CORRELATION OF THE AQUARIUM GOLDFISH TOXICITIES
OF SOME PHENOLS, QUINONES, AND OTHER BENZENE
DERIVATIVES WITH THEIR INHIBITION OF
AUTOXIDATIVE REACTIONS

BY TORALD SOLLMANN

(From the Laboratory of Pharmacology of the School of Medicine of Western Reserve
University, Cleveland)

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INTRODUCTION

In the course of some investigations on the effects of certain chemicals on
goldfish, it was noted that the toxicity of hydroquinone and of tertiary butyl
catechol in the aquarium water is about a hundred times higher than that of
phenol; while their toxicity on injection into mammals and into fish is at most
twice as high. Tertiary butyl catechol and hydroquinone show a high toxicity
for the small entomostracan Daphnia magna, forty and a hundred times
that of phenol. This suggests that the aquarium toxicity of these poisons is
qualitatively different from that of phenol and from their action when ad-
ministered to mammals or injected into fish. Hydroquinone and analogous
substances are noted for their powerful inhibitory action on autocatalytic
“chain reactions,” such as the oxidative changes that lead to rancidity in fat;
the “aging” of rubber; the polymerization of rubber; and the “cracking” of
gasoline. The high “toxicity” of hydroquinone to these reactions is illustrated
by the observation of Moureau and Dufraisse (1927) that one molecule of
hydroquinone prevents the oxidation of 40,000 molecules of acrolein. The
effective quantities are so minute that the anticatalytic actions are presumably
exerted on some critical points in the autocatalytic chain, perhaps by the reso-
nance of the quinone structure, involving oxidation or reduction in some cases
and not in others (Milas, 1929, 1932). This could have analogies, although
perhaps only superficial, with biologic processes. If so, there should be corre-
lation between the chemical reactivity of these substances in vitro and their
biologic reactivity, as expressed for instance in their aquarium toxicity. Such
correlation would not prove that the particular inhibition of autocatalytic
activity is the direct cause of the biologic response; they could both be mani-
festations of an underlying property of the substance, such as intramolecular
mobility. The correlation would, however, point out directions for further
inquiry along these lines. Correlation of the biologic action with the chemical
reactivity would be a step nearer to the essence of the action, than attempts at
correlation with the chemical constitution, which after all must operate by
modifying the chemical reactivity of the substance.
The following experiments are the initial part of an exploration of the practical usefulness and limitations of this line of inquiry, by attempting the correlation of a variety of such autocatalytic reactions with fish toxicity on the one hand, and with their oxidation potential on the other. The aquarium method offers the special advantage that the relatively large volume of the solution, which is several hundred times that of the fish, would keep the concentration in the blood and tissues of the animal uniform and constant for the entire duration of the experiment, provided that the substance is sufficiently stable in aqueous solution; and that the substance is absorbed from the aquarium more rapidly than it is destroyed in a 2 day exposure of the fish. It should therefore give more absolute values than are obtained when the concentration in the animal declines continuously by excretion and destruction, after the drug is injected. It should be remembered, however, that the aquarium toxicity may depend on local actions, especially on the gills, and need not be identical with the systemic toxicity.

Methods

Goldfish, generally 3.5 to 6.5 cm. in length (exclusive of the tail fin), weighing 3 to 10 gm. were placed singly in glass aquaria filled with 2 liters of Cleveland city water which had stood for a day in the same room as the storage aquarium, so as to guard the fish against sudden change of temperature. The agent under investigation was added and the symptoms observed during the 1st day and the fatality noted to the end of the 2nd or 3rd day and sometimes later. The solution was renewed daily if the substance was liable to change. Death after 48 hours was so exceptional that this period was selected as the time for the fatality calculation. The “approximate fatal concentration” was taken as the geometric mean between the largest concentration that was survived for 48 hours, and the smallest concentration that was fatal in this time for practically all fish.

Since the aquarium toxicity of the different substances spreads so widely (the most toxic in the present series being about 2500 times more potent than the least toxic) it sufficed for the purpose of preliminary exploration to determine the fatal concentration with a rather wide margin, so that the lowest fatal concentration was up to 4 times higher than the highest non-fatal concentration. This wide spread is well beyond the limit of ordinary biologic variation, so that it is not necessary to use many animals. The effects practically always fall smoothly into series with the dosage, even when only one animal is used at each dose level. It is also well beyond the effects of variations of the room temperature of the aquarium water (18–25°C.).

