THE STIMULATING EFFECT OF GLYCOLS AND THEIR POLYMERS ON THE TARSAL RECEPTORS OF BLOWFLIES*

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INTRODUCTION

Previous studies of the rejection thresholds of the blowfly Phormia regina Meigen for series of aliphatic alcohols have revealed a very high degree of correlation between the concentration at rejection and those chemical properties which are related thermodynamically to one another (3).

Because the technique of testing requires an aqueous solution, it had not been possible to extend the study of the relationship between chemical structure and stimulation beyond a chain length of eight carbon atoms. This difficulty has now been overcome in part by the use of water-soluble high polymers of the glycol series. The present study was undertaken, therefore, to examine the relation between stimulating effect and chemical properties with compounds having chain lengths up to C2n and to compare the effects of compounds containing ether linkages with the effects of straight saturated hydrocarbon chains.

The experimental technique was identical with that followed in earlier studies (3). For the purpose of statistical analysis the raw data were resampled in the following manner. A table with as many columns as there were concentrations tested was constructed for each compound. The specimens were now sampled, five at a time, in the order in which they had appeared for testing. For the first group of five, the number rejecting at the highest concentration was recorded in the table; for the next group, the number rejecting at the next lower concentration; and so on, in rotation, until all flies used in the test had been recorded. The percentages rejecting at each concentration were calculated from the totals in the columns, converted to probability units, and plotted against the logarithms of the respective concentrations. The most probable value of the log concentration rejected by 50 per cent of the flies was then determined according to the procedure described by Bliss (1) and has been used for comparing the responses of the population to the different compounds tested. The basis for this treatment of the results is considered in the discussion below.

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Ethylene, diethylene, triethylene, tetraethylene, dipropylene, ethylhexylene (2-ethyl hexanediol-1,3), and polyethylene glycols together with data on their properties were supplied through the courtesy of Carbide and Carbon Chemicals Corporation. Tripropylene glycol and three polypropylene glycols were received through the courtesy of the Dow Chemical Company. Propylene glycol was obtained from Eimer and Amend, trimethylene from Eastman, and decamethylene from Paragon Testing Laboratories. Octanediol-2,3 was supplied by the Orlando Laboratory of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, through the cooperation of the Chemical-Biological Coordination Center of the National Research Council.

Ethylene, trimethylene, and tetraethylene glycols were further purified by redistillation at reduced pressure. With the first there was no significant difference between thresholds obtained with the crude and with the purified product. Trimethylene and tetraethylene glycols, however, gave significantly higher thresholds after distillation, and there is reason to suspect that the former still contained a certain amount of physiologically active impurities. These results suggested the desirability of testing the purity of each of the compounds used exclusive of the polymers; hence boiling point determinations were made.

Polyethylene and polypropylene glycols are mixtures of polymers, and the numerical designation of each mixture represents the average molecular weight. Molecular size distribution within such a mixture follows a Poisson distribution (6, 7). Attempts at fractionation for the purpose of isolating some of these species were not entirely successful; hence, threshold values for higher members of these series refer to mixtures rather than to single compounds.

RESULTS

Three homologous series were studied, the methylene, ethylene, and propylene glycols. The first series was represented by ethylene glycol, trimethylene glycol, pentanediol-1,5, hexanediol-1,6, and decanediol-1,10. Since no homologous 7, 8, or 9 carbon diols were obtainable, 2-ethyl hexanediol-1,3, and octanediol-2,3 were substituted to give points of reference between C6 and C10. The second series was represented by ethylene glycol through polyethylene glycol 6000; the third, by ethylene, propylene, dipropylene, and tripropylene glycols and by three polypropylene glycols with average molecular weights of 400, 750, and 1200. The data for the glycols are summarized in the following equations, which describe the regression of log concentration (Y) rejected by 50 per cent of the flies on log No. of carbon atoms (X). In making these calculations the points for the individual compounds have been given equal values irrespective of the number of measurements by which each was determined. Data for the corresponding alcohols are given for comparison.

