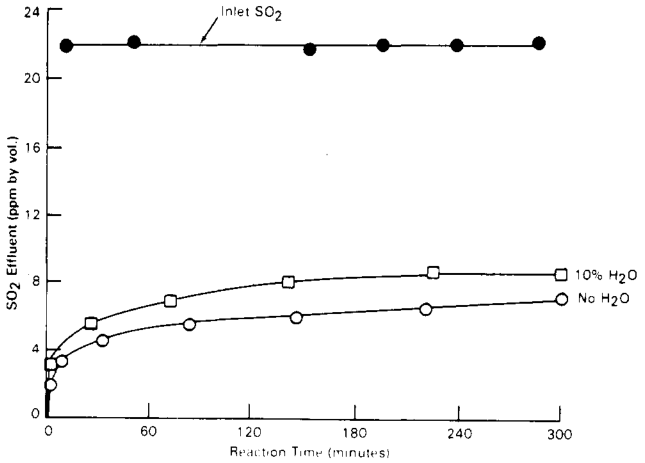

Figure 11. Effect of H_2O on SO_2 by Pt/Al_2O_3 at 5.1% O_2 and 540 °C

thought. Impurities in the Al_2O_3 support can catalyze SO_2 oxidation, and Pt and Pd apparently can catalyze the disproportionation of SO_2 to form surface sulfate species.

SO_2 and SO_3 adsorb on different Al_2O_3 sites, and they adsorb independently of each other. Consequently, it was possible to determine the relative quantities of sulfur stored on Al_2O_3 as SO_2 and as SO_3.

Acknowledgments

The experiments were conducted by J. Ulicny and D. Fournier.

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Chloroform and Chlorophenol Production by Decarboxylation of Natural Acids during Aqueous Chlorination

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■ Naturally occurring carboxylic acids of several structural types reacted in dilute solution with aqueous hypochlorite to afford decarboxylation products. Incorporation of chlorine into the residual organic molecule occurred. Citric acid was efficiently converted to chloroform at pH 7 by a pathway probably involving 3-ketoglutaric acid as an intermediate; in acidic or alkaline solution, yields of $CHCl_3$ were lower. Several other enolizable keto acids (including all three isomers of resorcylic acid) were likewise precursors of $CHCl_3$ or of substances which could thermally be converted to $CHCl_3$; yields varied widely. Two substituted benzoic acids common to natural waters, *p*-hydroxybenzoic acid and vanillic acid, were decarboxylated by hypochlorite with the production of chlorophenols.

Although the association of chlorophenols with unpleasant odors and tastes in drinking waters was recognized many years ago, fundamental studies on the kinetics and mechanisms of aqueous chlorination reactions have been sparse until recent years. It has now become evident that treatment of drinking and wastewaters with chlorine leads to the formation of a variety of organochlorine compounds. Chloroform ($CHCl_3$), other haloforms, chlorinated phenols and phenolic acids, and chlorinated quinones, benzoic acids, and heterocyclic compounds are only a few of the many structural types which have been identified (*1–4*). Much of the organically bound chlorine has not been fully characterized; a large fraction is associated with macromolecular organics (*5*).

Chlorine dissolved in water ("aqueous chlorine") exists principally as hypochlorous acid (HOCl) between pH 3.4 and 7.5, and as the hypochlorite anion OCl^- at higher pH values. The reactions of aqueous chlorine with organic molecules fall into three categories, addition, substitution, and oxidation. Chlorohydrins are produced by the addition of HOCl to olefinic double bonds, and chlorine can also be incorporated by substitution reactions into activated aromatic nuclei, amines, and enolizable ketones. Many oxidative reactions of hypochlorite are known, but until recently they have been neglected in discussions of water chlorination since it has been assumed that chlorine was not incorporated into organic molecules during their oxidation.

