ENZYMES AND CATALYSTS FOR PURIFICATION OF INDUSTRIAL WASTE WATER

James W. Fulton

Water Resources Research Institute
Oklahoma State University
Stillwater, Oklahoma

June 30, 1966

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ABSTRACT

The purpose of this study is to initiate research in the use of catalyst and enzyme systems for industrial waste water purification. Records have been assembled on the known undesirable waste water impurities. The literature has been searched for enzyme and bacteria systems which are known to decompose toxic materials. The decomposition of phenolic compounds has been emphasized, as an example. Suggestions for further research have been given.

INTRODUCTION

Catalysts and enzymes have been the object of intensive research during the past thirty years. In particular, industrial applications of these reaction rate accelerators have grown markedly since World War II. Today our fundamental knowledge and technical ability have advanced to a point where these catalysts might be applied to the purification of industrial waste water.

Trace quantities of enzymes and catalysts can accelerate reactions to surprising rates. One molecule of catalase, for example, can bring about the decomposition of 5,000,000 molecules of hydrogen peroxide in one minute at 0°C. This is an extreme example, of course, but does give hope that enzymes exist which could be used in industrial waste waters and could be applied in small enough quantities to be biologically harmless and economically feasible.

Furthermore, these enzyme and catalyst systems can be highly specific. This characteristic is useful because only the desired reaction is accelerated and unwanted side reactions are not promoted. The explanation for this behavior is given by Emil Fischer's famous "lock-and-key" hypothesis. That is, an enzyme and its substrate molecule must fit together in somewhat the same manner as a key fits into a lock. Any deviation and the key will not activate the lock. This characteristic would allow the removal of specific, undesirable impurities from industrial waste water without danger to other compounds.

It is proposed, therefore, that instead of using micro-organism to degrade industrial waste, it might be possible to employ the enzymes directly, thus bypassing the stage of growth of bacteria. This would shorten the treatment time and also make possible the removal of specific toxic chemicals which are not now removed.

The first recovery of an enzyme was made in 1833 by Payen and Persoz (48) when they found that an alcohol precipitate of malt extract contained a thermolabile substance which converted starch into

sugar. This substance, which we now call amylase, was named by them diastase. After that time, quite a bit of study was devoted to chemical reactions brought about by living cells. An important step took place in 1897 when Büchner was able to extract enzymes from yeast cells. Until his discovery, the only work with enzymes was done by using living cells such as bacteria and yeast. Büchner showed that his extract, which no longer consisted of living material, brought about the same fermentation as the living yeast cells.

Sumner succeeded in crystallizing the enzyme urease in 1926 and Northrop prepared crystalline pepsin in 1930 (45). Since then well over 100 of the more than 700 known enzymes have been crystallized. Today enzymes available commercially are quite expensive, but their cost might be lowered with improved techniques and greater production (eg., 1964 prices (57): catalase, $0.80/gm; chymotrypsin, $6.25/gm; tyrosinase, $38/gm).

In order to be able to apply enzyme catalysis to the purification of industrial wastes, factors affecting the specificity of the enzyme must be determined. Upon deduction of these facts, one might select a particular enzyme system that would catalyze the decomposition of each impurity. Or, if no suitable enzyme system were available, one might "manufacture" suitable enzymes.

These enzymes could then be used in a "chemical reactor" which takes the industrial effluent stream as its feed stream. The toxic chemical in the effluent stream would be rapidly degraded to a harmless product.

ENZYME ACTION

The behavior and activity of enzymes is not yet understood. The extreme activity, specificity, and sensitivity of these catalysts are well known, but no correlations or general laws have been developed which can be used to explain or predict enzyme activity. However, research in this area is impressive and is growing stronger all of the time. The next decade should produce major breakthroughs in this field of study.

In general, the mechanism of enzyme action involves the formation of a unique and highly specific enzyme-substrate complex. As with all catalysts, this "activated complex" is much more reactive than the original component, and the reaction rate is greatly accelerated. The orientation and juxtaposition of the enzyme with respect to the substrate can account for some of the reaction rate increase. These steric factors alone, however, cannot explain the enormous rates of enzyme-catalyzed reactions.

A number of theories on enzyme activity have been suggested, and these are well presented in the literature. Several of these are reviewed here.

BAYLISS'S THEORY (4): The increased rate of reaction is due to an increase of active mass owing to a physical absorption of the reactants by enzyme.

STRAIN THEORY (17): This theory applies to hydrolytic enzymes. Each substrate molecule combines with the enzyme at two points. The combined groups are considered to be so spaced that the two parts of the substrate molecule are stretched, thereby weakening the bond in the substrate and rendering it more susceptible to hydrolysis.

QUASTEL'S THEORY OF DEHYDROGENASE ACTION (50): Activation is explained in terms of a polarization of the substrate molecule by a local electric field due to charges on the enzyme surface.

THEORIES OF ENZYME HYDROLYSIS: (a) Taylor's theory (64) assumes a polarization of the bond to be hydrolysed. The polarization is due to the combination between enzyme and substrate by valency bonds, setting up an electronic strain which is conveyed along the substrate molecule by induction to the link to be activated. (b) Scott's theory (56) states that the negatively charged carbonyl oxygen combines by hydrogen bonding with an electrophilic group in the enzyme, which is the essential SH group.

This decreases electron density at the carbonyl oxygen and the approach of hydroxyl ions to the carbon is facilitated. (c) Wilson, Bergmann and Nachmansohn's theory (69) proposes that the enzyme combines with the carbon atom, not with the oxygen atom of the carbonyl group. (d) Swain and Brown's theory (62) involves catalysis by means of a simultaneous electrophilic and nucleophilic attack by two groups in the catalyst. (e) Roswell's "dipositive bond" theory (54) depends on the production of similar and not opposite charges at the two ends of the bond to be hydrolysed. The cause of the weakening of the bond is seen in the electrostatic repulsion between these two like charges. Once the charges have been formed the bond should be ruptured heterolytically by mutual repulsion of the two positively charged atoms composing the bond.

CHAIN REACTION THEORY (13): The first step, chain initiation, is the formation of free radicals by homolytic fission (by symmetrical rupture of a bond), leaving one of the electrons of the bond on each of the atoms concerned. The main reaction, chain propagation, then takes place by the alternation of two steps, each consisting of an attack on a molecule by a free radical, producing a new molecule and another free radical.

ELECTRON CONDUCTION THEORY (9): A kinetic theory of enzymatic oxidation-reduction has been developed considering the enzyme particle to catalyze the oxidation-reduction of two different substrates at two different enzymatic sites on the same particle, with conduction of electrons between the two sites through the enzyme particle. Electron conduction across the solid-liquid interfaces at the two sites is assumed to obey the voltage-current laws valid for electrodes in solution.

ENZYMES AND BACTERIA FOR DECOMPOSITION OF HARMFUL COMPOUNDS

A list of the harmful compounds from industrial wastes have been gathered by Klein (32) and have been reproduced for convenience in Appendix A of this report.

A search of the available literature has revealed a few harmful compounds which are known to be decomposed by enzymes or bacteria.

