Toxicities of "Excessively" Chlorinated Organic Compounds

by BERNICE M. KATZ and GLENN M. COHEN
Department of Biological Sciences
Florida Institute of Technology
Melbourne, Fla. 32901

It is now recognized that chlorine can combine with a large variety of organics present in waste waters to form stable organochlorinated compounds (JOLLEY 1973). Moreover, the possible entry of these stable organochlorines into supplies of drinking water has prompted the monitoring of these compounds in many localities (EPA 1974, HARRIS 1974). JOLLEY (1973) identified seventeen stable organochlorine compounds in chlorine-treated secondary effluents. GEHRS et al. (1974) demonstrated that two of these compounds, 5-chloruracil and 4-chlororesorcinol, significantly decreased the hatchability of non-water hardened carp eggs in concentrations as low as 0.001 mg/1.

At present the chlorinated pesticides provide the most useful guide to the formation of toxic organochlorine compounds. Reviewing the literature, we found that toxicity is generally related to the number of chlorines added to the parent compound, and moreover, some of these reactions that form the most toxic polychlorinated compounds can occur spontaneously at ambient temperatures and without catalysts (BROOKS 1974). For example, in the presence of "excess" chlorine, terpene forms toxaphene, benzene forms endrin under UV (sunlight), and phenol forms pentachlorophenol. These spontaneous reactions are of particular concern because conditions for excessive chlorination occur more commonly than is presently recognized, especially at the outfall from cooling towers and sewage treatment plants (PARKER 1975).

For the foregoing reasons, we began the present investigation by using high concentrations of chlorine rather than low. We tested several classes of common compounds at ambient temperatures before and after excessive chlorination to determine whether such treatment could transform relatively innocuous substances into toxic ones or potentiate pre-existing toxicities.

MATERIALS AND METHODS

Mosquito fish (Gambusia affinis) served as the test fish and were obtained from the first stabilization pond of The West Melbourne Sewage Treatment Plant, West Melbourne, Florida. Fish measuring 2.5 to 4.5 cm were used and experimental groups consisted of a mixture of males, pregnant and non-pregnant females in random proportions because these provided a more representative index of toxicity. Prior to experimental use, tap water (pH 7) was

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dechlorinated by exposure to sunlight. Compounds to be tested were added in one part per million (ppm) concentrations and allowed to react with an excess of chlorine (5-10 ppm). The chlorine concentration was adjusted within this range of 5-10 ppm to satisfy the chlorine demand of the compound and to ensure that a residual chlorine remained. This solution was then aerated at least overnight at room temperature ($20\pm2^{o}C$) to remove the residual chlorine and thereby leave only the chlorinated compound. When no further residual chlorine could be detected by the orthotolidine method as outlined by the AMERICAN PUBLICH HEALTH ASSOCIATION (1971), the toxicity of the remaining chlorinated compound(s) was tested on the mosquito fish to determine whether a new compound of greater toxicity than the original had formed.

The static bioassay was used for the measure of acute toxicity and consisted of one gallon bottles, each containing twenty fish and continuously aerated. Because of seasonal variability among test groups and in the chlorine demand of the tap water, two controls accompanied the testing of all compounds: one in tap water and the other in the compound itself prior to chlorination. Relative toxicities were tabulated by plotting cumulative deaths during a 6 day period. For this preliminary screening, the concentrations of the toxicants were not varied and, accordingly, LC50s were not computed. These tests were conducted from June, 1974 to June, 1975.

RESULTS

As summarized in Table I, excessive chlorination potentiated, reduced, or did not affect acute toxicity of the compounds tested. Those compounds that remained innocuous after excessive chlorination are illustrated in Fig. 1. Because these compounds displayed mortalities almost identical to controls, they were grouped together as the "Unaffected" in Table I. On the other hand, phenol and tryptophan, neither of which is toxic ordinarily at 1 ppm, became exceedingly toxic when excessively chlorinated (Figs. 2 and 3). When tryptophan and phenol were chlorinated at lower ratios, their toxicities were not as high (Figs. 2 and 3). Except for tryptophan, the aromatic amino acids tested herein (See Table I) remained innocuous after chlorination.

