9. J. Luft, *J. Biochem. Biophys. Cytol.* **9**, 409 (1961).
10. A. Loud, *J. Cell Biol.* **37**, 27 (1968).
11. E. Weibel, W. Staubli, H. Gnagi, S. Hess, *ibid.* **42**, 68 (1969).
12. A. Jones, D. Schmucker, J. Mooney, R. Adler, R. Ockner, *Gastroenterology* **71**, 1050 (1976).
13. T. Peters, Jr., B. Fleischer, S. Fleischer, *J. Biol. Chem.* **246**, 240 (1971); H. Schachter, I. Sabbal, R. Hudgin, L. Pinteric, E. McGuire, S. Roseman, *ibid.* **245**, 1090 (1970); A. Koga, *Z. Anat. Entwicklungsgesch.* **135**, 156 (1971); A. Cooper, A. Jones, R. Koldinger, R. Ockner, *Gastroenterology* **66**, 574 (1974).
14. A. Jones, D. Schmucker, R. Adler, R. Ockner, J. Mooney, in *The Liver: Quantitative Aspects of Structure and Function*, R. Preisig, J. Bircher, G. Paumgartner, Eds. (Cantor, Aulendorf, West Germany, 1976), pp. 36–51.
15. L. Grinna and A. Barber, *Biochim. Biophys. Acta* **288**, 347 (1972); *Exp. Gerontol.* **10**, 319 (1975); D. Hawcroft and P. Martin, *Mech. Ageing Dev.* **3**, 121 (1974).

16. Supported in part by Research Section, VAH; NIH grant HL-16363-02; and Liver Center grant NIAMDD P-17-AM 18520.

11 March 1977; revised 9 May 1977

Combustion of Several 2,4,5-Trichlorophenoxy Compounds: Formation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Abstract. *Grass and paper coated with several compounds containing the 2,4,5-trichlorophenoxy moiety have been subjected to combustion. By using compounds that had been purified to achieve low background amounts of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) together with an efficient cleanup and analysis of the residue, it was possible to detect as little as 0.001 microgram of TCDD in the combustion products of 0.5 gram of the 2,4,5-trichlorophenoxy material. Small self-supported fires converted about 10^{-6} of the 2,4,5-trichlorophenoxy material to TCDD.*

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been used for the control of vegetation for more than 25 years. A contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been reported to persist and accumulate in the environment (*1*, *2*). It has also been suggested that the combustion or burning of vegetation sprayed with 2,4,5-T could result in the formation of TCDD (*2*). To accurately assess the potential hazards, if any, from the continued use of herbicides containing the 2,4,5-trichlorophenoxy linkage in areas that might be subject to burning, we conducted a series of combustion experiments. In each case we attempted to take advantage of the most appropriate technology and analytical methodology to separate any TCDD formed as a result of the combustion of the 2,4,5-trichlorophenoxy compound from that present as an impurity in the herbicides themselves. We used a multiple cleanup procedure followed by gas chromatography–mass spectrometry (GC–MS) for the determination of TCDD as a trace impurity in the herbicide and its formulations (*3*).

At a symposium on various aspects of chlorinated dibenzo-*p*-dioxins (*4*), several references were made to the formation of chlorinated dioxins by thermal condensations or rearrangements (*5*, *6*). Langer (*5*) reported that, in controlled heating experiments, 0.13 percent of 2,4,5-T was converted to TCDD when the potassium salt was heated at temperatures up to 400°C for up to 43 hours. Buu Hoi *et al.* (*2*) reported much higher amounts of TCDD formed when several 2,4,5-trichlorophenoxy species were pyrolyzed. However, some question remains regarding this latter work since the mass spectrum taken to indicate TCDD is not consistent with other published mass spectra (*7*). Nilsson *et al.* (*8*) have described pyrolysis experiments on chlorinated phenoxyphenols which resulted in the formation of up to 6 percent dioxin condensation product.

In our work, we have used conditions as "natural" as feasible: a cellulosic combustible material burning at its own rate with good air feed; an amount of the 2,4,5-trichlorophenoxy moiety applied to the surface at a concentration of 12 pounds of active ingredient per acre (13.5 kg/ha); an inert collection system to avoid the catalytic effects of walls and surfaces; and an analytical scheme that would permit examination of all the combustion products with high sensitivity and specificity. The combustion takes place in an open-bottom 500-ml flask with the combustible sample contained in a quartz mesh basket; a 15-cm petri dish is mounted below the basket to catch any small fragments of ash.

The combustion flask is connected to a series of four gas-absorber traps by means of glass ball-and-socket joints. The entire apparatus is constructed on the center line of the ball joints, so that any section or sections of the apparatus can be inverted by simple rotation. Gas trap A is cooled with an ice bath, and all other gas traps are cooled with Dry Ice. To attain good collection efficiency, each trap is filled with smooth glass beads (3 mm). Air is drawn through the system with a vacuum pump connected at the outlet of trap D. In the analysis we used low-resolution GC–MS with a cleanup procedure described by Hummel and Shadoff (*9*).