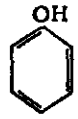
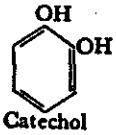
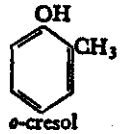
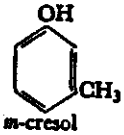
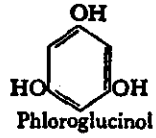
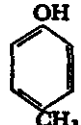
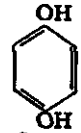
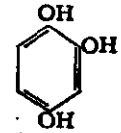
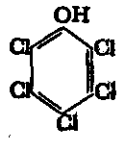
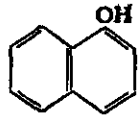
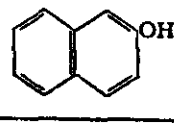
Harmful Compounds		Comments	Reference
1.	Acetaldehyde	Acetobacter sub-oxydans T.P.N. and D.P.N., as co-enzymes	30
2.	Acetic Acid	Oxidation by bacteria	16
3.	Anthracene	Degraded by gram-negative mobile rod. This organism possessed the enzymes to oxidize even salicylic acid and catechol.	53
4.	Benzene	Oxidized by pseudomonas aeruginosa, mycobacterium rhodochrous	10 41
5.	Benzoic Acid	Oxidation by A. Chroococcum and A. Beyerinckii; Rhodopseudomonas palustris; azeobacter	55
6.	Carbohydrate	Oxidation by pseudomonas fluorescens	43
7.	Catechol	Metapyrocatechase; catechol 2, 3-oxygenase	44,66
8.	O-chloro-m-cresol	Bacteria	63
9.	O-cresol	Potato phenol oxidase; Lactoperoxidase	29,47
10.	p-cresol	Potato phenol oxidase; Lactoperoxidase	29,47
11.	Denitrification of nitrate	Corynebacterium nephridii	25

Harmful Compounds		Comments	Reference
12.	2:4-Dichloro and 4-chlro-2-methyl-phenoxy-acetic acid	Flavobacterium and achromobacter	21,60
13.	Heparin	Bacterial Enzymes - flavobacterium heparium	49
14.	Hydrogen sulfide	Oxidation by thiobacteriaceae, thiobacteriaeae, S. natans, achromatium and leucothrix	1,14, 16,65
15.	m-Hydroxy-benzoic acid	Degraded by pseudomonas	68,70
16.	2-Methylnaptha-lein	Oxidation by pseudomonas aeruginosa	52
17.	Napthalene	Soil pseudomonas	20,23, 42,67
18.	Nicotinic acid	Pseudomonas fluorescens	5
19.	Nitrile oxidation	Nitrobacter	39
20.	Oxalic acid	Oxalate oxidase (mosses)	12,16
21.	Phenanthren	Genus pseudomonas	53
22.	Phenol	Phenolase Lactoperoxidase	18,19,22, 24,47, 58,63
23.	Phenylacetic acid	Aspergillus niger, penicillium chryogenum	27,33
24.	Phenylalanine	Vibrio O/1 Lactobacillus cassei	11,15, 33,68
25.	Protocate-chuic acid	Pseudomonas protocatechuic oxygenase	10,51
26.	Salycylic acid	Azeobacter	66,70
27.	Thiocyanate	Thiobacillus, thiocyanoxidans	8,24
28.	Tyrosine	Pseudomonas, psudomonas fluorescens	28,61

ENZYMES FOR DECOMPOSITION OF PHENOLIC COMPOUNDS

Phenols and phenolic compounds are common constituents of many important trade waste, and this section concerns some enzymes which degrade phenolic compounds. This particular series of chemicals was chosen to give a specific example of decomposition possibilities with enzymes.

Typical examples of phenols

Monohydric phenols	*Dihydric phenols*	*Trihydric phenols*
Phenol, C_6H_5OH or 	 Catechol	 Pyrogallol
Cresols, $CH_3C_6H_4OH$ (3 isomers)  *o*-cresol		
 *m*-cresol	 Resorcinol	 Phloroglucinol
 *p*-cresol	 Quinol	 Hydroxyquinol
Xylenols, $(CH_3)_2C_6H_3OH$ (6 isomers)		
Pentachlorophenol 		
α-naphthol (1-hydroxy-naphthalene) 		
β-naphthol (2-hydroxy-naphthalene) 		

Pyrocatechase

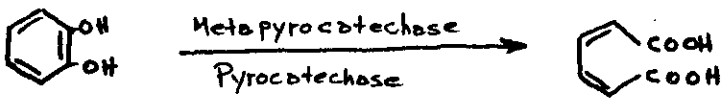
In 1955, Hayaishi and collaborators (26) showed that the cleavage of catechol by the enzyme pyrocatachase involved the utilization of molecular oxygen, not the oxygen of water. Ring openings characteristically consume two atoms of oxygen, and the reaction mechanisms is

Pyrocatechase shows absolute specificity for both catechol and oxygen. Pyrocatechuic acid and other dihydroxybenzoic acids, homogentisic acid, pyrogallol, dopa, trans-5,6-dihydroxycycle-hexadiene, and p-aminocatechol are among the compounds that fail to serve as oxygen acceptors with this emzyme.

The best-studied enzyme is that obtained from a strain of Pseudomonas that can use trytophan as a carbon source. The 100-fold purified enzyme from Pseudomonas adapted to tryptophan appears to be homogeneous. It has a molecular weight of about 80,000. The activity is rapidly reduced to zero as the pH drops below 7, is maximum between pH 7 and pH 10; then drops rapidly at higher pH value. The affinity for catechol is very great.