The concentration is expressed as parts per million (p.p.m.), corresponding to milligrams per liter, or 2 mg. per aquarium, or 250 mg. per kg. of fish of the average weight of 8 gm. The p.p.m. concentration is preferred for convenience in calculating the fatal concentration. The difference from the molecular concentration would generally fall within the spacing of the dosage.
The geometric mean is the square root of the product of two numbers. For instance, if the fish survive 25 p.p.m. but die at 100 p.p.m., the geometric mean of survival concentration is \(\sqrt{25 \times 100} = 50\) p.p.m. The relationship between these values is such that the smaller number multiplied by a constant, and the larger number divided by the same constant, equal the geometric mean. This constant or "spread" factor, expressed as \((X - \bar{X})\) indicates the degree of approximation. It may be obtained by dividing the geometric mean into the

**TABLE I**

Correlations with Aquarium Fish Toxicities
(Arranged in descending sequence of toxicities)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Fish toxicity</th>
<th>Data</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone</td>
<td>0.287</td>
<td>1.15</td>
<td>27</td>
</tr>
<tr>
<td>(\rho)-Methylaminophenol (metol, elon)</td>
<td>0.5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2,2,4-Trimethyl ((\rho)-phenylisopropyl)1,2-dihydroquinoline (akanol)</td>
<td>1.8</td>
<td>1.8</td>
<td>4</td>
</tr>
<tr>
<td>(\rho)-Aminophenol</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2-Mercaptopentanethiole (capta)</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hydroquinone monomethyl ether (agerite alba)</td>
<td>2.5</td>
<td>1.67</td>
<td>5</td>
</tr>
<tr>
<td>(\rho)-Phenyl-(\rho)-napthylamine (agerite powder)</td>
<td>4.4</td>
<td>1.36</td>
<td>6</td>
</tr>
<tr>
<td>Aziline hydrochloride</td>
<td>5.5</td>
<td>1.18</td>
<td>3</td>
</tr>
<tr>
<td>(\rho)-Isoproxy diphenylamine (iso)</td>
<td>5.7</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td>(\rho)-Phenylethylamine (agerite powder)</td>
<td>5.74</td>
<td>1.74</td>
<td>6</td>
</tr>
<tr>
<td>Pyrocatechol</td>
<td>14</td>
<td>1.4</td>
<td>3</td>
</tr>
<tr>
<td>Pyrocatechin</td>
<td>15</td>
<td>1.9</td>
<td>5</td>
</tr>
<tr>
<td>(\rho)-Hydroxybenzoglycerine</td>
<td>20</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Phenol</td>
<td>28.9</td>
<td>1.15</td>
<td>8</td>
</tr>
<tr>
<td>(4')-Chloro-3,5-dihydroxy diphenyl sulfone</td>
<td>35</td>
<td>1.15</td>
<td>4</td>
</tr>
<tr>
<td>(4',5)-dimethyl-2-mercaptopentanethiole</td>
<td>56</td>
<td>1.4</td>
<td>3</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>57.4</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td>(p)-Oxydiphenylamine</td>
<td>&gt;40</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2,4-Diaminobenzyl hydrochloride (amidol)</td>
<td>80</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hydroquinone monomethyl ether</td>
<td>200</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>630</td>
<td>1.6</td>
<td>4</td>
</tr>
</tbody>
</table>

\(P\) (probability of accidental correlation) | 0.09 | 0.20 | 0.30 | 0.265 | 0.39
larger number. In the above example, the spread factor is \( 100 \div 50 = 2 \); and the approximate fatal concentration would be expressed as 50 p.p.m. \((X \div 2)\). The relative aquarium toxicities and the inhibitory effects of the substances on various autooxidative reactions were compared according to the rank order method of Friedman (1937), viz.

\[
\chi^2 = \frac{12}{mp(p + 1)} \sum (\text{rank totals})^2 - 3n(p + 1)
\]

Where \( n \) is the number of series being compared (i.e., two in this investigation); \( p \) is the number of items being ranked in each series.

