Survey of Polychlorodibenzo-*p*-dioxin Content in Selected Pesticides

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One-hundred-twenty-nine samples of 17 different pesticides derived from chlorophenols were examined for polychlorinated dibenzo-*p*-dioxins by electron capture gas chromatography (ec–gc). The method of cleanup involved a concentrated sulfuric acid extraction of impurities from hexane and a mild nitration of the chlorophenol extracts. Seventy-six percent of the samples analyzed contained less than 0.1 μg/g of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the technical material, whereas 7% contained between 0.1 to 1.0 μg/g, and 9% contained greater than 10 μg/g TCDD. No TCDD was detected in the 20 tri-, tetra-, or pentachlorophenol samples examined. However, high levels of other dioxins were found. All samples which contained more than 1.0 μg/g of dioxin by ec–gc were confirmed by gas chromatography using a flame ionization detector (fid–gc), a microcoulometric detector (mc–gc), *p* values, uv irradiation, and/or gas chromatography/mass spectrometry (gc–ms). Samples of phenoxy herbicides from current production contained less than 0.5 μg/g TCDD.

Polychlorodibenzo-*p*-dioxins may be contaminants in chlorophenols or in pesticides which use chlorophenols in the manufacturing process. Chlorinated dibenzo-*p*-dioxins are hazardous materials and may cause skin eruptions, teratogenesis, and are toxic to animals at low levels. The dioxins are formed usually when the reaction temperature for making *o*-chlorophenol by hydrolysis exceeds 160°C under pressure. The reaction may be an alkaline hydrolysis of polychlorobenzene or a chlorination of phenol to form a polychlorophenol.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, Figure 1) is the dioxin that may be formed when the reaction temperature for making 2,4,5-trichlorophenol from tetrachlorobenzene exceeds 160°C (Schultz, 1968). In chlorinating phenol to make tetra- or pentachlorophenol, heat must be supplied to the reaction mixture in order to maintain the mix as a melt. If too much heat is supplied, hexa-, hepta-, and/or octachlorodibenzo-*p*-dioxins may be formed. Since little or no heat is required to form di- and trichlorophenols by chlorination, there is little likelihood that di-, tri-, or tetrachlorodibenzo-*p*-dioxins will be formed in this process.

Interest in dioxins originated in 1957 from outbreaks of "chick edema (CE) disease." The disease was characterized by hydroparicardium in chickens. In 1958 a toxic substance, isolated from an unsaponifiable fraction of feed fats which caused CE, was identified (Cantrell *et al.*, 1969) by single crystal X-ray crystallography as 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HCDD). In 1970, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was implicated as a potential teratogen in pregnant rats (Courtney *et al.*, 1970). Later tests indicated that the teratogenesis may have been caused by 27 ± 8 ppm of TCDD present as contaminant in the 2,4,5-T.

In order to assess possible environmental contamination with TCDD or higher chlorinated dioxins through pesticidal materials, samples of 17 pesticides based on use of chlorophenols in their manufacturing process were collected from U.S. Department of Agriculture's former Pesticide Regulation Division (PRD) laboratories. Additionally, samples of 2,4,5-T from one manufacturer, covering a 3-year period, were collected. A method was developed for the analysis of dioxins in the 17 different pesticides. The method developed and results of the survey are reported in this paper.

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PROCEDURE

Materials. No attempt should be made to analyze for dioxins without proper safety procedures. The following solvents were distilled in glass or were of pesticide grade quality: hexane, methanol, petroleum ether, acetonitrile, benzene, and diethyl ether.

The following apparatuses were required: chromatography columns (450 × 19 mm i.d.) with Teflon stopcock, gas chromatograph (gc), preferably equipped with a Ni^{63} detector (flame ionization or microcoulometric detectors also desirable for confirmation steps), glass gc columns (1.83-m × 4-mm), a sun lamp with maximum output at 310 nm, and thin-layer chromatography (tlc) apparatus.