There are a few references to oxidative decarboxylation in chlorination reactions in aqueous solution. The presence of chlorophenols (*4, 6*) and chlorinated quinones (*7*) in pulp bleaching liquors has been demonstrated. These compounds are largely produced through oxidative cleavage of lignin side chains (4-substituted guaiacols and catechols) by aqueous chlorine in strongly acid solution. Among the model compounds studied was *p*-hydroxybenzoic acid, which under these conditions was rapidly converted to 2,4,6-trichlorophenol

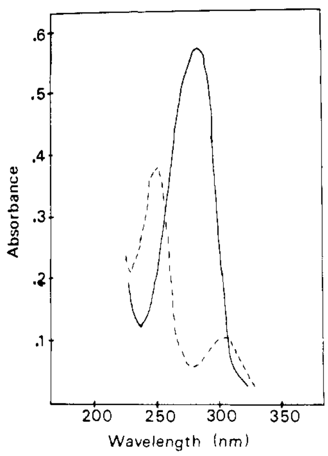
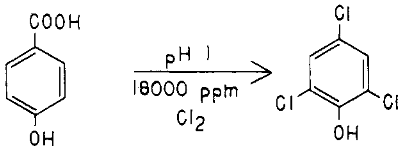


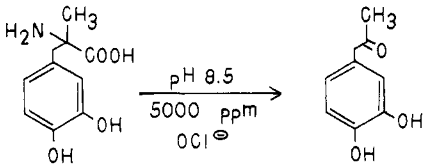
Figure 1. Absorption spectra of *p*-hydroxybenzoic acid (solid line) and *p*-chlorophenol (dashed line) at pH 12.0. Concentration, 1×10^{-5} M. Other chlorophenols and chlorinated derivatives of *p*-hydroxybenzoic acid had virtually identical absorption maxima, but absorbances varied

(Reaction 1) (*8*). Amino acids have been oxidatively decar-



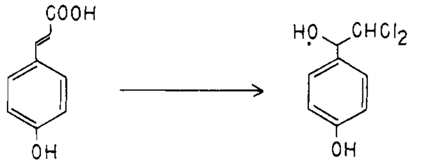
(1)

boxylated to carbonyl compounds; in one instance, α-methylDOPA was converted to a ketone by reaction with NaOCl (Reaction 2) (*9*). The carboxyl group of *p*-coumaric acid was



(2)

replaced during aqueous chlorination under unspecified "large-scale" conditions, with the formation of chlorinated styrene derivatives (Reaction 3) (*10*). The conditions used in



(3)

these reactions are not typical of those encountered in water treatment, and it was of interest to determine whether similar reactions might proceed under milder conditions. We report that several common natural carboxylic acids are readily attacked by hypochlorite in dilute solution, with loss of CO_2 and the incorporation of chlorine into the residual molecule.

Experimental

Materials. Most organic acids and phenols were obtained commercially and recrystallized. Hypochlorite solution was Clorox hypochlorite bleach, nominally 5.25% NaOCl (verified iodometrically). Buffered solutions were made up in deionized, charcoal-filtered water using sodium phosphate salts. Petroleum ether was Fisher pesticide grade material, checked for electron-capturing contaminants before use. 5-Chlorovanillic and *p*-dimethylaminobenzoic acids were synthesized by silver oxide oxidation from the corresponding aldehydes (*11*). 4-Chloro-2-methoxyphenol was synthesized from SO_2Cl_2 and guaiacol (*12*).

Chloroform Production from Pure Acids. The organic acid, dissolved in 50 mL of 0.05 M phosphate buffer, was treated at ambient temperature with a tenfold molar excess of hypochlorite solution. At intervals, a 10-mL portion was removed and extracted with 5 mL of petroleum ether. From the organic layer, 1.0 μL was injected onto a Chromosorb-101 GC column (stainless steel, 2.4 m × 3 mm i.d.) held isothermally at 165 °C. Argon–methane (95:5, 30 mL/min) was used as a carrier gas. Chloroform, which emerged at 5.8 min, was detected using a ^{63}Ni electron capture detector; its identity was confirmed by mass spectroscopy. Peak height was measured and compared to standards. A blank, not containing an organic acid, was always run. In a few experiments, the aqueous reaction mixture was injected directly, without petroleum ether extraction, to measure "potential" $CHCl_3$ (*13*).