1. Alcohols through 1-butanol
   \[ Y = 0.22825 - 1.7573(X - 0.345) \]
   variance of \(a\) = 0.0088; variance of \(b\) = 0.0592
2. Alcohols from butanol to 1-octanol, inclusive
   \[ Y = -2.552 - 13.5152(X - 0.80625) \]
   Variance of \(a\) = 0.0023; variance of \(b\) = 0.3247

3. Diols through hexanediol-1,6
   \[ Y = 0.35175 - 2.3040(X - 0.56375) \]
   Variance of \(a\) = 0.0068; variance of \(b\) = 0.1938

4. Hexanediol-1,6 to decamethylene glycol
   \[ Y = -1.181 - 9.8900(X - 0.88908) \]
   Curve based on only two points

5. Polyethylene glycols, pure compounds
   \[ Y = 0.39175 - 1.8555(X - 0.646) \]
   Variance of \(a\) = 0.0016; variance of \(b\) = 0.0303

6. Polyethylene glycol mixtures
   \[ Y = -0.1900 - 0.5359(X - 1.35467) \]
   Variance of \(a\) = 0.0002; variance of \(b\) = 0.0016

7. Ethylene glycol through dipropylene glycol
   \[ Y = 0.5323 - 2.4592(X - 0.5187) \]
   Variance of \(a\) = 0; variance of \(b\) = 0

8. Polypropylene glycol mixtures
   \[ Y = -2.5370 - 4.2642(X - 1.4533) \]
   Variance of \(a\) = 0.0144; variance of \(b\) = 0.3407

The type formula for the above is \(Y = a + b(X - \bar{x})\), where \(a\) has the numerical value of \(\gamma\) and where \(x\) and \(\gamma\) are the mean values of empirical \(X\) and \(Y\) respectively.

Results for the glycols are summarized in Table I, and for the first eight primary normal alcohols in Table II.

**DISCUSSION**

(a) The Distribution of Acceptance and Rejection Thresholds in Insect Populations.—An acceptance or rejection threshold may be defined as the least concentration of a chemical required to cause (or prevent) the manifestation of some response selected by the investigator and interpreted as acceptance or refusal. Despite precautions taken in the determination it is commonly observed that not all individuals of a given species respond alike to a single concentration of the test agent. Over a certain critical range, at least, some specimens will accept while others reject; and although with a small group of individuals it is possible usually to extend the range in both directions (unless solubility interferes) until 100 per cent acceptance or refusal is obtained, increasing the number of insects sampled generally requires a further extension of range in order to achieve 100 per cent response. These facts raise questions as to the accuracy and significance of threshold determinations, and as to the most suitable procedures for their measurement.

In studying with *Phormia* the rejection of a large number of unacceptable compounds, a definite relationship between concentration of the test material and the distribution of thresholds has been noted regularly in samples of flies selected at random from a population of known age which had been reared...
under standard conditions. For such samples, the scattering of thresholds is, within the limits of experimental error, normal with respect to the logarithm of concentration. The same sort of relationship is apparent also in the results of other entomologists who have reported sufficient numbers of tests to permit a comparable analysis. As evidence we present examples recalculated from the

