Analytical and Confirmative Techniques for Dibenzo-*p*-dioxins Based upon Their Cation Radicals

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A large number of analytical methods for the detection of chlorinated dibenzo-*p*-dioxins have been reported in the past few years (*1–8*); generally, these methods involve the use of gas–liquid chromatography (GLC) or GLC coupled with mass spectrometry (MS). Detection limits of about 1 ng have been reported for GLC–MS techniques with the demonstrated capability of detecting these compounds in the picogram range by use of special techniques. One of the disadvantages of such techniques, at least for confirmative purposes, appears to be the inability to distinguish between positional isomers by MS as well as the inaccessibility to many analysts of the expensive equipment involved.

We were therefore interested in developing the potential of some new confirmative techniques for identification of the various chlorinated dibenzo-*p*-dioxins. To support this work we first synthesized a series of chlorinated dibenzo-*p*-dioxins (*9*). We then obtained the visible and electron spin resonance (ESR) spectra of the cation radicals of these materials, and finally we attempted to assess the usefulness of these data for confirmation of the presence of chlorinated dibenzo-*p*-dioxins. The results of these studies are described below.

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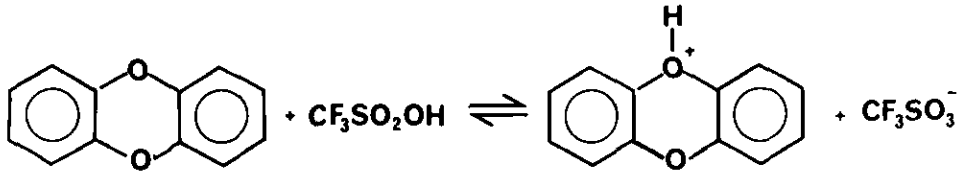
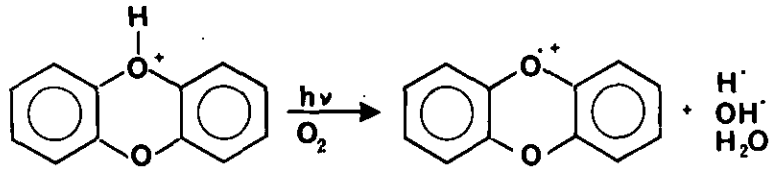
At the time we initiated these studies it was well known through the extensive and detailed published work of Tomita (*10–12*) and others (*13, 14*) that many substituted dibenzo-*p*-dioxins, when dissolved in strong acids such as H_2SO_4 or $SbCl_5$, are converted into cations which form highly colored cation radicals on oxidation by various oxidizing agents (KNO_3, H_2O_2, etc.). This phenomenon was the basis for a proposed color test for the dibenzo-*p*-dioxin nucleus. Unfortunately certain highly chlorinated dibenzo-*p*-dioxins, for example, the octachloro derivative, were reported not to produce the colored cation radicals. It was our thesis at the time that this color reaction might be made the basis of a suitable screening and confirmative procedure for the chlorinated dibenzo-*p*-dioxins and that under the proper conditions, all of the substituted derivatives of dibenzo-*p*-dioxin would give the test.

We quickly observed that the color reaction was quite specific; we found, for example, that no color was developed with chlorinated biphenyls, diphenyl ethers, dibenzofurans, xanthenes, or xanthones. In the case of the chlorinated dibenzo-*p*-dioxins, the ease of formation of the colored species was highly dependent upon the total number and position of the chlorine atoms present, and upon the solvent and oxidizing agent used (*15*). We found that tri-

fluoromethanesulfonic acid (TFMS acid) was an especially useful solvent for these studies (*16*). A mechanism illustrating the formation of the cation radicals in TFMS acid is shown in eqs. (1) and (2). It was observed with this acid that many of the lesser halogenated derivatives quickly developed the color in this solvent without the addition of an oxidizing agent such as is required when concentrated H_2SO_4 is used. With the higher chlorinated derivatives we found it necessary to add an oxidizing agent (KNO_3) or to irradiate with ultraviolet light in order to generate the free radicals.



(1)



(2)

Table 1. Visible absorption spectra of chlorinated dibenzo-*p*-dioxins.