Chloroform Production from Microbial Cell Contents. Basal medium (10 g of NaOAc, 3 g of $[NH_4]_2SO_4$, 3 g of KH_2PO_4, 250 mg of $CaCl_2$, 250 mg of $MgSO_4$, 10 mg of inositol, 2.2 μg of biotin, 1 mg of calcium pantothenate, 1 mg of pyridoxine-HCl, 1 mg of thiamin-HCl, 1 L of distilled water) was inoculated with 0.2 mL of turbid suspension of fine surface sediment obtained from White Clay Creek, Chester County, Pa. The mixture was shaken aerobically at 28 °C for 3 days. Microscopic observation of the culture revealed several microbial forms, principally coccoid bacteria and filamentous fungi. An aliquot of the culture was removed and filtered through a tared glass fiber ultrafilter; the filter was dried and weighed to determine total microbial biomass. The remainder of the culture was centrifuged; the pellet was washed three times in pH 7 phosphate buffer (to remove medium) and disrupted ultrasonically in this buffer. The suspension was centrifuged and filtered (0.45-μm membrane filter); the filtrate was passed over a column of anion exchange resin (Bio-Rad AG-1-X2, formate form, 1 × 6 cm). The column was eluted with two volumes of 0.1 M HCOOH ("weak acid" fraction) and then two volumes of 16 M HCOOH ("strong acid" fraction). These conditions have been shown to separate dicarboxylic from tricarboxylic acids (*14*). The two fractions were divided into two equal portions and evaporated at room temperature. One portion was esterified for determination of carboxylic acids by GC (*15*), and the other was redissolved in 5.0 mL of pH 7 phosphate buffer. A small portion of this solution was reserved for total organic carbon determination (Dohrmann DC-54), and the remainder was chlorinated by adding 50–100 μL of Clorox and allowed to stand overnight. Finally, a 2.0-mL portion of the reaction mixture was extracted with 2.0 mL of petroleum ether for chloroform determination.

Production of Chlorophenols and Chlorinated Aromatic Acids. Methods for chlorination and analysis of aromatic acids were described previously (*16*). In brief, an aqueous solution of the sodium salt of the acid (5×10^{-4} M) was treated with equimolar NaOCl at 20–25 °C. Products were extracted from the acidified reaction mixture with EtOAc and analyzed by GC.

In kinetic experiments, the acid (1.5×10^{-5} M) was treated with 5× excess NaOCl in 0.2 M phosphate buffer at 8 °C. The reaction was quenched by addition of excess $Na_2S_2O_3$ to an aliquot portion; its UV spectrum was determined after adjustment of pH to 12 ± 0.5. From the intensities of the peaks at 280 and 244 nm (Figure 1), the concentrations of acids (including both chlorinated and unchlorinated species) and chlorophenols were determined.

Hypochlorite concentrations were determined by the FACTS method (*17*).

Mass spectra of chlorinated aromatic acids and chloro-

Table I. Chloroform Production from Naturally Occurring Carboxylic Acids

acid	concn, M	pH	time, h	yield $CHCl_3$, %
2,4-dihydroxybenzoic	7.5×10^{-4}	9.6	0.33	92[a]
2,6-dihydroxybenzoic	7.5×10^{-4}	8.0	0.75	90[a]
3,5-dihydroxybenzoic	7.5×10^{-4}	7.8	0.75	78[a]
3-ketoglutaric	7.5×10^{-4}	8.1	0.75	94.4[a]
citric	1.0×10^{-4}	7.0	2	4.4[a]
	2.0×10^{-4}	7.0	2	8.9[a]
	7.5×10^{-4}	7.0	2	77.9[a]
	7.5×10^{-4}	5.5	2	21.2[a]
	7.5×10^{-4}	9.3	2	<1[a]
isocitric	7.5×10^{-4}	8.0	0.75	<1[a]
fumaric	5.0×10^{-4}	8.0	18	23[b]
maleic	5.0×10^{-4}	8.0	4	14[b]
	7.5×10^{-4}	8.0	22	<1[a]
malic	5.0×10^{-4}	8.0	24	30[b]
	7.5×10^{-4}	8.0	120	1.1[a]
oxaloacetic	7.5×10^{-4}	8.0	20	1.7[a]
salicylic	7.5×10^{-4}	8.0	20	2.1[a]

[a] By extraction with petroleum ether. [b] By direct aqueous injection.

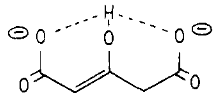
phenols were determined by Shrader Laboratories (Detroit, Mich.).

Results and Discussion

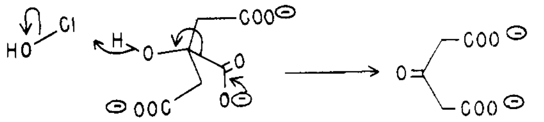
Chloroform Production. Experiments on the production of $CHCl_3$ from several naturally occurring carboxylic acids are summarized in Table I. All three carboxylic acids related to resorcinol (2,4-dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, and 3,5-dihydroxybenzoic acid) gave good yields of $CHCl_3$. Resorcinol itself and several simple substituted derivatives have already been shown to be efficient precursors of $CHCl_3$ (*18*); the active site for chlorination is the carbon atom flanked by phenolic hydroxyl groups. In this context, it is interesting that 2,6-dihydroxybenzoic acid, in which this carbon atom bears a carboxyl substituent, is approximately as good a precursor of $CHCl_3$ as are the other two isomers, or resorcinol itself. The conversion of this compound to $CHCl_3$ probably involves decarboxylation. Both phenolic hydroxyls are required for $CHCl_3$ production, as shown by the result for salicylic acid (2-hydroxybenzoic acid).