Metapyrocatechase

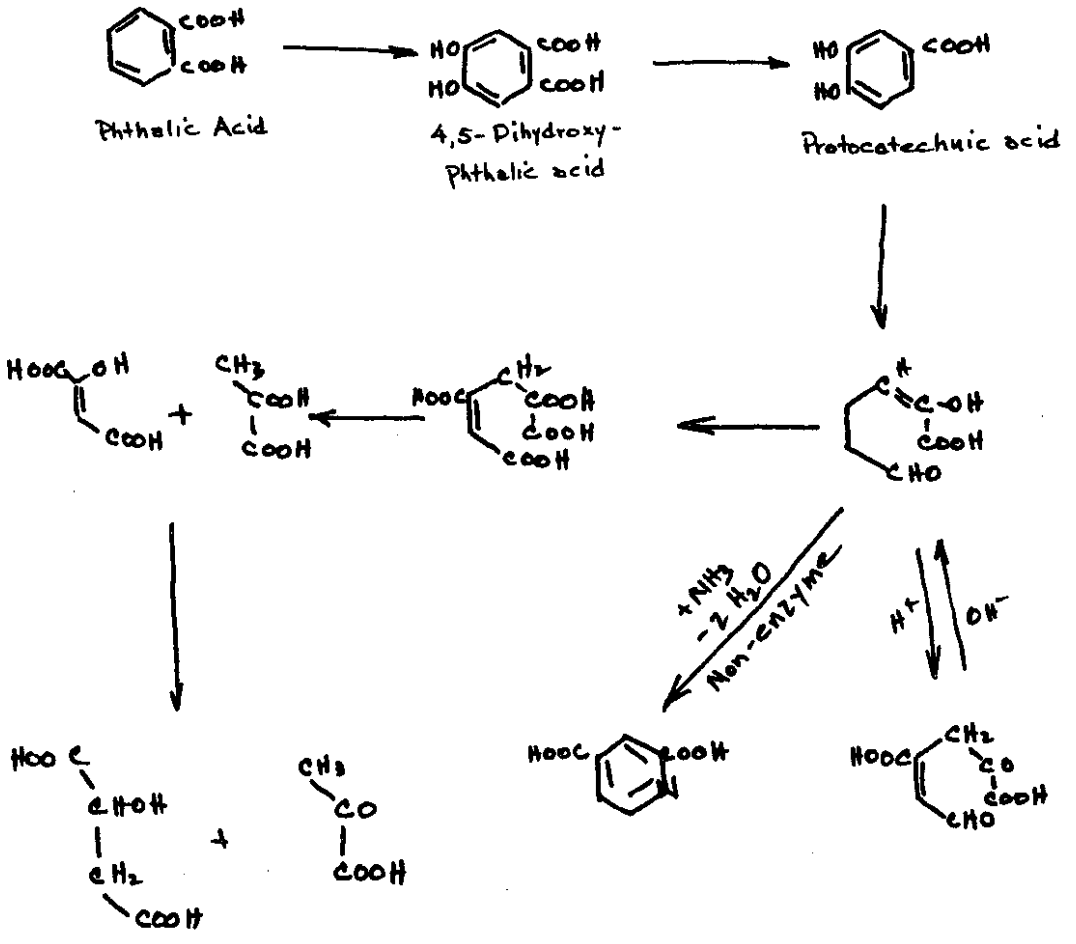
Metapyrocatechase, a catechol-cleaving enzyme, was purified about 17-fold from cell-free extracts of a strain of Pseudomonas (35). This enzyme catalyzed the oxygenation of catechol to 2 - hydroxymuconic semialdehyde. The reaction scheme is as follows:



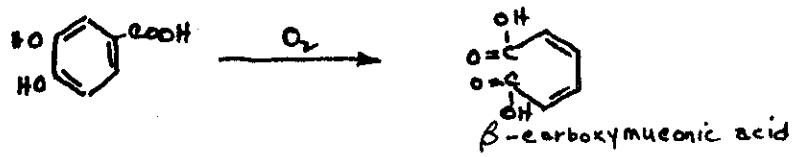
Optical pH of this enzyme is 7.5 and the Michaelis constant for catechol was calculated to be 0.5×10^{-6}M. No inhibition was observed with p-chloromercuribenzuate and metal-binding agents but about 62 and 99% inhibition were observed with 10^{-5}M $Ag^{++}$and Hg^{++} ions, respectively.

Protocatechuic Oxidase

Protocatechuic oxidase is one of the catechol oxygenases. Protocatechuic acid, 3,4-dihydroxybenzoic acid, has been known as a constituent of plants, and was an intermediate in the oxidation of p-hydroxybenxoic acid by another bacterium, Pseudomonas fluorescens. The degradation of phthalic acid proceeds via protocatechuic acid in a pathway involving unusual reaction which was detected by Ribbons and Evans (11). The reaction pathway is shown as follows:



The oxidation of protacatechuic acid by an enzyme from Pseudomonas fluorescens was studied by Stainer and Ingraham (59) as follows:



β-carboxymuconic acid

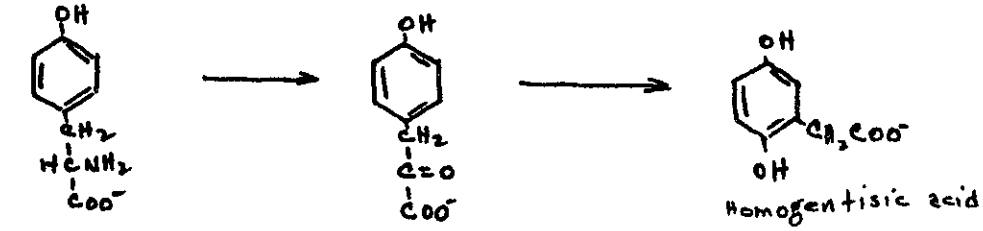
This enzyme converts protocatechuic acid to β-carboxymuconic acid, with the uptake of two atoms of oxygen and was identified by MacDonald, et al. (40).

Protocatechuic oxidase was found to be quite specific; catechol, hydroxyhydroquinome, 2,3-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, o-, m-, and p-hydroxybenzoic acids, and several esters of protocatechuic acid were not oxidized, although catechol and the dihydroxybenzoic acids were found to be competitive inhibitors. Methylene blue failed to replace oxygen in this reaction.

Homogentisic Oxidase

Homogentisic oxidase is an enzyme that catalyzes a step in the principal pathway of tyrosine degradation. The enzyme is found in both microbes and mammals.

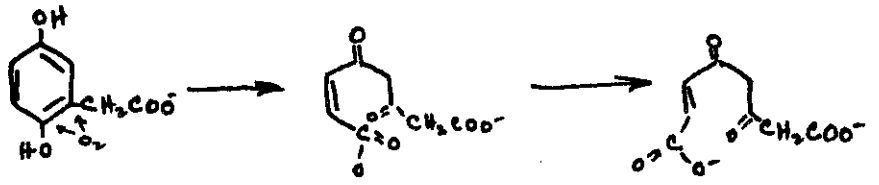
Tyrosine is oxidized only after transamination converts it to p-hydroxyphenylpyruvic acid (34). An oxidative decarboxylation then results in homogentisic acid formation in a single step involving migration of the side chain.



L-Tyrosine

Homogentisic acid

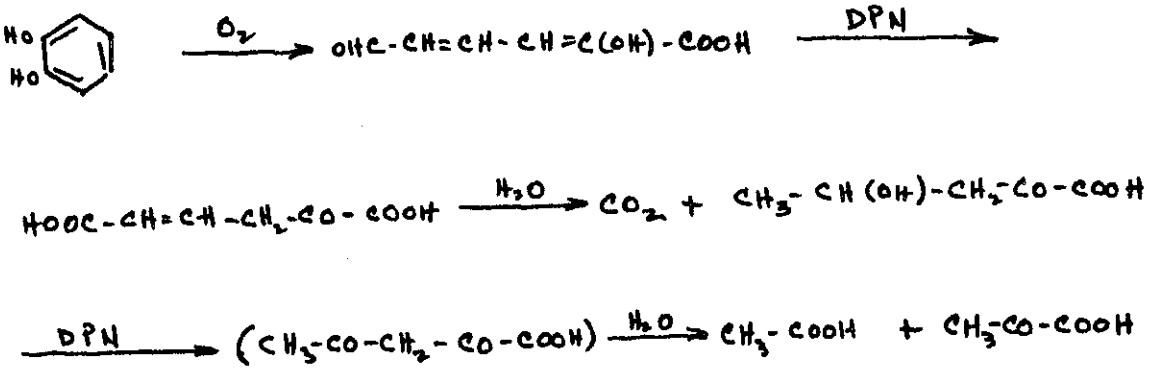
In the study of the nature of the reaction catalyzed by homogentisic oxidase, it was found that the primary product is an isomer, maleylacetoacetic acid, and that is converted enzymatically to the compound of Ravelin and Crandall (34) as follows:



The formation of maleylacetoacetate requires the consumption of two atoms of oxygen, and the two processes occur simultaneously.