### TABLE I

**Response of Phormia to Glycols in 0.1 M Sucrose**

<table>
<thead>
<tr>
<th>Glycol</th>
<th>No. of C atoms</th>
<th>Log molar concentration rejected by 50% per cent ± 2.575 s.e.</th>
<th>$a$ ± s.e.$^*$</th>
<th>$b$ ± s.e.$^*$</th>
<th>$\bar{X}$ ± s.e.$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene</td>
<td>2</td>
<td>1.076 ± 0.046</td>
<td>4.981 ± 0.087</td>
<td>4.888 ± 0.590</td>
<td>1.072 ± 0.254</td>
</tr>
<tr>
<td>Trimethylene</td>
<td>3</td>
<td>0.359 ± 0.151</td>
<td>5.188 ± 0.182</td>
<td>3.144 ± 0.481</td>
<td>0.419 ± 0.100</td>
</tr>
<tr>
<td>Pentanedio-1,5</td>
<td>5</td>
<td>0.056 ± 0.146</td>
<td>5.070 ± 0.154</td>
<td>2.736 ± 0.422</td>
<td>0.030 ± 0.100</td>
</tr>
<tr>
<td>Hexanediol-1,6</td>
<td>6</td>
<td>0.084 ± 0.129</td>
<td>4.996 ± 0.148</td>
<td>2.969 ± 0.393</td>
<td>0.083 ± 0.100</td>
</tr>
<tr>
<td>2-Ethyl hexanediol-1,3</td>
<td>8</td>
<td>1.654 ± 0.141</td>
<td>5.019 ± 0.209</td>
<td>3.785 ± 0.693</td>
<td>1.649 ± 0.100</td>
</tr>
<tr>
<td>Octanediol-2,3</td>
<td>8</td>
<td>1.884 ± 0.103</td>
<td>4.849 ± 0.230</td>
<td>2.278 ± 0.661</td>
<td>1.950 ± 0.100</td>
</tr>
<tr>
<td>Decanediol-1,10</td>
<td>10</td>
<td>-2.278$^*$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diethylene</td>
<td>4</td>
<td>0.374 ± 0.157</td>
<td>5.489 ± 0.171</td>
<td>3.069 ± 0.454</td>
<td>0.531 ± 0.140</td>
</tr>
<tr>
<td>Triethylene</td>
<td>6</td>
<td>0.171 ± 0.119</td>
<td>5.346 ± 0.138</td>
<td>3.126 ± 0.378</td>
<td>0.282 ± 0.144</td>
</tr>
<tr>
<td>Tetraethylene</td>
<td>8</td>
<td>0.054 ± 0.101</td>
<td>4.989 ± 0.142</td>
<td>3.625 ± 0.480</td>
<td>-0.016 ± 0.171</td>
</tr>
<tr>
<td>Propylene</td>
<td>3</td>
<td>0.621 ± 0.228</td>
<td>5.502 ± 0.375</td>
<td>5.010 ± 2.367</td>
<td>0.721 ± 0.123</td>
</tr>
<tr>
<td>Dipropylene</td>
<td>6</td>
<td>0.100 ± 0.148</td>
<td>5.172 ± 0.137</td>
<td>2.648 ± 0.321</td>
<td>-0.035 ± 0.150</td>
</tr>
<tr>
<td>Tripropylene</td>
<td>9</td>
<td>0.599 ± 0.109</td>
<td>5.301 ± 0.307</td>
<td>2.590 ± 0.386</td>
<td>-0.710 ± 0.175</td>
</tr>
<tr>
<td>Polypropylene 400</td>
<td>13.5$^*$</td>
<td>-1.233 ± 0.403</td>
<td>5.095 ± 0.532</td>
<td>2.797 ± 1.704</td>
<td>-1.201 ± 1.00</td>
</tr>
<tr>
<td>Polypropylene 750</td>
<td>31.5$^*$</td>
<td>-2.528 ± 0.193</td>
<td>5.377 ± 0.365</td>
<td>2.443 ± 0.525</td>
<td>-2.374 ± 1.00</td>
</tr>
<tr>
<td>Polypropylene 1200</td>
<td>54.5$^*$</td>
<td>-3.848 ± 0.352</td>
<td>4.903 ± 0.133</td>
<td>0.996 ± 0.328</td>
<td>-3.946 ± 1.00</td>
</tr>
<tr>
<td>Polyethylene 200</td>
<td>8$^*$</td>
<td>0.024 ± 0.178</td>
<td>5.314 ± 0.133</td>
<td>2.110 ± 0.409</td>
<td>0.173 ± 0.100</td>
</tr>
<tr>
<td>Polyethylene 300</td>
<td>12$^*$</td>
<td>-0.088 ± 0.094</td>
<td>5.407 ± 0.128</td>
<td>3.827 ± 0.524</td>
<td>0.098 ± 0.152</td>
</tr>
<tr>
<td>Polyethylene 400</td>
<td>18$^*$</td>
<td>-0.117 ± 0.336</td>
<td>5.431 ± 0.280</td>
<td>2.701 ± 1.341</td>
<td>0.042 ± 0.100</td>
</tr>
<tr>
<td>Polyethylene 600</td>
<td>26$^*$</td>
<td>-0.255 ± 0.266</td>
<td>5.282 ± 0.320</td>
<td>3.301 ± 1.386</td>
<td>-0.170 ± 0.118</td>
</tr>
<tr>
<td>Polyethylene 1000</td>
<td>44$^*$</td>
<td>-0.327 ± 0.119</td>
<td>5.041 ± 0.257</td>
<td>3.548 ± 1.642</td>
<td>-0.320 ± 0.170</td>
</tr>
<tr>
<td>Polyethylene 1540</td>
<td>68$^*$</td>
<td>-0.456 ± 0.073</td>
<td>4.808 ± 0.138</td>
<td>5.028 ± 0.934</td>
<td>-0.494 ± 0.109</td>
</tr>
<tr>
<td>Polyethylene 4000</td>
<td>182$^*$</td>
<td>Accepted in saturated solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene 6000</td>
<td>272$^*$</td>
<td>Accepted in saturated solution</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$ The 4th, 5th, and 6th columns of the table give the calculated values for $a$, $b$, and $\bar{X}$ in the equation $Y = a + b(X - \bar{X})$, which is the regression of per cent flies rejecting, $Y$, expressed as probits, on log concentration, $X$. s.e. = standard error.