Several other naturally occurring acids having β-keto groups or which could be converted to β-keto acids by simple reactions with hypochlorite were tested for $CHCl_3$ production. It was anticipated that the methylene group between the carbonyl and carboxyl groups would rapidly be enolized and readily attacked by hypochlorite. Subsequent decarboxylation would afford a dichloromethyl or trichloromethyl ketone which could readily be converted to chloroform by unambiguous processes.

3-Ketoglutaric acid (acetonedicarboxylic acid) reacted rapidly with hypochlorite at near-neutral pH, giving virtually quantitative yields of $CHCl_3$ within a few minutes. The high reactivity of 3-ketoglutaric acid may be explained by increased enol stability conferred by the two carboxylate groups available for hydrogen bonding:



In nature, 3-ketoglutaric acid is produced by enzymatic oxidative decarboxylation of citric acid (*19*). We showed that the reaction probably also occurs in hypochlorite solutions; citric acid gave high yields of $CHCl_3$ when chlorinated in near-neutral solutions (78% at pH 7). The reduced yields noted in acidic or alkaline media may be explained by a rate-determining oxidative decarboxylation (Reaction 4) requiring



(4)

the presence of the citrate trianion ($pK_a = 6.40$) and hypochlorous acid ($pK_a = 7.49$). The optimum pH for this reaction would be that at which the concentrations of these two species are maximal, namely (6.40 + 7.49)/2, or 6.94. Isocitric acid, which would not afford a highly stabilized enol on decarboxylation, produced little or no $CHCl_3$ when chlorinated. Simple β-keto or β-hydroxy acids (oxaloacetic, malic) were also not good precursors of $CHCl_3$. Fumaric and maleic acids, having α,β unsaturation (which could give chlorinated β-keto acids by addition of HOCl to their double bonds and oxidation), afforded compounds which could be converted to $CHCl_3$ by heating (direct aqueous injection), but little or no free (petroleum ether extractable) $CHCl_3$.

Table II. Production of $CHCl_3$ from Ultrasonically Disrupted Microorganisms (Total Biomass Production, 2.7 g)

fraction	total C, µg	citric acid, µg	$CHCl_3$ produced, µg
strong acid	7760	313	20
weak acid	2170	N.D.[a]	N.D.

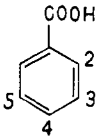
[a] N.D., none detectable.

Citric acid occurs in tap water, in natural waters, and in effluents; levels as high as 10.2 mg/L (5.3×10^{-5} M) have been reported (*20–22*). Although exudates of stems and roots of higher plants contain citric acid (*23*), the principal source in waters is probably the metabolic activity of the aquatic microflora. To determine whether cellular contents or exudates of aquatic microorganisms could produce $CHCl_3$ when chlorinated, we grew a mixed population of microorganisms isolated from a surface sediment in a defined medium containing acetate as the sole carbon source. The harvested cells were sonically disrupted, and the supernatant was partitioned by ion exchange chromatography into a strongly acid fraction (containing citric acid and other polycarboxylic acids) and a less acidic fraction. The two fractions were chlorinated separately; $CHCl_3$ was produced only from the strongly acid fraction (Table II). Citric acid was presumably the source of much of the $CHCl_3$. The yield of $CHCl_3$, based on citric acid, was 10%.

The 3-ketoglutaric and citric acid results may also be relevant to the formation of $CHCl_3$ from natural dissolved organic polymers ("humic acids", "fulvic acids"). Enzymatic degradation of lignin and related polyphenols, probable precursors of these materials, affords ring-opened products containing β-keto acid groups (*24*). These structures may persist in humic substances; a polycarboxylic acid, similar in some respects to homopolymerized maleic anhydride, has been identified as a major constituent of soil fulvic acid (*25*).