Radical	Absorption peak, nm
Dibenzo-*p*-dioxin (unsubstituted)	655.0
1-Chloro	708.0
2-Chloro	674.5
2,7-Dichloro	720.0
2,8-Dichloro	762.0
2,3-Dichloro	742.0
1,2,4-Trichloro	706.0
1,3,6,8-Tetrachloro	733.0
2,3,7,8-Tetrachloro	845.0
1,2,3,4-Tetrachloro	758.0
1,2,3,4,7-Pentachloro	790.0
1,2,4,6,7,9-Hexachloro	750.0
1,2,3,4,7,8-Hexachloro	841.0
Octachloro	855.0

The wavelength of maximal absorption of the various chlorinated dibenzo-*p*-dioxins was found to be highly dependent on the position of chlorine substitution (Table 1). It can be seen that the three tetrachloro isomers can be readily distinguished, solely on the basis of the position of the absorption band. Indeed, the evidence is quite strong that only those compounds substituted in the 2, 3, 7, and 8 positions will absorb in the 845–855 nm range. However, since we do not have a variety of pure standards of the more highly chlorinated materials, we are unable to say with certainty that such materials would not absorb in the range expected for the toxic 2,3,7,8-tetrachloro isomer. It is of interest to point out, however, that of the 26 possible pentachloro, hexachloro, and heptachloro isomers, only 5 compounds are fully substituted in the 2, 3, 7, and 8 positions.

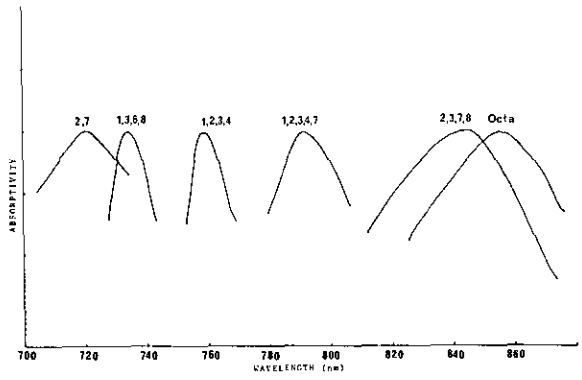


FIGURE 1. Absorption spectra of the cation radicals of various chlorinated dibenzo-*p*-dioxins.

Typical spectra of a series of chlorinated derivatives are shown in Figure 1, illustrating the ease with which these materials may be recognized solely on the basis of the position of the absorption maxima.

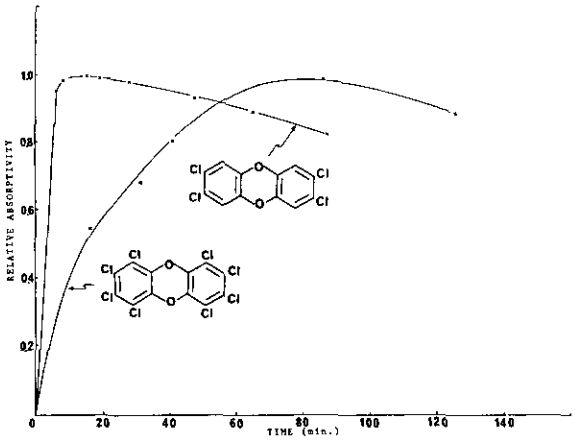


FIGURE 2. Rate of formation of various chlorinated dibenzo-*p*-dioxins in TFMS acid with added KNO_3.

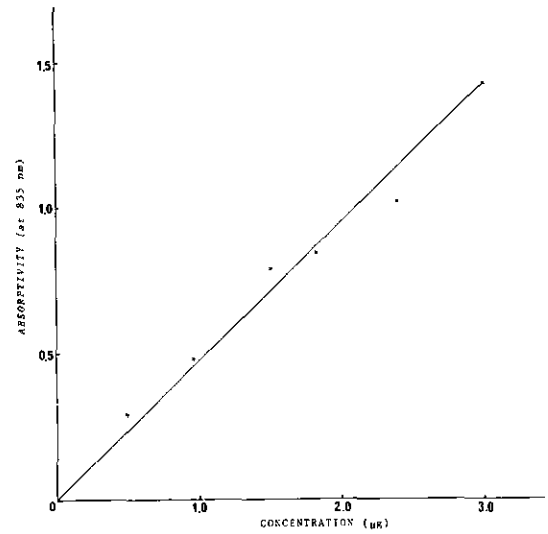


FIGURE 3. Absorptivity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in TFMS acid with added KNO_3 as a function of concentration.

The octachloro derivative was of special interest to us, because it illustrates well one of the important variables with which one must deal in carrying out this type of work, that is, time. We have observed that, whereas the 2,3,7,8-tetrachloro and the octachlorodibenzo-*p*-dioxins absorb maximally in the same range (845–855 nm), the rate of formation of the respective cation radicals has considerable variation (Fig. 2). Thus the formation of a maximum concentration of radicals occurs in ca. 10 min for the tetrachloro isomer, whereas about 60 min is required in the case of the octachloro isomer. This in theory provides a convenient way of estimating the presence of each of these species independently on the same sample.