The 2,4,5-trichlorophenoxy materials used for the combustion were obtained either from regular production or from the Ag-Organics Research Laboratory of Dow Chemical Company. Each was purified to reduce the residual TCDD content by the cleanup described in (*3*) (ion exchange or silica gel), and then the matrix was recovered. Samples of grass from a field that had been treated with Esteron 245 (a registered Dow formulation of 2,4,5-T esters), at 12 pounds of 2,4,5-T equivalent per acre, were obtained immediately after spraying and also 1 week later. The Esteron 245 was not purified in the manner described above. These grass samples were frozen, ground, blended, and stored in polyethylene bags prior to analysis. The herbicides were dissolved in methanol or acetone and applied uniformly over the surface of laboratory filter paper (Whatman No. 1, acid-washed). A 5-g portion of grass was air-dried at a slight vacuum (500 torr) at 50°C for 1 hour, and the

Table 1. Combustion of material containing 2,4,5-trichlorophenoxy species.

Sample	TCDD produced* (μg)	TCDD conversion† (% by weight)
0.41 g of sodium 2,4,5-trichlorophenoxy acetate on 1350 cm^2 of paper	0.05	1.2×10^{-5}
0.125 g of 2,4,5-T on 450 cm^2 of paper	0.03	2.4×10^{-5}
0.46 g of butyl 2,4,5-trichlorophenoxy acetate on 1350 cm^2 of paper	0.2	4.3×10^{-5}
0.32 g of sodium 2,4,5-trichlorophenate on 1350 cm^2 of paper	0.2	3×10^{-5}
0.10 g of 2,4,5-trichlorophenol on 450 cm^2 of paper	0.05	5×10^{-5}
0.15 g of butyl 2,4,5-trichlorophenoxy acetate on 450 cm^2 of paper	0.05	3.3×10^{-5}

*After correction for the TCDD content of the trichlorophenoxy starting material: 0.1 part per billion (ppb), 2 ppb, and 6 ppb for 2,4,5-T, trichlorophenol, and butyl 2,4,5-trichlorophenoxy acetate, respectively. †Expressed as the percentage conversion of the 2,4,5-trichlorophenoxy species to TCDD.

sample was placed between two sheets of filter paper. The filter paper was then crumpled into a small, loose ball, placed in the apparatus, and ignited with a single match. The complete combustion of 450 cm^2 of paper required about 5 minutes; an additional 15 minutes was needed to cool to room temperature.

We extracted the combustion products in situ by closing the valve to the vacuum, inverting the combustion flask, adding 20 ml of 50 percent benzene in methanol, and warming the outside of the flask with an electric heat gun to reflux the solvent. After several minutes, the vacuum valve was opened slightly and the solvent drawn into trap A. Trap A was then heated gently to reflux and then inverted, and the contents were transferred to trap B. In this manner, the contents of each trap were extracted and transferred three successive times with the solvent. The final extracts were then subjected to gentle evaporation and the final cleanup and analysis.

Temperatures were measured with a thermocouple at two locations during combustion: (i) above the flame and at the top of the combustion flask where the temperature was 600° to 800°C and (ii) at the sides of the flask where the temperature was 350° to 600°C. There was some variability in the amount of smoldering and in the amount of "tars" produced and collected in trap A. No significant amount of interference was observed in the GC–MS signals recorded; we observed just a slightly elevated general base line, probably due to small amounts of a large number of carbonaceous materials not entirely removed during the cleanup.

Under these experimental conditions we observed that a small amount of TCDD was produced in addition to that present as a trace contaminant in the 2,4,5-trichlorophenoxy species. Table 1 summarizes the results of the combustion of several species. An equivalent amount of each compound was burned per unit surface area (0.5 mmole of the 2,4,5-trichlorophenoxy moiety per 450 cm^2, or 12 pounds of 2,4,5-T per acre); thus the amounts of TCDD formed are comparable between compounds on a molar and on a weight percent conversion basis.

Table 2. Combustion of grass treated with Esteron 245 herbicide (12 pounds per acre).

Sample (5 g)	2,4,5-T initially present* (μg)	TCDD produced† (μg)	TCDD conversion‡ (% by weight)
Sampled immediately after treatment	5700	0.009	0.00016
Sampled 1 week after treatment	3400	0.005	0.00015

*Expressed 2,4,5-trichlorophenoxy acetic acid. †After correction for the TCDD present before burning: 0.002 μg and 0.0007 μg for the 0-day and 1-week sample, respectively. ‡Expressed as the percentage conversion of the 2,4,5-T species to TCDD.