TABLE I

EFFECTS OF EXCESSIVE CHLORINATION OF PARENT COMPOUNDS

Increased Toxicity	Unaffected		Decreased Toxicity
phenol	urea	egg albumin	tannic acid
tryptophan	ornithine	starch	
intact fish	histidine	cholesterol	
	phenylalanine	RNA (yeast)	
	tyrosine	uracil	
	leucine	Alconox(R) (detergent)	
	proline	linear alkene sulfonate	

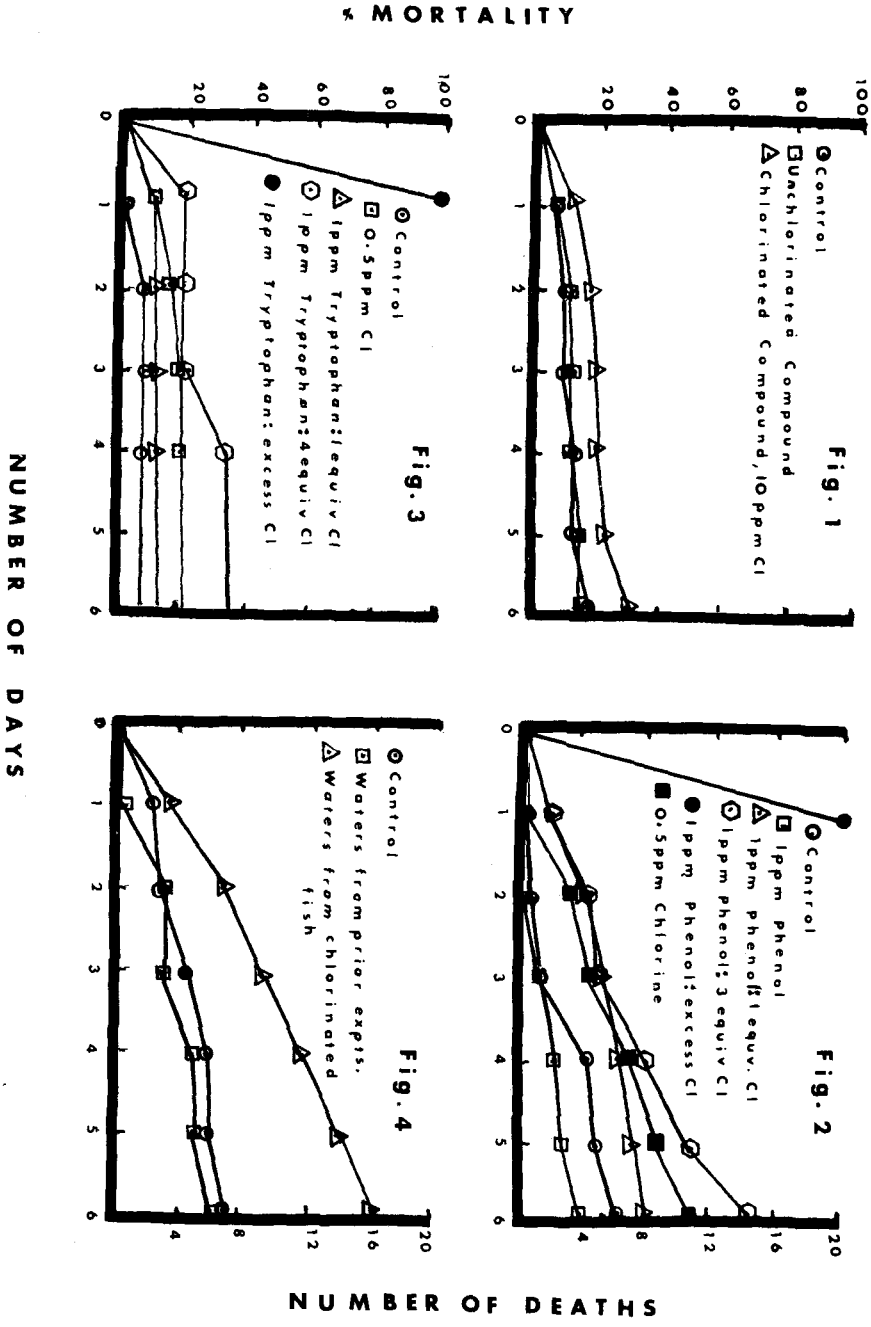
% MORTALITY
Control
Unchlorinated Compound
Chlorinated Compound, 10 ppm Cl
Fig. 1
Control
0.5ppm Cl
1ppm Tryptophan: 1equiv Cl
1ppm Tryptophan: 4equiv Cl
1ppm Tryptophan: excess Cl
Fig. 3
Control
1ppm Phenol
1ppm Phenol: 1 equiv. Cl
1ppm Phenol: 3 equiv Cl
1ppm Phenol: excess Cl
0.5ppm Chlorine
Fig. 2
Control
Waters from prior expts.
Waters from chlorinated fish
Fig. 4
NUMBER OF DAYS
NUMBER OF DEATHS

Fig. 1. "Unaffected" toxicities of compounds after excessive chlorination. These compounds remain innocuous after excessive chlorination at 10 ppm.