On closer examination of the results of our studies of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, we found that the absorptivity at 835 nm could be correlated with the amount of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin present. A linear plot was obtained (Fig. 3) in the range of 0.25–3.0 μg of pure material. A minimum detection limit based on a signal-to-noise ratio of 5/1 was estimated to be ca. 250 ng.

We next turned our attention to the ESR spectra exhibited by the cation radicals of the various chlorinated dibenzo-*p*-dioxins. We found that extremely well resolved spectra could be obtained in TFMS acid with the lower chlorinated dibenzo-*p*-dioxins; with the more highly chlorinated derivatives the hyperfine structure was no longer observed; however, each derivative gave a distinctive, identifiable spectrum so that a potential confirmative technique was at hand.

Figure 4 shows the ESR spectrum of dibenzo-*p*-dioxin in TFMS acid without addition of an oxidizing agent. Notice the well resolved five-line pattern with ^{13}C substructure. Such a spectrum is observed only on degassed samples in the absence of oxidizing agents. In Figure 5 the spectra of two of the tetrachloro isomers are displayed; again

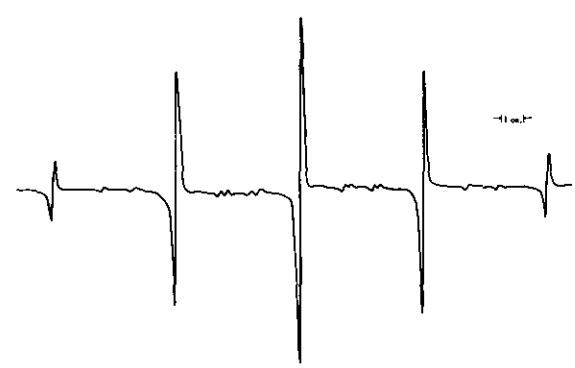


FIGURE 4. ESR spectrum of dibenzo-*p*-dioxin in TFMS acid with dilute concentration.

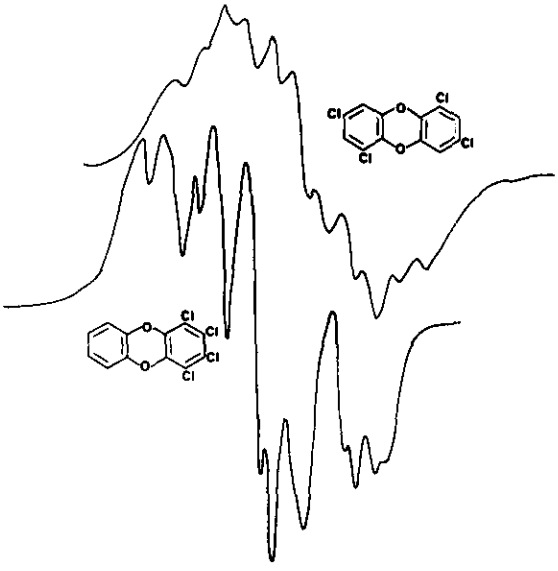


FIGURE 5. ESR spectra in TFMS acid with ultraviolet irradiation.

this type of hyperfine structure can be observed only on degassed samples. In these cases ultraviolet irradiation was used to generate the free radicals.

If the sample is not degassed, such hyperfine splitting is lost (Fig. 6). Hyperfine splitting is also lost when an oxidizing agent (KNO_3) is added. Loss in fine structure, however, does not prevent the accurate determination of which isomer is present, since the position of the resonance field (g factor) relative to Fremy salt or dibenzo-p-dioxin itself varies with structure. In addition, each isomer exhibits a characteristic line

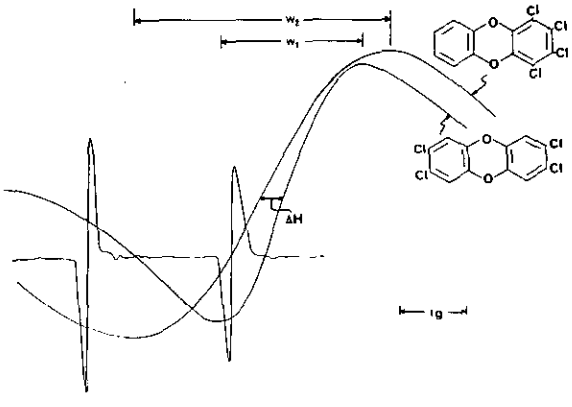


FIGURE 6. ESR spectra of tetrachlorodibenzo-p-dioxins in TFMS acid with added KNO_3.

width (W), a property which also may be used to identify a particular isomer.