Table 3. Recovery of TCDD from combustion apparatus.

Paper sample (cm^2)	TCDD (μg)		Comments
	Added	Found	
900	0.001	0.0012	TCDD added to extracted combustion products*
1350	0.001	0.0012	TCDD added to apparatus walls before extraction†
900	None	n.d.‡	

*TCDD added to an extract of the collected combustion products. †TCDD added to the apparatus after combustion. The apparatus was then heated to 600°C and air was pulled through for 10 minutes. ‡Not detected at a detection limit of 0.00015 μg of TCDD.

Table 2 illustrates the results of a similar series of experiments in which we used grass that had been treated with Esteron 245 herbicide. We analyzed the grass for both 2,4,5-T and TCDD before burning. The grass was then subjected to the same combustion process already described. The last column in Table 2 shows the fraction of 2,4,5-T converted to TCDD. Perhaps the most meaningful way to express these conversions is in parts per trillion (ppt) of TCDD formed per parts per million (ppm) of 2,4,5-T burned. The average of all experiments is 0.6 ppt of TCDD formed per 1 ppm of 2,4,5-T burned.

Although these experiments do not duplicate all possible burning conditions, they strongly suggest that only a very small fraction of 2,4,5-T (or other 2,4,5-trichlorophenoxy species) is converted to TCDD. When these data are combined with other observations on the degradation of both large and small amounts of 2,4,5-T and TCDD (*10*), the TCDD burden added to the environment by the combustion of natural materials treated with 2,4,5-trichlorophenoxy species is no larger than 1 ppt of TCDD per ppm of 2,4,5-T residue burned.

As a test of the efficiency of our apparatus for the collection and recovery of any TCDD formed during combustion, we carried out several controlled additions of TCDD (Table 3). When 0.001 μg of TCDD was added to the extracted combustion products, it was completely recovered. The use of the methanol-benzene mixture facilitates the extraction of TCDD from these oxidized, tarry materials. In another experiment, 0.001 μg of TCDD was deposited on the inside walls of the combustion flask after the combustion of three circles of filter paper. The combustion flask was then heated with a heat gun to a wall temperature at 650°C while air was drawn through the apparatus. As before, all of the added TCDD was recovered (Table 3). The absence of any interferences from either the paper or earlier combustions was demonstrated by the combustion and analysis of two circles of blank, untreated paper. Under these conditions, the detection limit for TCDD was about 0.00015 μg. The high recovery of TCDD (100 percent for 0.0010 μg of TCDD) and the consistent results obtained suggest that this type of apparatus is extremely useful for the accurate examination of the combustion products of a variety of chemicals (*11*).

R. H. STEHL
L. L. LAMPARSKI

Analytical Laboratories,
Dow Chemical U.S.A.,
Midland, Michigan 48640

References

1. R. W. Baughman and M. Meselson, paper presented at the 162nd national meeting of the American Chemical Society, Washington, D.C., September 19[illegible] D. G. Crosby, K. W. Moilanen, A. S. Wong, *Environ. Health Perspect.* **5**, 259 (1973); G. Saint-Ruf, *Naturwissenschaften* **59**, 648 (1972); *Food Chem. News* **13** (No. 47), 20 (1972).
2. N. P. Buu Hoi, G. Saint-Ruf, P. Bigot, M. Mangane, *C. R. Acad. Sci. Ser. D* **273**, 708 (1971).
[illegible] W. B. Crummett and R. H. Stehl, *Environ. Health Perspect.* **5**, 15 (1973).
4. See the September issue of *Environ. Health Perspect.* **5** (1973).
[illegible] H. Langer, *ibid.*, p. 3.
[illegible] A. S. Kende and J. J. Wade, *ibid.*, p. 49.
7. F. P. Boer, M. A. Neuman, F. P. van Remoortere, P. P. North, H. W. Rinn, *J. Am. Chem. Soc.* **94**, 1006 (1972).
8. C. A. Nilsson, K. Andersson, C. Rappe, S. Westermark, *J. Chromatogr.* **96**, 137 (1974).
9. R. A. Hummel and L. A. Shadoff, paper presented at the 170th national meeting of the American Chemical Society, Chicago, September 1975.
10. A. L. Young, "Ecological studies on a herbicide-equipment test area" (report AFATL-TR-74-12, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1974); R. H. Stehl, R. R. Papenfuss, R. A. Bredeweg, R. W. Roberts, paper presented at the 162nd national meeting of the American Chemical Society, Washington, D.C., September 1971.
11. J. H. Petajan, K. J. Voorhees, S. C. Packham, R. C. Baldwin, I. N. Einhorn, M. L. Grunnet, B. G. Dinger, M. M. Birky, *Science* **187**, 742 (1975).

1 September 1976; revised 29 June 1977