The following compounds were examined for polychlorodibenzo-*p*-dioxins: (2,4-dichlorophenoxy)acetic acid, 2,4-D; 4-(2,4-dichlorophenoxy)butyric acid, 2,4-DB; 2-(2,4-dichlorophenoxy)propionic acid, 2,4-DP; (2,4,5-trichlorophenoxy)acetic, 2,4,5-T; 2-(2,4,5-trichlorophenoxy)propionic acid, silvex; 2-(2,4-dichlorophenoxy)ethyl sodium sulfate, sesone; 3,6-dichloro-*o*-anisic acid, dicamba; pentachlorophenol, PCP; tetrachlorophenol; trichlorophenol; 2,4-dichlorophenyl *p*-nitrophenyl ether, nitrofen; *O*-(2,4-dichlorophenyl) *O*-methyl isopropylphosphoramidothioate, DMPA; 2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate, erbon; tris[2-(2,4-dichlorophenoxy)ethyl]phosphite, 2,4-DEP; *O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothionate, ronnel; 2,4,5,4′-tetrachlorodiphenyl sulfone, tetradifon; and *O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate, VC-13, Nemacide.

The cleanup procedure consisted of three steps which were dependent on the nature of impurities found in and the complexity of the sample.

Step A. Technical Salts and Acids of Phenoxy Acids. One-hundred milliliters of MeOH was added to 3.00 g of herbicide and 10 ml of 5 *N* KOH contained in a 1000-ml separatory funnel. Two-hundred milliliters of H_2O and 100 ml of hexane were added after 20 min and shaken. The aqueous phase was reextracted with 100 ml of hexane and the extracts were combined. The hexane extracts were washed twice with 100 ml of 1 *N* NaOH, twice with 1 *N* HCl, and twice with 100 ml of H_2O. The extracts were dried over anhydrous Na_2SO_4, transferred to a 250-ml beaker, and evaporated to *ca.* 10 ml. The sample was transferred to a pre-

Table I. Relative Retention Times of Several Chlorinated Hydrocarbon Insecticides and Polychlorodibenzo-*p*-dioxins on Five Gas Chromatography Columns[a]

	Relative retention times[b] on columns				
	5% OV-225	1.5% OV-17 + 2.0% QF-1 (1:1)	5% OV-17	5% UCW-98	15% QF-1 + 10% DC-200 (1:1)
Aldrin	1.00 (2.17)[c]	1.00 (1.80)	1.00 (0.91)	1.00 (1.26)	1.00 (4.2)
p,p'-DDE	2.63	2.50	1.98	1.67	1.90
p,p'-TDE	5.84	3.62	3.19	2.14	2.64
p,p'-DDT	5.62	4.17	*d*	2.70	3.14
Endrin	3.69	2.78	3.08	1.90	2.64
Endrin, Δ keto	15.76	6.89	8.57	3.25	5.48
2,7-CDD[e]	1.29	1.00	1.21	0.83	1.02
2,3,7-CDD	1.94	1.67	2.09	1.51	1.64
tri-CDD	2.30	1.83	7.10	1.57	1.83
tetra-CDD[f]	2.86	2.50	2.31	2.22	2.38
tetra-CDD[f]	3.27	2.52	2.33	2.24	2.52
2,3,7,8-CDD	4.33	3.44	3.41	2.86	3.19
penta-CDD	8.29	6.56		5.08	5.48
hexa-CDD (1)	11.61	9.28	7.18	7.06	7.26
hexa-CDD (2)	13.13	10.56	8.24	7.94	8.09
hexa-CDD (3)	16.45	12.44	11.10	9.97	9.45
hepta-CDD (1)	23.32	18.39	13.19	13.09	13.26
hepta-CDD (1)	26.96	21.17	15.82	14.68	14.88
octa-CDD	46.31	36.28	23.41	24.52	24.05

[a] Column conditions: length, 1.8-m × 4-mm glass; solid support, Chromosorb W, 80–100 mesh; injector, 240°C; column, 220°C; detector, Ni^{63}, 310°C; flow rate, 80–100 ml/min. [b] ±1%. [c] Time in minutes in parentheses. [d] Not resolved from TDE. [e] Chlorodibenzo-*p*-dioxin = CDD. [f] More than one isomer was present in the tri-, tetra-, hexa-, and heptachlorodibenzo-*p*-dioxins. Known isomers have the positions numbered.