Fig. 2. Potentiated toxicity of phenol after excessive chlorination. Low ratios of chlorination are not as effective as high ratios for increasing acute toxicity.

Fig. 3. Potentiated toxicity of tryptophan after excessive chlorination. Low ratios of chlorination are relatively ineffective for increasing acute toxicity.

Fig. 4. Formation of toxic compound from body of fish after excessive chlorination of 10 ppm. The compound does not form in detectable quantities at low chlorination levels of 1 ppm. Chlorination of excrement does not form this compound.

Fish were placed in excessively chlorinated tap (10 ppm) and quickly died. The excessive chlorine apparently reacted with the fish bodies to form one or more stable and highly toxic organochlorine compounds as evidenced by the continued toxicity of the test water after the elimination of the residual chlorine (Fig. 4). In contrast, the re-use of water in which fish had died after exposure to a lower level of chlorine (1 ppm) and from which the residual chlorine had been eliminated, did not produce a comparable increase in toxicity (Fig. 4). In addition, waters in which fish had died from natural causes were also tested for the possible presence of toxic compounds. In all cases mortalities corresponded to the control (Fig. 4).

In contrast to tryptophan and phenol (Figs. 2 and 3), we found that excessive chlorination of tannic acid reduced toxicity (Fig. 5). However, tannic acid retained its ordinarily high toxicity after exposure to the lower chlorine level of 1 ppm.

Preliminary stability studies on test waters containing either phenol or tryptophan that had been excessively chlorinated the previous week indicated that the former retains its toxicity but the latter does not. Thus the observations that test waters containing excessively chlorinated phenol lost their potencies as successive batches of fish were placed in them indicated indirectly that the fish absorbed this compound rather than that it decomposed.

Gas chromatographic identification of chlorinated compounds that formed or the specific bonding sites were not determined for this preliminary report. The detection of a toxic substance was based on mortalities.

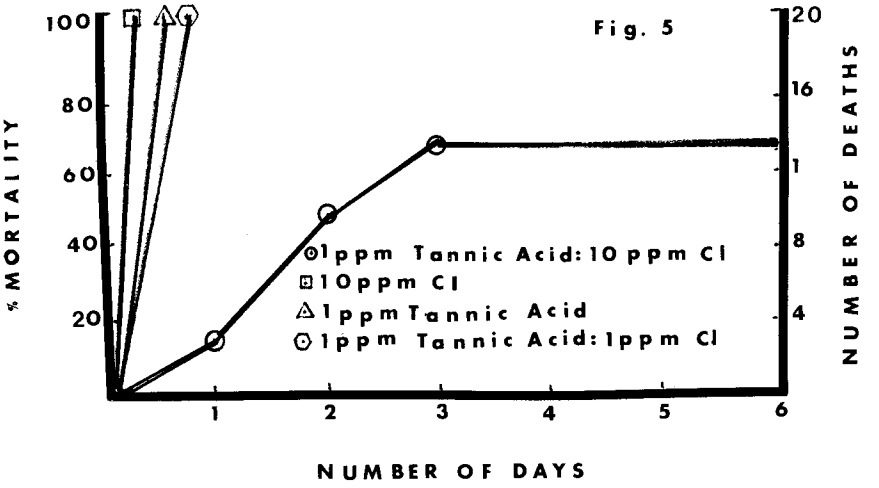

Fig. 5. Reduced toxicity of tannic acid after excessive chlorination. Low ratios of chlorination were almost completely ineffective in reducing toxicity.

DISCUSSION

Chlorine is a highly reactive oxidizing agent that possesses the potential for combining with a wide variety of organic compounds. This reactivity, long exploited commercially to synthesize several exceedingly toxic organochlorines which are themselves environmental problems because of their seepage into waterways (JONES 1964), poses the additional environmental hazard of forming the same toxic compounds under natural conditions in the presence of the appropriate pollutants. This spontaneous formation of toxicants may account in part for their presence in unexpectedly high concentrations in receiving waters of certain areas. For example, excessive chlorine reacts with benzene, an industrial waste to form endrin; with terpene, a natural pine oil product, to form toxophene; and with phenol, also an industrial waste, to form pentachlorophenol (BROOKS 1974).