In Table 2 are tabulated the g factors for a series of chlorinated dibenzo-p-dioxins. Such data are extremely useful in characterization of the various isomeric chlorinated dibenzo-p-dioxins.

Table 2. g Factors and linewidth for the chlorinated dibenzo-p-dioxins in TFMS acid.

$|\Delta H = H_1 - H_0|$ $\nu = 9507.5$ GHz

H_0 Dibenzo-p-dioxin	H_1 Chlorinated dibenzo-p-dioxin		
	g factor	ΔH, oe	Linewidth, oe
Dibenzo-p-dioxin	2.0038	—	0.090
1-Chloro	2.0027	1.82	3.26
2-Chloro	2.0034	0.67	2.13; 4.91
2,3-Dichloro	2.0018	3.31	4.34
2,7-Dichloro	2.0026	2.17	3.33
2,8-Dichloro	2.0024	2.31	2.45
1,2,4-Trichloro	2.0029	1.50	4.34
1,2,3,4-Tetrachloro	2.0017	3.47	4.23
2,3,7,8-Tetrachloro	2.0020	3.10	3.16
1,3,6,8-Tetrachloro	2.0025	2.13	3.79
1,2,3,4,7-Pentachloro	2.0019	3.18	3.42
1,2,3,4,7,8-Hexachloro	2.0017	3.60	3.68
1,2,4,6,7,9-Hexachloro	2.0024	2.29	4.86
Octachloro	2.0016	3.76	3.39

A calibration curve was constructed by using the toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (Fig. 7). The plot of amount of dioxin versus relative peak height of the resonance lines was found to be linear, the lower detection limit being estimated to be ca. 1 ng.

We next turned our attention to the application of these techniques to the confirmation of the presence of chlorinated dibenzo-p-dioxins in phenols and a toxic fat sample. Using the extraction procedures outlined by Firestone et al. (*1*) we were able to show that all fractions which were positive by GLC for chlorinated dibenzo-p-dioxins also yielded strong ESR signals and intense visible absorption. We were unable to characterize the dioxins in these samples completely, however, because of the lack of the appropriate pentachloro, hexa-

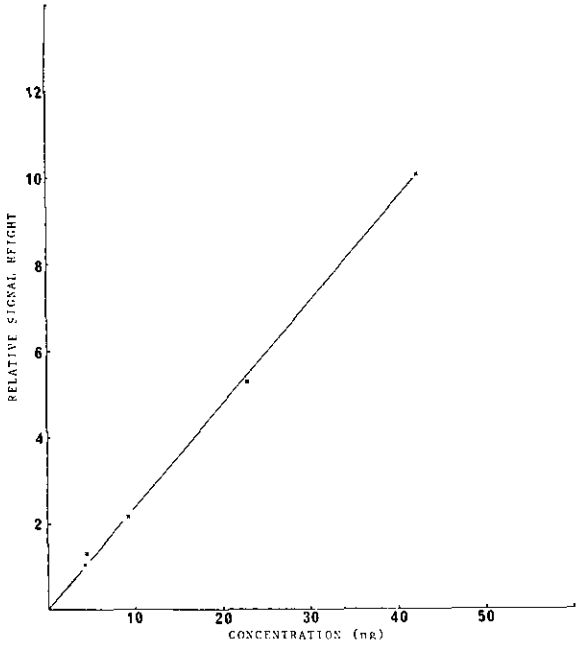


FIGURE 7. ESR calibration curve for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in TFMS acid with added KNO_3.

chloro, and heptachloro standards. We were able to establish the absence of appreciable amounts of the 2,3,7,8-tetrachloro derivative on the basis of the position of the absorption maxima and the rate at which the color developed.

In summary, we feel that both the visible and ESR spectra are highly suited for confirmation of the presence of chlorinated dibenzo-*p*-dioxins, provided a suitable separation technique is at hand and provided pure standard materials are available. Thus one may readily differentiate between the four tetrachlorodibenzo-*p*-dioxins on the basis of either their visible or ESR spectra, provided the pure isomer is available. We suggest that two unique possibilities present themselves; one would be a separation technique involving preparative high-pressure liquid chromatography; the other might be preparative gas chromatography in which the sample is actually collected in the ESR tube. Both of these techniques are under consideration.

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