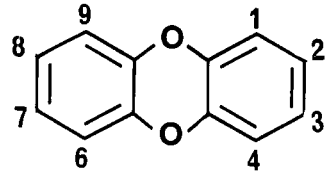

Figure 1. Structure of dibenzo-*p*-dioxin. Chlorines may be attached at the 1,2,3,4,6,7,8, and/or 9 positions to yield chlorodibenzo-*p*-dioxins

washed (petroleum ether) 15-g Al_2O_3 column (450 × 19 mm i.d.) and eluted with 100 ml of petroleum ether followed by 50 ml of 5% (v/v) diethyl ether in petroleum ether, which were discarded. One-hundred milliliters of 50% (v/v) diethyl ether in petroleum ether was collected, evaporated just to dryness, and adjusted to volume with hexane. The amount of each dioxin present was determined.

Step B. Low Organic Matter Soils, Phenol-Based Insecticides, Oil, and Ester Formulations of Phenoxy Herbicides. The following steps were performed prior to the Al_2O_3 column in Step A. The extract was shaken with concentrated H_2SO_4 until the acid was clear (not yellow or cloudy after standing *ca.* 10 min), washed with H_2O, and passed through a $NaHCO_3$–Na_2SO_4 column (1 cm of each layered in a 19 mm i.d. column). The eluant was evaporated to *ca.* 10 ml and continued with the Al_2O_3 column in Step A.

Step C. Chlorophenols, High Organic Matter Soils, and Other Samples Difficult to Clean Up. After completing parts A and B of the cleanup procedure, the eluant from the Al_2O_3 column was evaporated just to dryness, cooled to *ca.* 0°C on an ice bath, and 10 ml of 1:1 (v/v) HNO_3:H_2SO_4 mixture was added. The mix was gradually warmed to room temperature and added to 50 ml of ice water in a separatory funnel after 15 min. The beaker was rinsed with 5 × 10 ml of hexane. The combined hexane rinses were shaken for 1 min, washed with H_2O, and the hexane was drained through a $NaHCO_3$–Na_2SO_4 column. The volume was adjusted for analysis.

Quantitation was made on a 5% OV-225 column using electron-capture gas chromatography (ec-gc). Analysis was made with temperature programming from 230 to 260°C at 4°C/min and a 16-min hold at the final temperature.

Confirmation Techniques for Dioxins. If a positive response was found by ec–gc, the *p* value was determined for the dioxin between hexane and acetonitrile on a portion of the extract. The *p* value equals final concentration in hexane/initial concentration in hexane (Beroza and Bowman, 1965). Another portion of the extract was irradiated for 16 hr under a sun lamp (maximum output, 310 nm) at a distance of 30 cm since TCDD and the lower dioxins are destroyed under these conditions.

The remainder of the extract was condensed for confirmation on different gc columns using different detectors. The columns preferred were 1:1 (w/w) mix of 1.5% OV-17 plus 2.0% QF-1 and 5% UCW-98, both on Chromosorb W, 80–100 mesh. Flame ionization and microcoulometric detection were with these detectors. The ultimate confirmation was provided by a Perkin-Elmer Model 270 gc-mass spectrometer (gc–ms). There was very little fragmentation since the parent peak (*m/e* 322) was the parent ion and *m/e* 257 and 194 were the only fragments over 20% in relative abundance for the tetradioxin.