$^\dagger$ Estimated from 6 per cent rejection of the saturated solution (0.0016 $^\dagger$), by assuming a value of 3 for $b$.

$^\pp$ Average number of carbon atoms.
work of Weis (21) with the butterfly, *Pyrameis*; von Frisch (10) with bees; and
Frings (8, 9) with the American roach (Fig. 1). We are indebted to Dr. Frings
for his kindness in arranging and making available his original data. Our own
data for various glycols are shown in Figs. 2 and 3.

Recognition of this relationship has served as a useful guide in planning ex-
periments and in the treatment of results. The procedure we have followed is
essentially that advocated by Bliss (1) for dealing with dosage-mortality data.
Separate samples of 1 to 3 day old flies taken at random from the culture are
tested at each of six or seven concentrations within the critical range, and the
percentages accepting or rejecting converted into probability units. A plot of
these against log concentration yields a linear regression. The slope and posi-
tion of the regression line, and the most probable value of the concentration

<table>
<thead>
<tr>
<th>TABLE II</th>
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<tbody>
<tr>
<td>Response of Phormia to Normal Alcohols in 0.1 m Sucrose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>No. of C</th>
<th>Log molar concentration rejected by 50 per cent</th>
<th>± s.e.</th>
<th>± s.e.</th>
<th>± s.e.</th>
<th>± s.e.</th>
<th>± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1</td>
<td>0.782 ± 0.205</td>
<td>4.928 ± 0.223</td>
<td>2.809 ± 0.062</td>
<td>0.757 ± 0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>2</td>
<td>0.377 ± 0.152</td>
<td>5.179 ± 0.248</td>
<td>4.304 ± 1.254</td>
<td>0.418 ± 0.120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Propanol</td>
<td>3</td>
<td>0.077 ± 0.048</td>
<td>5.064 ± 0.170</td>
<td>9.076 ± 1.799</td>
<td>0.084 ± 0.088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Butanol</td>
<td>4</td>
<td>-0.323 ± 0.066</td>
<td>5.363 ± 0.167</td>
<td>7.059 ± 1.348</td>
<td>-0.212 ± 0.146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>5</td>
<td>-1.122 ± 0.066</td>
<td>4.919 ± 0.191</td>
<td>7.507 ± 1.272</td>
<td>-1.132 ± 0.105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>6</td>
<td>-2.211 ± 0.136</td>
<td>4.991 ± 0.145</td>
<td>2.742 ± 0.491</td>
<td>-2.213 ± 0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Heptanol</td>
<td>7</td>
<td>-2.935 ± 0.189</td>
<td>4.848 ± 0.142</td>
<td>1.968 ± 0.370</td>
<td>-3.012 ± 0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Octanol</td>
<td>8</td>
<td>-3.940 ± 0.161</td>
<td>4.832 ± 0.150</td>
<td>2.445 ± 0.414</td>
<td>-4.008 ± 0.100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

required for 50 per cent response (or for some other chosen level), together with
their variances, are calculated by the usual statistical methods.

Since the distribution of thresholds is normal with reference to log concen-
tration, the use of the arithmetic mean for purposes of comparison (as in our
earlier papers) is invalid. Fortunately, if similar numbers and similar series of
concentrations have been used in the experiments, the arithmetic means will
stand in about the same order relative to one another as the geometric means,
so that the same qualitative conclusions are reached in both cases.

The treatment here adopted, in addition to providing a quantitatively more
accurate measure of the response of a population, has the advantage that it
permits the inclusion in comparisons of data on compounds which, on account
of low solubility or weak stimulating power, cannot be investigated over the
full range required for 100 per cent response. This is possible because the
slope and position of the regression line can still be determined within known
limits of accuracy on the basis of the results available from the range open to
Fig. 1. Distribution of acceptance and rejection thresholds for various insects as a function of concentration. Raffinose—acceptance by *Pyrameis atalanta* L., from data of Weis (21); sucrose—acceptance by *Pyrameis atalanta* L., from data of Weis (21); HCl—rejection by *Apis mellifera* L., from data of von Frisch (10); quinine·HCl—rejection by *Apis mellifera* L., from data of von Frisch (10); NH₄NO₃—rejection by *Periplaneta americana* L., from data of Frings (8).
Fig. 2. Distribution of rejection thresholds for Phormia for various glycols, as a function of concentration.
experimentation. Thus, for example, in our tests with ethylene glycol about one-quarter of the flies regularly accepted the undiluted compound containing 0.1 M sucrose, but this fact has not interfered with the calculation of the most probable value for the concentration rejected by 50 per cent of the population.