**TABLE II**

**Rank Correlations with Oxidation Potential**

(Arranged in ascending sequence of \( E_a \))

<table>
<thead>
<tr>
<th>Substance</th>
<th>Oxidation potential</th>
<th>Aquarium fish toxicity</th>
<th>Photographic reduction potential</th>
<th>Oil stabilization</th>
<th>Gasoline induction period</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Methylaninophenol (metol, elon)</td>
<td>0.603</td>
<td>2</td>
<td>2.5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.609</td>
<td>8</td>
<td>3.5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.631</td>
<td>1</td>
<td>1.5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>( p )-Aminophenol</td>
<td>0.636</td>
<td>3.5</td>
<td>4.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>( p )-Phenylenediamine</td>
<td>0.710</td>
<td>6</td>
<td>1.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pyrocatechol</td>
<td>0.742</td>
<td>7</td>
<td>2.5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2-Mercaptobenalothiazole</td>
<td>0.785</td>
<td>3.5</td>
<td>4.5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>0.799</td>
<td>13</td>
<td>4.5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>( p )-Hydroxyphenylglycinic</td>
<td>0.833</td>
<td>9</td>
<td>5.5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hydroquinone monomethyl ether</td>
<td>0.848</td>
<td>12</td>
<td>6.5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>1.043</td>
<td>11</td>
<td>5.5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Phenol</td>
<td>1.089</td>
<td>10</td>
<td>6.5</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Aniline hydrochloride</td>
<td>1.135</td>
<td>5</td>
<td>8.5</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

\( P \) (probability of accidental correlation) . . . 0.09 1.53 0.10 0.099

This method1 provides a measure of the probability, \( P \), of obtaining through chance alone an observed degree of agreement between the two (or more) series of rankings. A small value of \( P \) indicates that factors other than chance are probably operating and implies a relationship between the two methods under

1 I am greatly indebted to Dr. G. F. Badger, not only for suggesting this method of analysis, but also for working out the computations and advising on their significance. Dr. José J. Estable assisted in many of the later experiments.
The values of $x^2$ in terms of $P$ are obtainable from the tables of Pearson (1930).

The data for the aquarium toxicity and various autocatalytic reactions are shown in Table I, as are also their rank order and their $P$ (the probability that the rank correlation could occur by chance). Table II gives the correlation of their ranking with the oxidation potential. The rank sequence in these tables shows the general correspondence and the outstanding exceptions.

**Correlations with Oxidation Potential**

Fieser (1930) published determinations of the "critical oxidation potentials" ($E_0$), the potential (in volts) at which the rate of oxidation of the reductant of an oxidation-reduction system becomes so small as to be just detectable. Data on both this $E_0$ and the aquarium goldfish toxicity ($FT$) are now available for thirteen benzene derivatives. In nine, or 70 per cent of these, the two properties differ in rank by $\frac{1}{6}$ or less (2 positions) of the total positions. Three, or 23 per cent differ by more than $\frac{1}{6}$ (4 positions), namely aniline, $E_0 13$, $FT 5$, difference $+ 8$; phloroglucinol, $E_0 8$, $FT 13$, difference $- 5$; pyrogallol, $E_0 2$, $FT 8$, difference $- 6$. Presumably some special factor enters for these substances. With pyrogallol and perhaps also with phloroglucinol, the toxicity would be lowered by oxidation of the aqueous solution in the aquarium. The relatively high toxicity of aniline may be due to a different mechanism of action, such as the formation of methemoglobin. The mercaptan benzothiazole ($E_0 7$, $FT 3\frac{1}{2}$) also introduces other chemical groups which are likely to have different actions.

The probability, $P$, for the entire series is 0.09, which is more than suggestive. If aniline is omitted, $P$ of the remaining twelve substances becomes 0.06, which is quite good.

**Correlation with Photographic Developers**

Lowe (1939) grades the effectiveness of substances commonly used for this purpose by their "reduction potential," defined by the amount of potassium bromide that must be added to the developer to produce a specified decrease in the activity of the chemical in question.