Two-dimensional tlc was used for cleanup and partial confirmation occasionally. The plate was developed first in acetonitrile followed by benzene. The spots were visualized under a uv lamp and were scraped off, extracted, and assayed by gc if desired. Infrared spectrometry was also used to confirm the identity of various chlorodioxins.

RESULTS AND DISCUSSION

Relative retention times of 13 dioxin isomers and a few selected chlorinated hydrocarbon insecticides are presented in Table I. The values were determined isothermally at

220°C. Because of the large value (rt_a = 46.31) for the octa-CDD, the 5% OV-225 column was normally used with temperature programming from 230 to 260°C at a rate of 4°C/min and a final temperature hold. Under these conditions, analysis was completed in about 40 min. The use of the three recommended columns (5% OV-225, 1.5% OV-17 plus 2% QF-1, and 5% UCW-98) prevented confusion of some chlorinated hydrocarbon insecticides in environmental samples with dioxins because relative retention times were sufficiently different.

p Values for dioxins are presented in Table II. Values for four specific isomers (2,7-, 2,3,7-, 2,3,7,8-, and 1,2,3,4,6,7,8,9-chlorodibenzo-*p*-dioxins) are given. Other values are for mixtures of isomers and were determined using total peak area or peak height. R_f values for all dioxin isomers by tlc in acetonitrile were 0.82 ± 0.08 and in benzene were 0.80 ± 0.02. The di-, tri-, tetra-, and hexadioxin were not separated from each other under our test conditions, but were separated from most interfering impurities in formulations. Care must be taken not to overload the spots or the material appears to stay on the origin. This behavior is presumably due to the low solubility of dioxins in most solvents.

In a further confirmatory step, dioxins were destroyed by uv light. The lower isomers (<4 Cl) were completely destroyed in 16 hr by irradiation (Crosby *et al.*, 1971) at concentrations less than 0.2 ppm in hexane. Nondestruction of the suspected di-, tri-, or tetrachlorodioxin is sufficient evidence that the peak is not a chlorodioxin. The higher the degree of chlorination, the more resistant the dioxins were to irradiation. Only 20% of the octa-CDD was destroyed in 16 hr at concentrations of about 0.3 ppm.

A total of 129 pesticide samples were analyzed for dioxins. Table III presents the occurrence and content of the dioxins in all samples. TCDD (tetra-) was found primarily in 2,4,5-T samples. Only one sample of silvex contained more than 0.5 ppm of TCDD. Detectable amounts of hexa-CDD were found in four 2,4,5-T samples, one 2,4-D, and two other samples other than the chlorophenols. Hepta- and octa-CDD also occurred in four other samples apiece in addition to their presence in chlorophenols. Most samples were made before 1970 and may not represent current production materials.

The TCDD concentration in selected pesticides is also presented in Table III. Twenty-two of 42 2,4,5-T samples collected contained less than 0.5 ppm of TCDD. Of the 20 samples containing more than 0.5 ppm of TCDD, 15 were obtained for the yearly survey of one manufacturer. The samples were from 1966–1970, with four samples usually collected each year. There was a tenfold drop in TCDD content by this manfacturer between 1968 and 1969. However, their technical 2,4,5-T still contained 2–3 ppm of TCDD in 1970. Four of the five remaining samples containing over 0.5 ppm of TCDD also came from this same manufacturer and were received from the PRD laboratories. Recent (1970) samples from another manufacturer contained <0.5 ppm of TCDD. No other samples tested had over 0.5 ppm of TCDD. Any sample could contain one or more different chlorodioxins.

The higher polychlorodibenzo-*p*-dioxin content of the same selected pesticides is also presented in Table III. Four samples of 2,4,5-T contained greater than 0.5 ppm of the hexa isomers. No higher dioxins were found in silvex or dicamba

Table II. *p* Values for Chlorodibenzo-*p*-dioxins in a 1:1 (v/v) Acetonitrile:Hexane Solvent System[a]

Isomer	*p* Value	std dev
2,7-CDD	0.76	±0.03
2,3,7-CDD	0.86	±0.03
2,3,7,8-CDD	0.51	±0.06
hexa-CDD	0.94	±0.03
hepta-CDD	0.90	±0.05
octa-CDD	0.90	±0.05

[a] Ratio of peak height in hexane solution before equilibration with hexane-saturated acetonitrile to peak height in hexane after equilibration.