In the present report we chlorinated at room temperature ($20\pm2^{o}C$) representative organic compounds likely to be present in agricultural, industrial or sewage wastes to determine whether such treatment could transform relatively innocuous substances into toxic ones or potentiate pre-existing toxicities. The tests were restricted to the demonstration of acute toxicity. We found that most of the compounds tested remained innocuous to the mosquito fish after exposure to chlorine (Table I and Fig. 1). The unaffected compounds constituted a diverse group, with no apparent chemical similarities to suggest a common mechanism for the lack to toxicity. On the other hand, several compounds became

extremely toxic after being excessively chlorinated. For example, we found that the amino acid tryptophan, ordinarily innocuous, forms a highly toxic compound after excessive chlorination (Fig. 3). Tryptophan has an aromatic group with a cyclic nitrogen in its structure that is thought to permit the addition of chlorine. Likewise, we duplicated the results from earlier reports (BROOKS 1974) that phenol becomes extremely toxic to fish upon excessive chlorination (Fig. 2). Though phenol is acutely toxic at the high levels of 9-75 ppm (JONES 1964, pg. 143), we deliberately chose the low and relatively non-toxic concentration of 1 ppm phenol in order to detect an acutely lethal transformation after excessive chlorination (Fig. 2). Lesser ratios (5:1 or <) of chlorine to phenol produced chlorinated phenols of intermediate toxicities (Fig. 2). Preliminary evidence indicates that pentachlorophenol but not chlorinated tryptophan is stable and retains its potency (See RESULTS); the stability of the commercial herbicide pentachlorophenol is well-known (BROOKS 1974). In the present investigation the demonstration that some aromatics such as tryptophan and phenol are transformed into highly toxic compounds underscores the significance of the findings of CARLSON et al. (1975) that chlorine is readily incorporated into aromatic systems under ambient conditions. However, they did not determine toxicities. On the other hand, since excessive chlorination does not render histidine, phenylalanine or tyrosine toxic, it is not presently known whether this toxic transformation is limited to only a few compounds or extends to many other chlorinated aromatics. The fact that chemical structure cannot be linked to potential toxicity means that the effects of chlorination will have to be determined empirically for each compound until a consistent pattern emerges.

Mosquito fish were added to excessively chlorinated tap water as a possible test for chemical groups most readily available for reaction. The fish died immediately. The chlorine reacted with the body of the fish to form one or more stable and highly toxic compounds that remained in the test waters. When another group of fish was placed in the same test water after the elimination of the residual chlorine from the previous experiment, the test water was highly toxic to the second group of fish as evidenced by subsequent mortalities (Fig. 4). We have not as yet identified the toxic substance(s) that formed, though it was unlikely to have been excretory material because excessive chlorination of test waters containing high levels of their excretory material but from which the fish had been removed did not produce this toxic compound. In addition, exposure of fish to 1 ppm chlorine did not produce this substance (Fig. 4).

Finally, we demonstrated that excessive chlorination can reduce the toxicity of some compounds. For example, tannic acid, one of the natural products from woody plants that enter into many receiving waters and also reaches high concentrations in effluents from paper mills (BETTS and WILSON 1966), is ordinarily quite toxic (Fig. 5). We found that after excessive chlorination the toxicity of tannic acid is approximately halved (Fig. 5) but is unaffected at the lower chlorine ratios.

The present study indicates that conditions for excessive chlorination are conducive and often available for the facile formation of acutely toxic compounds at ambient temperatures. Moreover, the foregoing emphasizes the importance of establishing an inventory of those compounds requiring only chlorination for this toxic transformation. Thereafter methods might be developed to eliminate these compounds from the various effluents or to modify current practices of excessive chlorination.

Erratum: The corrected means of cumulative toxicities in graphs ◘ and ◘ of Figure 1 are listed outside the parentheses and the incorrect values are within the parentheses.

	1	2	3	4	5	6
◘	0.233(0.255)	0.342(0.427)	0.692(0.986)	1.23(1.36)	1.92(3.7)	2.99(3.47)
◘	0.12(0.16)	0.142(0.266)	0.142(0.266)	1.85(4.02)	2.14(3.48)	2.56(4.81)

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