New Oxygenase

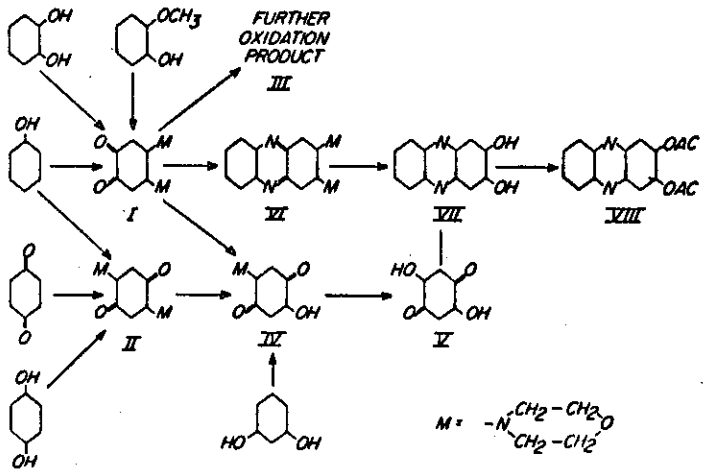
A new metabolic pathway of catachol by a new oxygenase from Pseudomonas was found by Nishizuka and Hayxishi (46). Catechol is enzymatically converted to stiochiometric quantities of pyruvate, acetate, and CO_2, and that d-hydroxymuconic semialdehyde, γ-oxalccrotonate and α-keto-γ-hydroxyvalerate are intermediates by this new oxygenase.



Reaction mixture 2.0 ml.	Catechol 20 µmoles D P N 4 µmoles Phosphate buff 10 µmoles (pH 8.0) Metapyrocatechase 24 mg enzyme protein	incubation 2 hrs. 35°	Pyruvate: 18.2 µmoles Acetate: 19.8 µmoles CO_2: 21.8 µmoles O_2 Consumption = 41.3 µmoles

The Phenolase Model of Brackman and Havinga

The mode of action of the enzyme has been studied extensively in model experiments by Brackman and Havinga (7). The oxidation of mono- and dihydricphenols of the benzene series, using morpholine-capric complex as catalyst is shown:

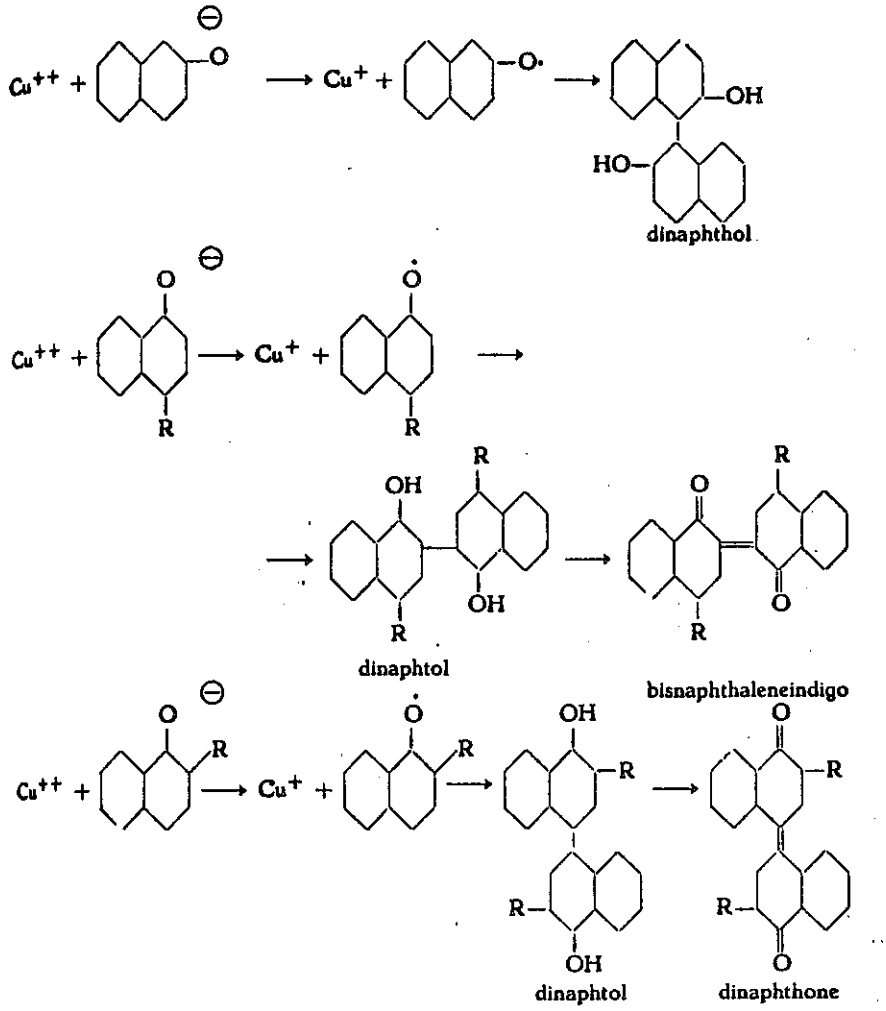


Proof of structure and interrelation of the oxidation products of phenol, catechol, guaiacol, resorcinol and hydroquinone.

The cresols and xylenols are also readily oxidized under the conditions used in the phenol oxidation. The structure of the products formed is subject to further investigation.

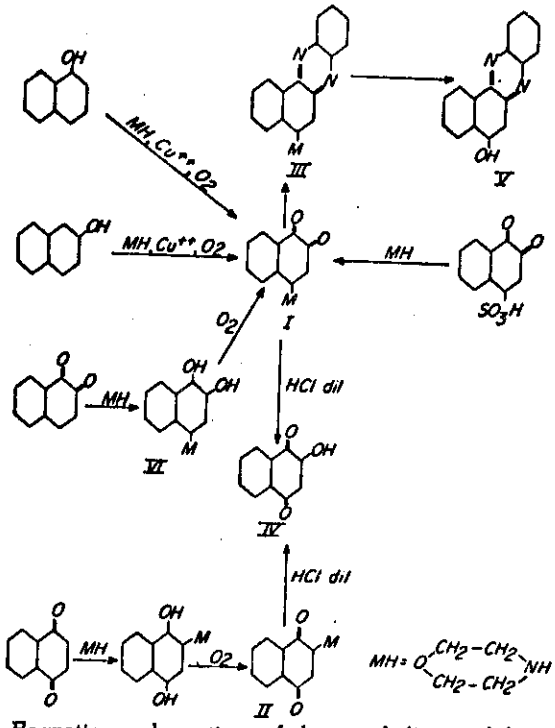
The catalytic oxidation of naphthols is similar to that of the phenols but with one important complication. Because of the relatively low critical oxidation potential of the naphthols as compared with the phenols, a direct oxidation by a cupric complex gives rise to the formation of naphthol free-radicals. These radicals partly dimerize and partly react with other products.