It is apparent from the literature on insect chemoreception that others must also have been aware that the rate of response is related to the logarithm of concentration, although we have found no definite statements to that effect. For instance, Eger (5) has recorded his results with lepidopterous larvae on a semilogarithmic scale, with percentages of rejection plotted against class units which are proportional to the logarithm of concentration, and has tacitly assumed a normal distribution by calculating the geometric means and their standard errors. Frings and O'Neal (9) expressed their data with Tabanus as geometric means which were determined by a method of graphical interpolation. That the situation is not peculiar to insects is indicated by the results of Krinner (12) with the minnow Phoxinus.

The underlying reason for the phenomenon described is not known. It is quite usual for the intensity of various expressions of a sensory response to be related approximately logarithmically to the intensity of the stimulus (here, concentration), and we have cited elsewhere (4) a number of examples in chemoreception where this appears to be the case. But at present we do not have any information on questions such as how the frequency of discharge of a single chemoreceptor and the number of units responding are dependent on stimulus intensity, nor can we judge whether these or yet other factors are at the base of differences between individual specimens.

(b) The Relative Stimulating Effectiveness of the Glycols.—For all series of homologous aliphatic compounds studied thus far it has been found that members of the series were rejected at logarithmically decreasing concentrations as the carbon chain increased in length. In every case only chains of CH₂ groups were involved. In the series represented by polyethylene and polypropylene glycols a different type of molecular chain than heretofore tested is formed by the presence of ether linkages. Members of the former, for example, are compounds of the general formula OHCH₂(CH₂OCH₂)ₙ CH₃OH. Nevertheless, these glycols as well as the straight diols conform to the previously established pattern (Fig. 4). In terms of molecular weight or number of carbon atoms the latter are slightly more stimulating than the ether-linked chains although the differences in stimulating effectiveness between the lower members of the three series are not statistically significant when the slopes of the lines of best fit are compared. The propylene series, members of which contain longer carbon chain links between oxygen atoms than do the polyethylene glycols, more nearly resemble the straight diols in stimulating power. Unfortunately physical data are not available for a complete analysis of this problem. It has been shown by Sauter (15, 16) and Fuller and Frosch (11) that the presence of ether or
glycol linkages in a chain causes distortion of the usual zigzag form with the consequent assumption of a helical form. Inasmuch as chain length appears to be of importance as far as stimulation is concerned, it is not improbable that an explanation of the differences between straight diols and polyethylene or polypropylene compounds may be associated with the difference in molecular shape.

At that point in the ethylene series where polymer mixtures were employed in lieu of pure compounds, which were unobtainable, there is a significant change in stimulating effectiveness. The mixtures are less stimulating than would be expected from their average molecular weight. The difference in slope of the pure and mixed members is 1.3197, about 7.4 X its standard error of 0.1786. A comparable relationship probably exists between the pure polypropylene glycols and mixed members. At the moment we have no satisfactory explanation of this phenomenon. Lovell and Hibbert (14) have stated that uni-
polymers may show outstanding differences in properties from a mixture of homologues with the same average molecular weight; however, no comparative data were given. Hence, while we can point to comparable physiological differences, we are unable to state whether the relation is direct or inverse.

Saturated solutions of polyethylene glycols 4000 and 6000 did not stimulate. Thus the highest effective member tested is polyethylene glycol 1540. The former solutions were 0.2 M and 0.1 M respectively. The effective concentrations, if the curve is extrapolated, fall above these values. Had the reverse been true, it would have indicated that the stimulative efficiencies of compounds at this point had begun to decrease. Data from other sources also militate against this possibility. Studies of the toxicity of single oral doses of glycol polymers to the rat, rabbit, and guinea pig (13, 17-19) have shown that the LD₅₀, if measured as molar concentration, decreases in a more or less regular manner with increasing molecular weight. In these studies it was possible to obtain a value for a polymer as high as 3600, and the curve shows no tendency to break at this point. It is noteworthy that toxicity data on mammals should parallel so closely rejection thresholds in insects. This may be construed as further evidence to support the idea that what is being measured in this and other cases is probably the rate of access of the compounds to the system rather than their final interaction with the processes under observation (cf. reference 3).