Chlorophenol Production. In order to react rapidly with hypochlorite, an electrophilic reagent, aromatic compounds require activation by electron-donating substituents (*26*). Because the carboxyl group withdraws electrons, simple aromatic carboxylic acids would not be expected to react by addition of hypochlorite to the ring; thus, benzoic acid (**1**, Table III) did not react appreciably with hypochlorite under our conditions. However, incorporation of a phenolic hydroxyl group allowed attack of hypochlorite; salicylic acid (**2**) and *p*-hydroxybenzoic acid (**4**) readily formed chlorinated addition products. 5-Chlorosalicylic acid was the principal product

Table III. Reactions of Substituted Benzoic Acids with Aqueous Hypochlorite[a]



no.	substituent 2	3	4	5	addition product (s)[b]	%	decarboxylation product(s)[c]	%
1	H	H	H	H	0		0	
2	OH	H	H	H	1	14	0	
3	H	OH	H	H	0		0	
4	H	H	OH	H	2	22	3	78
5	H	H	OCH_3	H	1	71	1[d]	<1
6	H	H	NH_2	H	2	82	0	
7	H	H	$N(CH_3)_2$	H	1	96	1[d]	4
8	H	OCH_3	OH	H	1	8	2	92
9	H	OH	OH	H	1	9	0	
10	H	Cl	OH	H	1	<1	2	97
11	H	Cl	OH	Cl	0		1	79
12	H	NO_2	OH	H	0		1	36
13	H	OCH_3	OH	OCH_3	1	16	0	

[a] Yields derived from GC peak areas, relative to total area of all peaks observed (including any residual starting material). [b] Number of products retaining the carboxyl group detected by GC. [c] Number of products shown by GC-MS to have lost the carboxyl group. [d] Structure not established by GC-MS; peak had shorter GC retention time than starting material.

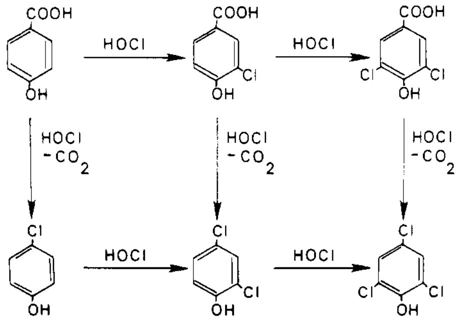


Figure 2. Summary of aqueous chlorination reactions of *p*-hydroxybenzoic acid

from **2**, whereas **4** gave a mixture of the 3-chloro and 3,5-dichloro acids **10** and **11**. *m*-Hydroxybenzoic acid (**3**), however, could be recovered practically unchanged from aqueous hypochlorite solutions, even when the reaction temperature was increased to 90 °C.

In addition to phenolic acids, benzoic acids containing alkoxyl, amino, or alkylamino substituents were sufficiently activated for addition reactions with hypochlorite (**5–9**, **13**).

A few phenolic acids also underwent efficient decarboxylation reactions with hypochlorite. A mixture of 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol was produced when *p*-hydroxybenzoic acid (**4**) was chlorinated. Because no phenol, 2-chlorophenol, or 2,6-dichlorophenol was detected among the products, it is unlikely that decarboxylation occurred without chlorination of the 4 position. The products of this reaction may be accounted for by a multistep scheme summarized in Figure 2. Decarboxylation probably occurs through 2,5-cyclohexadienone intermediates bearing Cl and COOH on the same carbon atom; similar stable compounds were identified in the chlorination of other para-substituted phenols (*27*). The chlorophenols were shown by TLC to be present in the reaction mixture, and were accordingly not artifacts of the workup or derivatization procedures.

Chlorinated phenolic acids were likewise readily decarboxylated; 3-chloro-4-hydroxybenzoic acid (**10**) gave a mixture of 2,4-dichlorophenol and 2,4,6-trichlorophenol, whereas only 2,4,6-trichlorophenol was produced from 3,5-dichloro-4-hydroxybenzoic acid (**11**).

A free phenolic hydroxyl group appeared to be required for efficient decarboxylation (cf. **5**), but incorporation of an additional methoxyl group into *p*-hydroxybenzoic acid (vanillic acid, **8**) still allowed chlorophenol production to occur. The products were 4-chloro-2-methoxyphenol and a dichloro-2-methoxyphenol. Kinetic studies at lower reactant concentrations, however, showed that the decarboxylation of vanillic acid was much slower than that of *p*-hydroxybenzoic acid.