The figure below shows the reaction with naphthol and the influence of substitents on the subsequent reactions of the free radical.



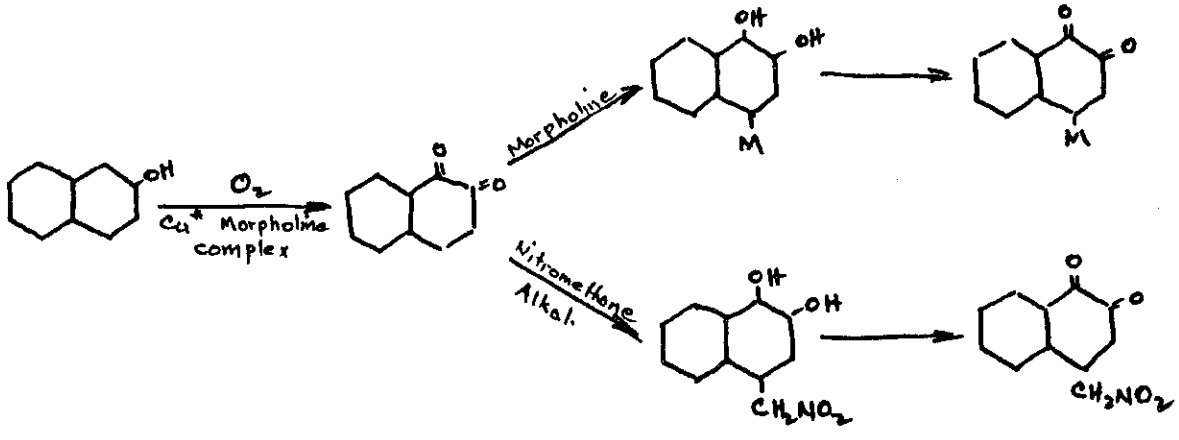
The oxidation of naphthol-2 with oxygen in a methanolic solution of morpholin and copper-acetate proceeds very rapidly at room temperature and the reaction product, 4-morpholino-naphthaquinone-1,2, precipitates in high yields (up to 73%).

The formation and reactions of the morpholino-naphthaquinones are as follows:

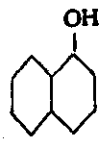
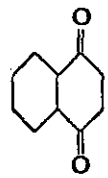
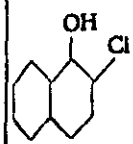
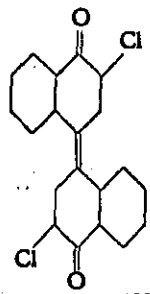
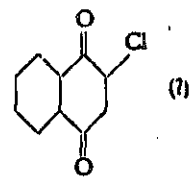
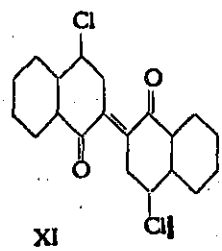
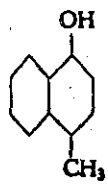
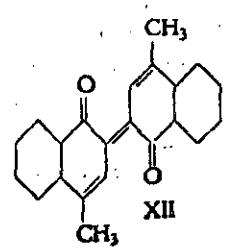


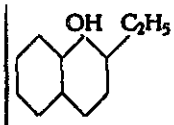
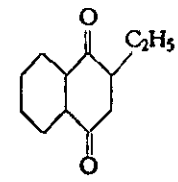
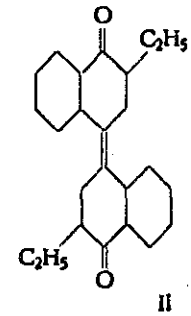
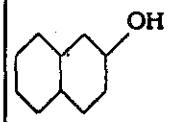
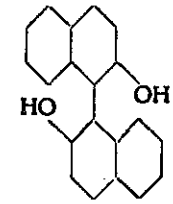
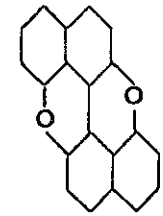
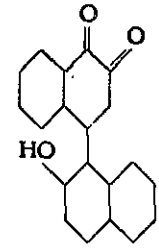
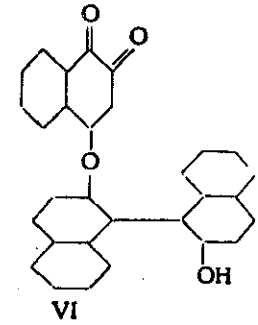
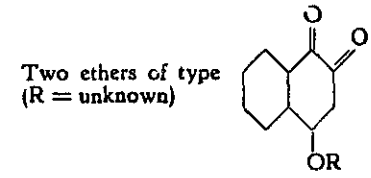
Formation and reactions of the morpholino-naphthaquinones

The competitive formation of 4-morpholino- and 4-nitromethyl-1,2-naphthaquinone from naphthol-2 is:

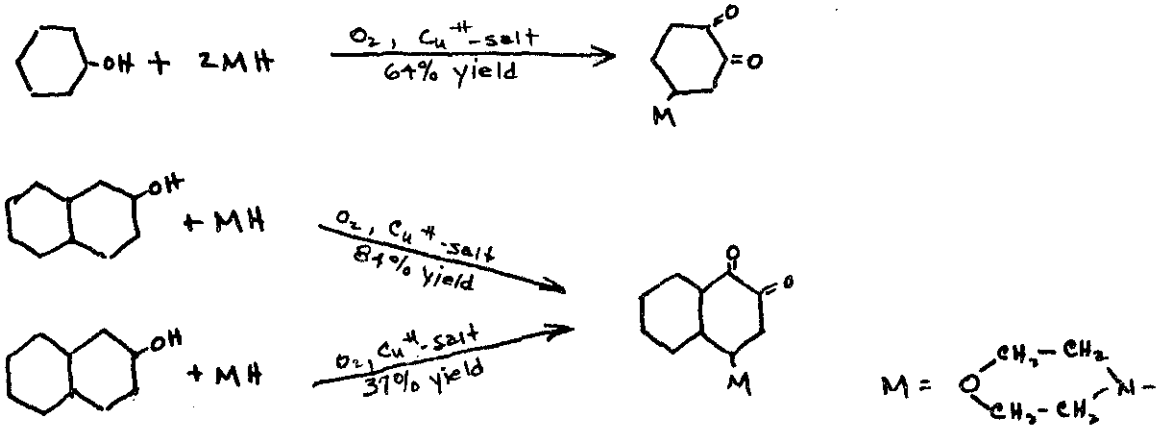


The reaction products obtained from some phenols are shown in the following table:

Naphthol	Catalyst	Products
	Cu-collidine	 Black crystals $(C_{30}H_{18}O_4)_x$
	Cu-pyridine	VII less VII; VIII more VIII
	Cu-collidine	 IX;  (?) X
	Cu-collidine	 XI
	Cu-collidine	Pale yellow products, probably derived from  XII

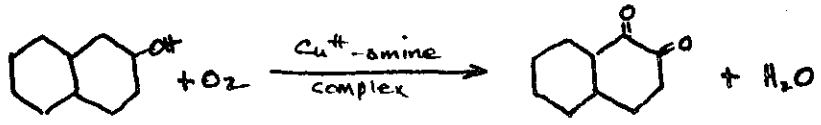
Naphthol	Catalyst	Products
	Cu-collidine	 I  II
	Cu-Collidine	 III  IV  V  VI  Two ethers of type (R = unknown)

They summarized the important reaction as:

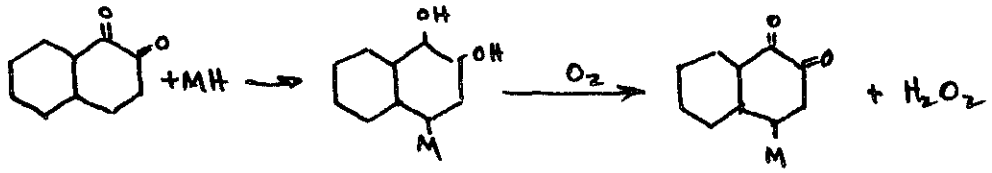


They argued that these reactions consist essentially of two phases:

(1) A conversion of the monohydric phenol into an orthoquinone.