Comparing the photographic reduction potential with the 8 compounds for which both data are available, five (62 per cent) differ by less than $\frac{1}{6}$ of the total positions (1½ places) in rank. Three (38 per cent) differ by more than $\frac{1}{6}$ (2½ places). The $P$ of the entire series is 0.20, which is at least suggestive.

Comparing the photographic reduction potential with the critical oxidation potential of the seven substances for which both data are available, three (43 per cent) differ by less than $\frac{1}{6}$ (1½ places) in rank. Two (29 per cent) differ by more than $\frac{1}{6}$ (2½ places). Excluding these two gives $P$ 0.109. $P$ of the entire series is 0.153, rather better than the correlation with fish toxicity.
Stabilization of Fatty Oils

Rancidity of fats is due to the autooxidation of a small amount of their unsaturated fatty acids. It involves the production of peroxides. There is a considerable "lag" or "induction period," during which the change is very slow; but when once started it proceeds progressively faster, by autocatalytic chain reactions. The long lag of natural fats is due to their content of antioxidant substances, not well identified, but some, at least, related to tocopherol (Olcott et al., 1936). The lag is overcome by the gradual destruction of these antioxidants by peroxidases (Boehm and Williams, 1945). The autooxidation may be very effectively inhibited by the addition of a variety of "stabilizing agents" (Jamieson, p. 26), especially phenolic substances and aromatic amines. The efficiency of these antioxidants can be rated quantitatively by various methods. Half a dozen series of determinations are available totaling nearly thirty substances, with fairly concordant ranking, although different criteria were used. These lists include six of the phenolic substances for which we have aquarium fish toxicity and oxidation potential data.

Correlation of the ranking of the fish toxicity with that of oil stabilization shows that of the six phenols, five (83 per cent) differ in rank by \( \frac{1}{6} \) (1 position) or less of the total positions. None differs by \( \frac{1}{6} \) (2 positions) or more. The \( P \) is 0.20 which is suggestive.

Correlation of the oxidation potential with the oil stabilization rankings shows that five (83 per cent) differ in rank by \( \frac{1}{6} \) (1 position) or less; none by \( \frac{1}{6} \) (2 positions) or more. \( P \) is 0.10 which is more than suggestive.

It appears therefore, that there is correlation between the aquarium fish toxicity and the inhibition of the autocatalytic oxidation of oils; and an even higher correlation between this and the oxidation potential.

Correlation with Inhibition in "Cracki~g" of Gasoline

Lowry et al. (1933) correlated the critical oxidation potential with the effectiveness of a number of agents in breaking the chain reactions of this thermal decomposition process by an accelerated oxidation test, and found a considerable agreement in ranking, with a few notable exceptions which could be explained by the fact that the higher temperature of their test probably produced changes in the inhibitors, especially phloroglucinol and hydroquinone.

Comparing the gasoline induction period and the aquarium fish toxicity of the nine compounds for which both data are available, three (33 per cent) differ by \( \frac{1}{6} \) (1½ positions) or less in rank. Four (44 per cent) differ by \( \frac{1}{6} \) (3 positions) or more. The \( P \) is 0.265, a rather poor correlation. Excluding the three extremes, aniline, hydroquinone, and pyrocatechol, improves \( P \) to 0.112.

The correlation of the gasoline induction period with the oxidation potential is much better. Of the nine substances, seven (78 per cent) differ by less
than \( \frac{1}{4} \) (1\% positions or less; two (22 per cent) differ by \( \frac{3}{2} \) (3 positions) or more. \( P \) is 0.099, which is more than suggestive. Excluding pyrocatechol and hydroquinone reduces \( P \) to 0.078.

**Antioxidants ("Age Resisters") of Rubber Industry**

The manufacture of rubber involves chain reactions at several points. The deterioration of the distinctive physical characteristics of rubber is due to oxidation, probably by the formation of an unstable peroxide from the isoprene units (Fieser and Fieser):

\[
\text{--CH}--\text{C}==\text{CH}--\text{CH}_2 \rightarrow \text{--CH}--\text{C}==\text{CH}--\text{CH}_2--\text{OOH}
\]