If two phenolic hydroxyl groups were present (**9**), chlorophenols were not detected. A phenolic acid with a nitro substituent (**12**) afforded only a decarboxylation product; no addition product retaining the carboxyl group was detected. Apparently, decarboxylation of a phenolic acid is sensitive to the nature of further ring substitution. Resonance stabilization of the phenolate anion generated by decarboxylation of a cyclohexadienone would be favored by electron-withdrawing substituents at the ring positions ortho to the phenolic hydroxyl.

At reactant concentrations and conditions more nearly typical of those encountered in the environment (pH 6.7, 8 °C), 10^{-5} M (1.5 ppm) *p*-hydroxybenzoic acid consumed 3 equiv (1.5 ppm) of hypochlorite in 15 min. Products of the reaction were assessed by UV spectroscopy. About 55% of the starting material was converted to chlorophenols; another 30% retained the carboxyl group. The rate of decarboxylation was maximal at pH 7.9 and was very slow below pH 6. Addition compounds retaining the carboxyl group were still produced at lower pHs, however.

Substituted benzoic acids are common in the environment; *p*-hydroxybenzoic acid has been identified in numerous studies of soils (*28–30*) and waters (*31–33*). It is probable that some of the chlorophenols of drinking and wastewaters are products of the chlorination of these aromatic acids, particularly at periods of high concentrations of natural dissolved organic matter (spring thaw, autumn leaf fall) (*34*).

Acknowledgment

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Determination of Several Industrial Aromatic Amines in Fish

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■ A procedure is described for the determination of selected industrial aromatic amines in fish. Ground fish tissue is digested with aqueous sodium hydroxide and extracted with benzene. The extract is washed with dilute acid and cleaned up using gel permeation chromatography. The amines are separated and quantitated using nitrogen-selective gas–liquid chromatography. Recoveries of *N*-ethyl-*N*-phenylbenzylamine, *N*-ethyl-*N*-(*m*-tolyl)benzylamine, *N,N*-dibenzylmethylamine, diphenylamine, and *N*-phenyl-1-naphthylamine from fish tissue fortified at levels of 20–100 ppb (μg/kg) averaged at least 80%. Recoveries of 1-naphthylamine and 3,3′-dichlorobenzidine were somewhat lower. Fish samples obtained from rivers near nine textile and dyestuff manufacturers known to use certain aromatic amines have been analyzed. 1-Naphthylamine was detected in fish from the Buffalo and Delaware Rivers downstream from two dyestuff manufacturers. *N*-Ethyl-*N*-phenylbenzylamine and *N*-ethyl-*N*-(*m*-tolyl)benzylamine were also detected in the Buffalo River fish.

The carcinogenicity of several industrial aromatic amines has stimulated interest in investigating possible environmental contamination by this class of compounds. In 1974, the Occupational Safety and Health Administration issued regulations on 14 chemical compounds that were either known or suspected human carcinogens (*1*), including aromatic amines such as 1- and 2-naphthylamine and 3,3′-dichlorobenzidine. These carcinogenic compounds could create a potentially serious problem should there be widespread contamination of the human food chain.

The Food and Drug Administration became concerned with the possible contamination of human foods by industrial aromatic amines because of their known or suspected carcinogenicity, relatively large production volumes, and potential for biomagnification. Quantities produced vary from approximately 584×10^6 lb/year for aniline to several million pounds/year for numerous other compounds (*2*). Many aromatic amines are used as intermediates for dyes and pigments, and as antioxidants and antiozonants in rubber products. These varied applications suggest many possible modes of entry into the environment, ranging from direct discharge to conversion of azo dyes to the precursor amines by bacteria.

Many workers have published detection schemes for various aromatic amines, using gas–liquid, high performance liquid, or thin-layer chromatography, sometimes coupled with ancillary techniques such as ultraviolet or fluorescence spectrometry (*3–12*). Most investigations into amines in fish or foods have centered around naturally derived volatile amines such as di- and trimethylamine, or more recently the *N*-nitrosamines (*13–16*). To this author's knowledge, however, there are no published methodologies or findings of unhalogenated industrial aromatic amines in aquatic organisms such as fish.

This study reports the presence of microgram/kilogram (parts per billion) quantities of selected industrial aromatic amines in fish tissue and an analytical technique for their detection and quantitation. The technique is aimed at detecting the less water-soluble aromatic amines which are expected to biomagnify or concentrate to higher levels in fish tissue than are found in the aqueous environment. The pres-