol = 3, tetrafluorophenol = 4, pentafluorophenol = 5.

The apparent partition coefficients of the compounds between cyclohexane (a solvent which does not form hydrogen bonds with the solute) and water (Table 3) are low; there is no correlation between them and the bactericidal activities which increase with increasing fluorine substitution (Fig. 3). However, with oleyl alcohol (a proton-attracting solvent), the antibacterial activities increase with the partition coefficients. The plot of toxic concentration against partition coefficient for oleyl alcohol (Fig. 5) is linear with a correlation coefficient of 0·990 (d.f. = 6; $P' < 0{\cdot}001$).

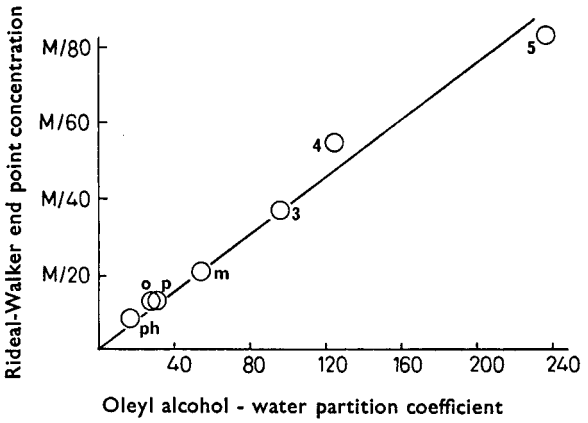

FIG. 5. Relation between toxic concentration of fluorophenols and their partition coefficients in oleyl alcohol-water. Symbols as in Fig. 4.

The plots of surface tension against concentration (not shown) for each phenol were concave to the axes. The surface tensions (Table 3) of equitoxic aqueous solutions of the bactericides were read from these plots. It is apparent that the concentration of each compound necessary to produce a 99·9% kill of *E. coli* in 50 min reduces the surface tension of water to the same order of magnitude. The mean surface tension of such equitoxic solutions is 60·3 mNm^{-1}, with limits of error ($P' = 0{\cdot}05$) of $\pm$ 2·0, and a coefficient of variation of 3·6%.

Table 3. *Partition coefficients and surface activities of phenol and fluorophenols. (Concentration of toxic solutions as in Table* 2)

Compound	Apparent partition coefficients at 25°		Surface tension of equitoxic solution at 25°
	Cyclohexane–water	Oleyl alcohol–water	mNm^{-1} (dynes/cm)
Phenol	0·1	15·6	60·4
o-Fluorophenol	0·7	24·8	58·8
m-Fluorophenol	0·2	54·1	63·0
p-Fluorophenol	0·1	29·9	58·6
Trifluorophenol	0·7	96·3	57·5
Tetrafluorophenol	—	123·1	60·3
Pentafluorophenol	0·3	236·8	63·2

Ferguson (1939) showed with a homologous series of normal primary alcohols that a nearly parallel relation existed between solubilities, toxic concentrations, concentrations reducing the surface tension of water to 50 mNm^{-1}, vapour pressures and partition coefficients. Fig. 6 summarizes our results for phenol and the fluorophenols.

An analysis of variance and a test for parallelism on the four regressions in Fig. 6 gave $F^3_{20} = 8{\cdot}57$. The tabulated value of F^3_{20} ($P' = 0{\cdot}05$) is 3·10; since the tabulated value is much less than that calculated, the lines may not be regarded as parallel. A similar calculation on the slopes of the regression lines for toxicity, partition coefficients and surface tension reduction, gave $F^2_{15} = 3{\cdot}28$ (tabulated value = 3·68; $P' = 0{\cdot}05$). The slopes of the regressions relating these three properties to the number of fluorine substituents are therefore not significantly different.

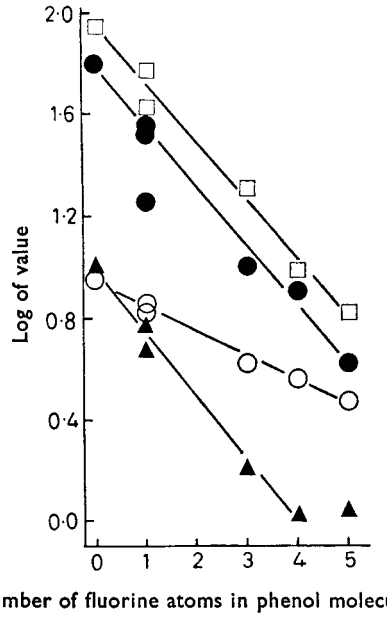

FIG. 6. Correlation of toxic concentrations (99·9% mortality in 50 min) and physical properties of fluorophenols with the number of fluorine atoms substituted in the phenol molecule. □ = molar toxic concentration, × 10^3. ● = 1/oleyl alcohol-water partition coefficient, × 10^3. ○ = molar solubility, × 10. ▲ = molar concentration to reduce surface tension of water to 60 mNm^{-1} (dynes/cm), × 10^2.