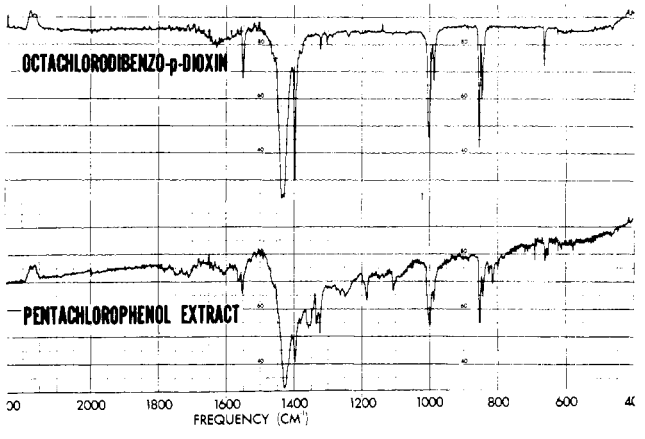

Figure 2. Infrared spectra of authentic octachlorodibenzo-*p*-dioxin and a cleaned-up pentachlorophenol extract. The material was incorporated in KBr and the spectra made on a Perkin-Elmer 625 infrared spectrometer

Table III. Number and Content of Polychlorodibenzo-*p*-dioxins in Selected Pesticides

Pesticide	ppm of -chlorodibenzo-*p*-dioxin: tetra <10	tetra <100	hexa <10	hexa <100	hexa <1000	hepta <10	hepta <100	hepta <1000	octa <10	octa <100	octa <1000	No. of samples contaminated	Total no. of samples tested
	Number found												
2,4,5-T	7[a]	13	3	1	0	ND[b]	...	...	ND	...	...	23	42
Silvex	1	0	ND	...	...	ND	...	...	ND	...	...	1	7
2,4-D (-DB,-DP)	ND	...	1	0	0	ND	...	...	ND	...	...	1	28
Dicamba	ND	...	ND	...	...	ND	...	...	ND	...	...	0	8
Chlorophenol													
tri-	ND	...	4	0	0	1	0	0	2	0	0	4	6
tetra-	ND	...	1	1	0	1	2	0	1	2	0	3	3
penta-	ND	...	0	7	0	0	4	6	0	4	6	10	11
Others	ND	...	1	0	1	3	0	1	3	1	0	7	24

[a] Any sample may contain one or more different dioxins. [b] ND = <0.5 ppm of any one chlorodioxin.

and only one sample of 2,4-D contained measurable amounts of the hexa isomers. The hexa isomers present presumably were there because of tetrachlorophenol impurities which condensed with each other. Higher dioxins were found in some samples of several other pesticides, including erbon, tetradifon, ronnel, sesone, and DMPA.

The dioxin content of the 20 chlorophenol samples is also presented in Table III. No TCDD was detected in any sample at levels above 0.5 ppm. However, the higher dioxins were plentiful. Trichlorophenol contained only small amounts of hexachlorodibenzo-*p*-dioxin and no sample contained over 10 ppm. Tetrachlorophenol contained less than 100 ppm of hexa-, hepta-, and octachlorodibenzo-*p*-dioxins, while six of 20 pentachlorophenol samples contained over 100 ppm of the hepta- and octachlorodibenzo-*p*-dioxin isomers. An infrared spectrum of a pentachlorophenol extract is presented in Figure 2. It is quite obvious that the extract contains octachlorodibenzo-*p*-dioxin when the spectrum is compared to the standard octachlorodibenzo-*p*-dioxin spectrum. Analysis by ec–gc indicated both hepta- and octachlorodioxins were present in the sample. This may account for the peak broadening. The reason for the high amounts of dioxin in chlorophenol is probably due to heat treatment during synthesis. As chlorination of phenol proceeds past the dichlorophenol stage, heat must be supplied in order to keep the reaction mixture in a melt condition. Since heat is being supplied in the presence of chlorophenols, the formation of higher chlorinated dibenzo-*p*-dioxins might be expected. The rate and time of melt heating probably governs the formation and amounts of the various dioxin isomers. If the chlorination temperature is raised too high too quickly, the lower chlorinated dioxins may be formed since the lower chlorophenols would be present and available for reaction with each other. If the temperature is raised too high after nearly all chlorophenol is in the penta form, octachlorodibenzo-*p*-dioxin would be the predominant impurity formed.