(2) A spontaneous addition of morpholine to quinone, followed by a rapid autoxidation of the catechol derivative formed.



The catalytic oxidation of phenol has two kinetic peculiarities which are of special importance for the deduction of the reaction mechanism; i.e., the induction period and the critical phenol concentration. Moreover, reactions of this type usually display copper and amine-concentration optima.

SUGGESTIONS FOR FURTHER WORK

The next step in this study should be experimental work to determine an enzyme system which might be active in decomposing an industrial waste impurity. Phenol would probably be the best choice for decomposition, because this is a common impurity and some information on its decomposition is available in the literature.

Once the correlations and general quantitative laws are available for a system, the engineering aspects could be studied; i.e., design of a "reactor" for the decomposition reaction.

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APPENDIX A

TOXIC MATERIALS IN INDUSTRIAL WASTES

Taken from Klein (32)

Types of industrial waste waters From M. B. ETTINGER, by courtesy of *Water and Sewage Works*

A. WASTES CHIEFLY MINERAL IN NATURE, OR PARTLY MINERAL AND PARTLY ORGANIC

1. Brine wastes
2. Mineral washing slurries and suspensions (e.g. stone sawing, sand and china clay washing)
3. Mine drainage (pit water from coal mines)
4. Pickle liquor wastes (e.g. iron and copper pickling, galvanizing, etc.)
5. Electro-plating
6. Water softening
7. Cooling water from condensers
8. Boiler blow-off
9. Inorganic chemical manufacturing wastes
10. Battery manufacture
11. Coal-washing
12. Inorganic pigments
13. Photographic wastes

B. WASTES CONTAINING CHIEFLY ORGANIC MATERIALS

I. Hydrocarbon wastes

1. Oil wells
2. Petroleum refining
3. Styrene manufacture
4. Co-polymer rubber plants
5. Butadiene plants
6. Natural rubber processing or reclaiming
7. Petrol stations, garages, engineering works

II. Miscellaneous organic chemical wastes

8. Munition plants (e.g. TNT, Tetryl, ammonium picrate, etc.)
9. Synthetic pharmaceuticals
10. Synthetic or man-made textile fibres (e.g. rayon, nylon, etc.)
11. Organic chemical manufacture
12. Paints and varnishes
13. Oil and grease processing and refining

III. Phenolic wastes

14. Gas plants and by-product coke plants
15. Tar distillation, road oil and creosoting plants
16. Chemical plants
17. Synthetic resin plants
18. Wood distillation
19. Dye manufacturing

IV. Biological wastes

(*a*) *Wastes from processing of biological materials and/or from biological processes*

20. Tanneries and leather trades
21. Pharmaceuticals (antibiotics, biologicals; e.g. penicillin)
22. Alcohol industries (brewing and distilling)
23. Miscellaneous fermentation industries
24. Glue, size and gelatine plants
25. Wool scouring
26. Textile manufacture (natural fibres; e.g. cotton, wool, silk, flax)
27. Floor-cloth manufacture
28. Paper manufacture
29. Laundries

(*b*) *Food processing wastes*

30. Canneries
31. Meat packing, slaughterhouse and related trades
32. Milk and dairy wastes
33. Corn products plants
34. Beet sugar factories
35. Cane sugar factories
36. Fish processing plants
37. Other food processing and dehydration plants

(*c*) *Farm wastes* (e.g. piggeries)

C. RADIOACTIVE WASTES

(e.g. atomic energy plants and experimental stations; hospitals and industries using radioactive isotopes)

Some chemical substances in industrial wastes

Substances	*Present in waste waters from*
Free chlorine	Laundries, paper mills, textile bleaching
Ammonia	Gas and coke manufacture, chemical manufacture
Fluorides	Scrubbing of flue gases, glass etching, atomic energy plants, fertilizer plants, metal refineries, ceramic plants, transistor factories
Cyanides	Gas manufacture, plating, case-hardening, metal cleaning
Sulphides	Sulphide dyeing of textiles, tanneries, gas manufacture, viscose rayon manufacture
Sulphites	Wood pulp processing, viscose film manufacture, bleaching
Mineral acids	Chemical manufacture, mines, iron and copper pickling, D.D.T. manufacture, brewing, textiles, battery manufacture, photoengraving
Acetic acid	Pickle and beetroot manufacture, acetate rayon
Citric acid	Soft drinks and citrous fruits
Organic acids	Distilleries, fermentation plants
Tartaric acid	Dyeing, wine making, leather manufacture, chemical works
Alkalis	Cotton and straw kiering, wool scouring, cotton mercerizing, laundries
Chromium	Plating, aluminium anodizing, chrome-tanning
Lead	Battery manufacture, lead mines, paint manufacture
Nickel	Plating
Cadmium	Plating
Zinc	Galvanizing, zinc-plating, viscose-rayon manufacture, rubber-processing
Copper	Copper-plating, copper pickling, cuprammonium rayon manufacture
Arsenic	Sheep-dipping, fellmongering
Silver	Plating, photography
Hydrogen peroxide	Peroxide bleaching of textiles, rocket motor testing
Sugars	Dairies, breweries, preserve manufacture, glucose and beet sugar factories, chocolate and sweet industries, wood processing
Starch	Food processing, textile industries, wall-paper manufacture
Fats, oils, and grease	Wool scouring, laundries, textile industries, petroleum refineries, engineering works
Phenols	Gas and coke manufacture, synthetic resin manufacture, textile industries, tanneries, tar distilleries, chemical plants, dye manufacture, sheep dipping
Formaldehyde	Synthetic resin manufacture, penicillin manufacture
Mercaptans	Oil refineries, pulp mills
Tannic acid	Tanning, sawmills
Nitro compounds	Explosives factories, chemical works
Hydrocarbons	Petro-chemical and synthetic rubber factories

Some typical alkaline and acid waste waters

Alkaline Wastes

Waste	pH	*Alkali(s) present*
Gas liquor	8–9	Ammonia
Kier liquor (kiering of cotton or straw)	12–14	Caustic soda, sodium carbonate, lime
Cotton mercerizing wastes	12–14	Caustic soda
Chemical manufacturing wastes.	variable	Caustic soda, sodium carbonate, lime, ammonia
Tannery wastes	up to 12	Lime
Wool scouring wastes (untreated)	9–10	Sodium carbonate