This change can be retarded by antioxidant catalysts, such as aromatic amines, phenols, and quinones, added in the ratio of about \( \frac{1}{2} \) to 1 per cent. These stabilizers of rubber are also effective for the stabilization of fats (Lea, p. 173). The B. F. Goodrich Chemical Company, kindly provided nine of these substances for comparison with their aquarium goldfish toxicity. Many were found poorly soluble in water, but fatal concentrations of the majority were obtained by first dissolving them in a little ethyl alcohol, well below its toxic concentration. Three of the substances were not fatal in saturated solution and were not counted, as their concentration was not known, but they were not important for the present purpose, as the successful tests included closely related substances. This left six substances shown in Table I. Four are highly toxic (1.8 to 5.7 p.p.m.): akanol, agerite alba, agerite powder, and iso. The other two are considerably toxic, 35 to >40 p.p.m. It appears that the age-resistant antioxidants generally have a high aquarium toxicity.

Quantitative data on the potency of these substances on age-resisting of rubber are not available in the literature. The review of Jacobs (1933) contains only one of the substances (\( n \)-phenyl-\( \beta \)-naphthylamine; agerite powder; neozone D, M.C.), and this ranks very differently according to conditions. Mr. Kehe of the Goodrich Company kindly gave the personal estimates shown in Table I with the caution that "these ratings are not very accurate and the differences indicated may be smaller or larger depending on a great number of other conditions." These estimates give a very poor correlation with the aquarium fish toxicity: Of the six compounds, three (50 per cent) differ in rank by \( \frac{3}{2} \) (1 position) or less of the total positions. Three (50 per cent) differ by \( \frac{1}{2} \) (2 positions) or more. The \( P \) is 0.39, which is poor.

Accelerators are added to the rubber "mix" to hasten the molecular rearrangement of vulcanization. Various inorganic and organic substances have this effect but they differ greatly in potency. Organic sulfur compounds are especially potent. The Goodrich Company furnished three of the compounds
for the aquarium tests, but one was not sufficiently soluble to be toxic. Of the other two, 2-mercaptobenzothiazole (captax), which contains a benzene ring, is highly toxic (2 p.p.m.); 28 times more toxic than 4,5-dimethyl-2-mercaptobenzothiazole.

**SUMMARY**

Hydroquinone when added to the aquarium water was found to be about a hundred times more toxic than phenol, to goldfish (and to *Daphnia magna*), but is only about twice as toxic when injected into fish or mammals. Tertiary butyl catechol shows a similar high toxicity in the aquarium, while the toxicity of catechol, resorcinol, and pyrogallol approaches more closely that of phenol.

As the substances of high aquarium toxicity are known to inhibit many oxidative and polymerizing autocatalytic "chain reactions," rank correlations were tabulated between the recorded inhibitory potency of various substances in these processes, and their aquarium toxicity for goldfish.

The correlation between aquarium fish toxicity and electric oxidation potential (*P* 0.09) is more than suggestive, and becomes still more so if explainable discrepancies are excluded. Antioxidant fat stabilizers show suggestive correlation with fish toxicity (0.20), and better with electric oxidation potential (0.10). The photographic reduction potential gives suggestive correlation with fish toxicity (0.20) and somewhat better with the oxidation potential (0.15). The gasoline induction period correlation is more than suggestive with the oxidation potential (0.099), but rather poor for fish toxicity (0.265). The rubber anti-aging potency gives only poor correlation (0.39) with fish toxicity. The reasons for these divergencies are not clear; they may perhaps be connected with the solvent properties of the substrate. As an example, Lea (p. 175) cites that 0.01 per cent of maleic acid prevents rancidity of fats, but is rendered ineffective by the presence of water.

Taken by themselves, no one of the *P* values is entirely convincing of the relationships stressed in this paper. However, the consistent finding of relatively small values of *P* lends considerable weight to the hypothesis that these chemicals act in a related manner; and that the chemical activity of a substance may furnish useful suggestions of its biologic potency, perhaps more so than the chemical constitution as such. The aquarium toxicity for goldfish is a convenient means of classifying the biologic potency.

**CONCLUSION**

There is considerable correlation between the aquarium fish toxicity and the antiautocatalytic potency of quinones, phenols, and related substances, in marked contrast to their toxicity on systemic administration.
REFERENCES

Jacobs, F., *Caoutchouc et Guatia-Percha*, 1933, 80, 16548, 16578, 16608.