In conclusion, TCDD has been present at levels above 0.5 ppm in the past, but was less than 0.5 ppm in the current production samples examined. Higher chlorodioxins are present predominantly in chlorophenols with the highest amounts present in pentachlorophenol. Thirty-eight percent of all samples examined contained at least one chlorodioxin, with many containing more than one.

LITERATURE CITED

Beroza, M., Bowman, M. C., *J. Ass. Offic. Anal. Chem.* **48,** 358 (1965).
Cantrell, J. S., Webb, N. C., Mabis, A. J., *Acta Crystallogr.* **B25,** 150 (1969).
Courtney, K. D., Gaylor, D. W., Hogan, M. D., Falk, H. L., Bates, R. R., Mitchell, I., *Science* **196,** 864 (1970).
Crosby, D. G., Wong, A. S., Plimmer, J. R., Woolson, E. A., *Science* **173,** 748 (1971).
Schultz, K. H., *Arbeitsmedizin-Socialmedizin-Arbeitshygiene* **3,** 25 (1968).

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The Insecticidal and the Anticholinesterase Activity of *meta*-Acylamidophenyl and *meta*-Thioureidophenyl *N*-Methylcarbamates

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Fifty-nine new *N*-methyl and *N,N*-dimethylcarbamates were synthesized and tested as insecticides and as inhibitors of fly and bovine cholinesterases. In spite of the high anticholinesterase activity exhibited by many of these compounds, only few chemicals have shown insecticidal activity. Furthermore, these compounds could not be synergized effectively by 2,3-methylenedioxynaphthalene. Structure–activity correlation revealed that maximal anticholinesterase activity is associated with a definite size of the alkyl substituent on the amide or the thiourea grouping. In all series tested, alkyl substituent with four to six carbon atoms produced the most potent cholinesterase inhibitors.

The insecticidal properties of *N*-methyl and *N,N*-dimethylcarbamic esters of various alcohols and phenols have been demonstrated by several investigators (Gysin, 1954; Kolbezen *et al.*, 1954). A systematic evaluation of the structure–activity correlation of various aromatic carbamates was published in a series of papers by Metcalf, Fukuto, and coworkers (*e.g.*, Metcalf *et al.*, 1960, 1962; Metcalf and Fukuto, 1965). This paper presents a discussion of two new groups of insecticidal carbamates, the *meta*-acylamidophenyl and the *meta*-thioureidophenyl *N*-methylcarbamates.

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MATERIALS AND METHODS

Chemicals. The *meta*-acylamidophenyl *N*-methylcarbamates were prepared according to Leuckart (1890) by heating the corresponding *meta*-hydroxyacylanilide with methyl isocyanate. Anhydrous alcohol-free ethyl acetate was substituted for toluene as solvent, and triethylamine was used as a catalyst.

The *meta*-thioureidophenyl *N*-methylcarbamates were prepared *via meta*-hydroxyphenyl isothiocyanate; the latter was synthesized by the procedure of Dyson and George (1924). It is noteworthy that this hydroxyphenyl isothiocyanate reacts exothermically with methyl isocyanate in the absence of a catalyst, a behavior which had not been previously noted with nonalkaline phenols.