It is of further interest that such large molecules as 1540 should stimulate the chemical senses. Not many large ones appear to do so. Von Frisch (10) recorded that bees accepted with slight hesitation a 1 M sucrose solution containing 1/5 per cent colocynthin (C₂₀H₅₀O₇) and that eupatorin (C₂₂H₂₇O₁₁) is tasteless or slightly bitter. Some proteins, e.g. casein, hen's egg albumin, horse serum albumin, and hemoglobin, are reported by Thorpe et al. (20) as eliciting the biting response in wireworms.

When glycols are compared with the corresponding alcohols, it is seen that the introduction of a second hydroxy radical renders the compound much less stimulating. Thus the median rejection threshold for ethylene glycol is 11.91 M as compared with 2.38 M for ethanol, and for trimethylene, 2.287 M as compared with 1.195 M for n-propyl alcohol. The effect of —OH substitution here agrees with that observed by Chadwick and Dethier (2) with aliphatic acids. From a comparison based on the lines of best fit it is seen that the alcohols corresponding to the first three diols average about four times as stimulating as the latter. Similarly calculated, the alcohols corresponding to the diols from hexanediol-1,6 to decamethylene glycol are more than one hundred times as stimulating.

That the position of the hydroxy substitution is of some importance is observed when trimethylene and propylene glycols are compared. As would be expected from a comparison of the corresponding alcohols, the straight chain
compound is more stimulating than its branched isomer. Position effect has been studied in a large series of compounds and is to be treated at greater length in a subsequent communication now in preparation.

Present analyses indicate the presence of a break in the curve describing the stimulating effectiveness of the methylene series (cf. Fig. 4). Reexamination of our results with the primary normal alcohols also has shown the existence of a definite sharp break at or beyond n-butanol. The slope of the curve for the lower alcohols is $-1.7573 \pm 0.2433$ (standard error); for the higher members it is $-13.5152 \pm 0.5698$. The line of best fit for the diols (ethylene glycol through hexanediol-1,6) has a slope of $-2.3040 \pm 0.4402$; i.e., not significantly different from that for the lower portion of the alcohol series. If the tentative threshold value given in Table I for decamethylene glycol is accepted, a line drawn between this point and that for hexanediol-1,6 has a slope of $-9.8900$. Although we hesitate to stress this determination of the portion of the curve for the higher diols in view of the uncertainty of the threshold value for decamethylene glycol, the results found with 2-ethyl hexanediol-1,3 and octanediol-2,3 strengthen the case for such an interpretation. These C₈ diols are not strictly homologous with the other members of this series; however, when a comparison is made of isomers such as propylene and trimethylene glycols or various alcohols (3), the difference in stimulating effectiveness is not great unless the isomers differ markedly in such properties as solubility, boiling point, etc. With reference to the polypropylene glycols, not enough unipolymers are presently available for testing to decide whether a similar break may also occur in this series. While it is possible to calculate a single line of best fit for the entire group it is unlikely that this represents the true situation. There is a fair probability that the slope of such a line ($-3.5028$) is too great for the range below C₄ and that unipolymers with more than twelve carbon atoms might be significantly more stimulating than the corresponding mixtures.

**SUMMARY**

The rejection thresholds of *Phormia regina* Meigen for twenty-four glycols have been determined. A definite relationship between the concentration of the test material and the distribution of thresholds has been noted regularly in samples of flies selected at random from a population of known age which had been reared under standard conditions. The scattering of thresholds is normal with respect to the logarithm of concentration. Recalculation of the data of other workers reveals the same sort of relationship with other species of insects and the minnow *Phoxinus*. The underlying reason for the phenomenon is not known.

The glycols in common with other series of homologous aliphatic compounds are rejected at logarithmically decreasing concentrations as the chain length is
increased. In general the straight chain diols are more stimulating than the corresponding polyethylene and polypropylene glycols. This difference is related in some manner to the presence of ether linkages in the latter. Polypropylene glycols, with chains of three carbon atoms between the ether linkages are more stimulating than polyethylene glycols, where the spacing is $\cdots\text{O}--\text{C}--\text{O}\cdots$. Unipolymers are more stimulating than mixtures of homologues with the same average molecular weights. Polyethylene glycol 1540 is the largest molecule of measured molecular weight known to stimulate chemoreceptors. The introduction of a second terminal hydroxyl group into the straight hydrocarbon chain reduces the stimulating effect. Alcohols corresponding to the first three diols average about four times as stimulating as the latter while those corresponding to the higher diols average more than one hundred times as stimulating.

REFERENCES