DISCUSSION

Chauhan & Walters (1967) showed that the log survivor-time curves for *Penicillium notatum* spores exposed to phenols became increasingly concave to the axes as the aqueous solubilities of the compounds decreased and, therefore, the oil-water partition coefficients increased. A similar trend (for example, Fig. 1) has been obtained with the fluorophenols as the series is ascended from the more water soluble unsubstituted phenol (Table 2) to the more lipid soluble pentafluorophenol (Table 3 and Fig. 5).

The concavity of the time-survivor curves may explain why tetra- and penta-fluorophenol are more active when compared with phenol at the 99·9% mortality level than by the Rideal-Walker test (Table 1). Thus, the initial reaction rate is rapid for the higher fluorophenols and decreases with increase in mortality level. Therefore, a given concentration will, relative to phenol, take longer to reach the lower survivor levels of the Rideal-Walker test than to attain a 99·9% kill.

The log survivor-time curves for the lowest concentrations of tetra- and penta-fluorophenol are concave to the axes to about the 5% survivor level; the rate of kill then increases and they become convex (Fig. 1). This apparent change in the rate of reaction is unlikely to be due to adsorption or penetration phenomena since, in each case, the second phase of rapid mortality does not commence until after about 60 min exposure. It is probable that the shape of the curves is determined by the distribution of resistance in the bacterial population.

The thermodynamic activities of equitoxic solutions of the fluorophenols (Table 2), decrease with increasing fluorine substitution and, therefore, with increasing molecular

weight of the compounds. A similar relation was found by James, Loveday & Plummer (1964) with *p*-halogenated phenols. These authors also investigated the series: phenol, *p*-cresol, *p*-ethylphenol and *p*-n-propylphenol. The activities remained almost constant as did those of the phenols examined by Allawala & Riegelman (1954). Both of these trends differ from that originally described by Ferguson (1939) and considered normal by Sexton (1963), namely that activities rise as a homologous series is ascended. Sexton (1963) considers this rise to be due to departure from ideality in the physical properties of high molecular weight substances. The comparatively lower molecular weights of the fluorophenols may be the reason why a similar trend was not found.

The partition coefficients between oleyl alcohol and water increase with toxicity, but those between cyclohexane and water do not (Fig. 5 and Table 3). These results substantiate the findings of Burton, Clarke & Gray (1964); only organic solvents which form hydrogen bonds with phenols should be used for partition coefficient measurements when the results are to be compared with bactericidal activities. The partition coefficients of the fluorophenols between oleyl alcohol and water represent their bulk phase distribution, and hydrogen bonding which occurs between the phenol and the lipid may assist transfer of the phenol molecule from the aqueous to the organic phase (Burton & others, 1964). Such a mechanism could occur at or above the cell membranes of organisms where a large amount of lipid is located (Gilby & Few, 1957; McQuillen, 1960; Martin, 1963). Correlations between increasing lipid solubility of phenols and both their uptake by *E. coli* and their bactericidal activities have been demonstrated by Judis (1964). It is possible that hydrogen bonding between phenols and the lipoprotein and lipopolysaccharide of the cell wall and membrane is part of the mechanism involved in the disruption of the permeability barriers of the cell which results in lysis and death.

The lipid content of Gram-negative cells is much greater than in Gram-positive organisms and a high lipid content has been implicated in resistance to disinfectants (Chaplin, 1952; Truby & Bennett, 1966; Hugo, 1967; Hugo & Franklin, 1968). It may be that the inner layers of the lipoprotein of lipid rich membranes are not so readily damaged and thereby permeability barriers are maintained.

Equitoxic solutions of the bactericides have approximately the same surface tension (Table 3) in agreement with the results of Traube & Somogyi (1921), Frobisher (1927) and Zissmann (1954, 1957). Such correlation is found only with surfactant solutions which are antibacterial. Thus James (1965) observes that reduction of surface tension is not in itself bactericidal, since non-ionic surface-active agents are non-toxic (Baker, Harrison & Miller, 1941; Hotchkiss, 1946).

The regressions relating log molar toxic concentration, partitioning (plotted as the reciprocal of the partition coefficient so as to obtain a line of negative slope) and surface tension reduction, to the number of fluorine substituents in the phenol molecule are parallel (Fig. 6). However, although there is a linear correlation between log molar solubility and log molar toxic concentration (Fig. 4), the reduction in solubility is not paralleled by the increase in toxicity (Fig. 6). It would appear that a better prediction of bactericidal activity may be obtained from partitioning data than from solubility determinations. Similar conclusions have been drawn by Hess & Speiser (1959) and McCulloch & Stock (1966).

Thus although the principle of Ferguson (1939) is of great value in determining whether toxic action is of a chemical or physical nature, i.e. whether death is due to

interference with cell biochemistry or results from non-specific structural damage, deviations from the ideal state of solutions and approximations produced by derivation of thermodynamic activities from solubility data, may result in better correlations of toxicity being obtained with some physico-chemical property other than solubility.

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