Acid Wastes

Waste	pH	*Acid(s) present*
Mine water	2·5–6·5	H_2SO_4
Battery factory wastes	1·0 or less	H_2SO_4
Iron pickle liquor	strongly acid	H_2SO_4, sometimes HCl, rarely H_3PO_4
Copper pickle liquor	strongly acid	H_2SO_4
DDT wastes	very strongly acid	H_2SO_4
Viscose rayon wastes	strongly acid	H_2SO_4
Wood pulp spent sulphite liquor	2–4	Sulphurous acid (H_2SO_3)
Chemical manufacturing wastes.	variable	H_2SO_4, HCl, HNO_3, H_3PO_4
Munition factory wastes	1–3	H_2SO_4, HNO_3
Wool scouring wastes ('cracked' with acid)	<4	H_2SO_4

Concentrations of phenols and other organic compounds present in coal carbonization effluents having adverse effects on taste of fish

Compound	*Fish tested*	*Toxicity to fish (threshold value)* p.p.m.	*Approximate concentration (threshold value) at which fish flesh is tainted* p.p.m.
Phenol	trout, carp	9·5	25
Cresols	trout, carp	10–15	10
Coke oven wastes	freshwater fish	3–5	0·02–0·1
Phenols in polluted river	minnows	0·08	0·02–0·15
1:3:4-xylenol	carp	10	5
1:3:5-xylenol	rudd	18	1
1:2:4-xylenol	rudd	5	1
Pyrocatechol	carp	15	2·5
Resorcinol	carp	35	30
p-toluidine	rudd	50	20
Pyridine	carp, rudd	160–200	5
Quinoline	carp	10	0·5–1
Naphthalene	rudd	—	1
α-naphthol	rudd	2	0·5
β-naphthol	carp, rudd	2	1
α-naphthylamine	rudd	6	3

Some important organic compounds toxic to fish and present in trade wastes discharging to streams

Class	*Typical examples*	*Occurrence*	*Approximate lethal concentration to fish* p.p.m.
Phenols	Phenol, C_6H_5OH Cresols, *o*-, *m*-, and *p*-$C_6H_4(CH_3)OH$	gas works effluents; coal-tar; chemical wastes; synthetic resin wastes	1–20
Tar bases	Pyridine, C_5H_5N	gas works effluents	1,000
	Acridine,	coal-tar	0·7–1·0
Hydrocarbons	Naphthalene, $C_{10}H_8$	gas works effluents; coal-tar	10–20
Aldehydes	Formaldehyde, H·CHO	synthetic resin wastes; penicillin wastes textile wastes	50 (kills trout in 1–3 days)
Cyanogen compounds	Cyanides, e.g. KCN, NH_4CN	plating wastes; gas liquor	0·04–0·1* (as CN)
	Cyanogen chloride CNCl	chlorinated gas liquor	0·08
Chlorinated hydrocarbons	DDT,  (dichloro–diphenyl–trichloroethane)	insecticidal sprays; wastes from manufacture of insecticides	>0·1 (toxic to goldfish)
	Gammexane, γ–$C_6H_6Cl_6$ (γ–hexachloro*cyclo*hexane)		0·035
Mercaptans	Methyl mercaptan, CH_3SH	wood pulp wastes (sulphate process); oil refinery wastes	1 (kills game fish in 2–6 h)

Lethal limits to fish for Synthetic Detergents and Soaps

This Table is based upon the data in HENDERSON, PICKERING and COHEN and *Water Pollution Research, 1955*.

Substance	*Fish tested*	*Lethal concentration* p.p.m.	*Water type*	*Exposure time* hours
Entire packaged detergents	fathead minnow	41–85	soft	96
" " "	" "	15–87	hard	96
Surface active agents				
alkyl benzene sulphonates	" "	4·5–23	soft	96
" " "	" "	3·5–12	hard	96
polyoxyethylene ester	fathead minnow	37	soft	96
" "	" "	38	hard	96
sodium lauryl sulphate	" "	5·1	soft	96
" " "	" "	5·9	hard	96
sodium tetrapropylene benzene sulphonate	rainbow trout	12	?	6
Builders				
sodium perborate	" "	920	?	24
sodium pyrophosphate	" "	1120 PO_4	?	24
sodium silicate	" "	> 256	?	24
sodium sulphate	" "	> 704	?	24
" "	fathead minnow	9000	soft	96
" "	" "	13,500	hard	96
sodium tripolyphosphate	rainbow trout	1120 PO_4	?	24
" "	fathead minnow	140	soft	96
" "	" "	1300	hard	96
Soaps				
household soaps	" "	29–42	soft	96
" "	" "	920–1800	hard	96
pure sodium stearate	" "	100	soft	96
" " "	" "	> 1800	hard	96

Lethal limits to fish for some important polluting substances

In this table the concentration values are the lowest at which definite toxic action is indicated by the data in the reference cited. Wherever possible the exposure time is given. It must not be assumed that lower concentrations are harmless and for further information the works cited should be consulted as many include survival curves or tables. Where no reference is given the figures are based on unpublished work by the writer. Most of the data is for temperatures between 15° and 23° C. Concentrations are parts per million unless otherwise stated. Exposure times have been approximated in some cases.

Substance	*Fish tested*	*Lethal concentration*	*Exposure time* hours	*Ref.*
Acetic acid	goldfish	423	20	1
Aluminium potassium sulphate (alum)	„	100	12–96	1
Aluminium nitrate	stickleback	0·1	144	15
Ammonia	goldfish	2–2·5 NH_3	24–96	1
„	perch	3 N	20	55
„	roach	3 N	5	55
„	rainbow trout	3 N	2	55
Ammonium chloride	„ „	500	17	79
Ammonium sulphate	„ „	1000	14	79
Amyl alcohol	goldfish	1	161	1
Aniline	minnow	200	48	
„	brown trout	100	48	
Arsenic compounds				
Sodium arsenite	minnow	17·8 As	36	79
Sodium arsenate	„	234 As	15	79
Barium chloride	goldfish	5000	12–17	1
„ „	salmon	158	?	80
Barium nitrate	stickleback	500 Ba	180	15
Bromine	goldfish	20	15–96	1
Butyl alcohol	„	250	7–20	4
Cadmium chloride	„	0·017	9–18	4
Cadmium nitrate	stickleback	0·3 Cd	190	15
Calcium hydroxide	goldfish	100 (pH 11·1)	?	81
Calcium nitrate	„	6061	43–48	4
„ „	stickleback	1000 Ca	192	15
Carbon dioxide	various spp.	100–200	?	2
Carbon monoxide	„ „	1·5	1–10	2
Chloramine	brown trout fry	0·06	?	82
Chlorine	rainbow trout	0·03	?	83
„	„ „	0·08	?	46
„	brook trout	0·5–1·5	?	84
„	goldfish	1	96	1
Chromic acid	„	200	60–84	1
Citric acid	„	894	4–28	1
Cobalt chloride	„	10	168	1
Cobalt nitrate	stickleback	15 Co	160	15
Copper nitrate	„	0·02 Cu	192	14
„ „	rainbow trout	0·08 Cu	20	72
„ „	salmon	0·18	?	80
Copper sulphate	stickleback	0·03 Cu	160	
Cupric chloride	goldfish	0·019	3–7	4

Substance	*Fish tested*	*Lethal concentration*	*Exposure time* hours	*Ref.*
Cresylic acid	goldfish	1	6–48	1
Cyanogen chloride	rainbow trout	0·1	?	85
Ethyl alcohol	goldfish	0·25 c.c./l.	6–11	4
Ferric chloride	stickleback	pH 4·8	144	15
*Herbicides**				
Aminotriazole	coho salmon	325	48	86
„	bluegill	10,000	48	86
Baron	chinook salmon	2·3	48	86
„	largemouth bass	4·6	24	86
Dowpon	coho salmon	340	48	86
Diquat	chinook salmon	28·5	48	86
„	rainbow trout	60	96	86
Diuron	coho salmon	16	48	86
Endothal	chinook salmon	136	48	86
F.98 (Acrolein)	„ „	0·08	24	86
„	rainbow trout	0·065	24	86
Hyamine 1622	coho salmon	53	48	86
Kuron	chinook salmon	1·23	48	86
„	largemouth bass	3·5	24	86
Monuron	coho salmon	110	48	86
„	various spp.	40	240	86
Omazene	chinook salmon	0·83	48	86
Phygon XL	largemouth bass	0·07	48	86
Simazine	chinook salmon	6·6	48	86
Sodium TCA	„ „	>870	48	86
Hydrochloric acid	stickleback	pH 4·8	240	15
„ „	goldfish	pH 4·0	4–6	1
(See also *Figure 30*)				
Hydrogen sulphide	„	10	96	1
*Insecticides**				
Aldrin	„	0·028	96	87
„	rainbow trout	0·05	24	60
BHC	goldfish	2·3	96	87
BHC (6·5 per cent gamma isomer)	rainbow trout	3	96	88
Chlordane	goldfish	0·082	96	87
„	rainbow trout	0·5	24	60
Chlorothion	fathead minnow	3·2	96	87
Co-ral	bluegill	0·18	96	87
DDT	goldfish	0·027	96	87
„	rainbow trout	0·5	24	60
„	„ „	0·32	36	63
„	salmon	0·08	36	63
„	„	0·072	?	89
„	brook trout	0·032	36	63
Dieldrin	goldfish	0·037	96	87
„	bluegill	0·008	96	87
„	rainbow trout	0·05	24	60
Dipterex	fathead minnow	180	96	87
Di-syston	bluegill	0·064	96	87
Endrin	goldfish	0·0019	96	87
„	carp	0·14	48	65

* The references cited should be consulted for the chemical names of these compounds, the degree of purity of the preparations used for the tolerance tests, and the methods employed for making the test solutions.

Substance	*Fish tested*	*Lethal concentration*	*Exposure time* hours	*Ref.*
Insecticides (cont.)				
Endrin	fathead minnow	0·001	96	87
EPN	" "	0·2	96	87
Guthion	bluegill	0·005	96	87
"	fathead minnow	0·093	96	87
Heptachlor	rainbow trout	0·25	24	60
"	goldfish	0·23	96	87
"	bluegill	0·019	96	87
Malathion	fathead minnow	12·5	96	87
Methoxychlor	rainbow trout	0·05	24	60
"	goldfish	0·056	96	87
OMPA	fathead minnow	121	96	87
Parathion	" "	1·4–2·7	96	87
Para-oxon	" "	0·33	96	87
Sevin	" "	13	96	87
"	bluegill	5·6	96	87
Systox	fathead minnow	3·6	96	87
Toxaphene	rainbow trout	0·05	24	60
"	goldfish	0·0056	96	87
TEPP	carp	0·1	?	90
"	fathead minnow	1·7	96	87
Lactic acid	goldfish	654	6–43	1
Lead nitrate	minnow	0·33 Pb	?	7
" "	stickleback	0·33 Pb	?	7
" "	brown trout	0·33 Pb	?	7
" "	stickleback	0·1 Pb	336	14
" "	goldfish	10	1–2	14
" "	rainbow trout	1 Pb	100	72
Lead sulphate	goldfish	25 Pb	96	6
Magnesium nitrate	stickleback	400 Mg	120	15
Mercuric chloride	"	0·01 Hg	204	15
Methyl alcohol	goldfish	0·25 c.c./l.	11–15	4
Naphthalene	salmon	3·2	?	80
"	perch	20	1	51
Nickel chloride	goldfish	10	200	1
Nickel nitrate	stickleback	1 Ni	156	15
Nitric acid	minnow	pH 5·0	?	7
Oxalic acid	goldfish	1000	1	1
Oxygen	rainbow trout	3 c.c./l.*		45
"	eel	1 c.c./l.*		45
"	coho salmon	2	720	41
Nascent oxygen	various species	0·033	?	91
Ozone	" "	0·01	?	91
Phenolic substances				
ortho-cresol	minnow	60	2	92
para-cresol	"	50	2	92
" "	rainbow trout	5	2	21
phenol	minnow	20	4	92
"	rainbow trout	6	3	21
"	perch	9	1	51
"	goldfish	10	72	1
Potassium chromate	rainbow trout	75	60	79

* These are said to be the minimum tensions at which the animal is able to extract its *normal* requirement of oxygen from the water.

Substance	*Fish tested*	*Lethal concentration*	*Exposure time* hours	*Ref.*
Potassium chromate	largemouth bass	195 Cr	68	93
Potassium dichromate	rainbow trout	57	72	79
„ „	goldfish	500	72	1
Potassium cyanide	„	0·4 CN	118	4
„ „	„	0·1–0·3	96	1
„ „	rainbow trout	0·13 CN	2	21
„ „	„ „	0·07 CN	70	18
Potassium nitrate	stickleback	70 K	154	15
Potassium permanganate	goldfish	10	12–18	1
„ „	various species	3–5	?	68
Pyridine	perch	1000	1	51
„	goldfish	1·87 c.c./l.	10–30	4
Quinoline	perch	30	1	51
Silver nitrate	stickleback	0·004 Ag	180	15
Sodium chlorate	goldfish	> 1000	120	1
Sodium chloride	„	10,000	240	1
Sodium cyanide	stickleback	1·04 CN	2	11
„ „	various species	1	?	20
Sodium fluoride	goldfish	1000	60–102	1
Sodium hydroxide (See also *Figure 30*)	„	pH 10·6	168	2
Sodium nitrate	stickleback	6000 Na	180	15
Sodium sulphide	„	4·5 S	2	11
„ „	brown trout	1 S	?	29
Sodium sulphite	goldfish	100	96	1
Strontium chloride	„	10,400 Sr	17–31	4
Strontium nitrate	stickleback	1500 Sr	164	15
Sulphuric acid	goldfish	pH 3·9	5–6	1
Tannic acid	„	100	180	1
„ „	salmon	4·8	?	80
Tartaric acid	goldfish	100	200	1
Zinc sulphate	stickleback	0·3 Zn	204	14
„ „	goldfish	100	120	1
„ „	rainbow trout	0·5	64	10

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