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Analysis of some older Scandinavian formulations of 2,4-dichlorophenoxy acetic acid and 2,4,5-trichlorophenoxy acetic acid for contents of chlorinated dibenzo-\(p\)-dioxins and dibenzofurans

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The polychlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs) are two classes of compounds that have caused much concern during recent years. They are known as highly stable contaminants in chlorinated phenols, phenoxy acids and in polychlorinated biphenyls (PCBs). In all, 75 and 135 positional isomers exist for the PCDDs and PCDFs, respectively.

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The dioxy problem was first observed in connection with the herbicide spraying program in Vietnam in the late 1960s. The 2,3,7,8-Tetra-CDD, the most toxic dioxin isomer, has been frequently found in 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) formulations. This dioxin is one of the most toxic compounds ever studied, with an LD_{50} (median lethal dose) value of 0.6 \(\mu\)g/kg (guinea pig). Several other isomers are also very toxic, but the toxicity is highly dependent on the structure of the isomer. 2,3,7,8-Tetra-CDD has been by far the most carefully investigated isomer, and it has also been found to have chronic, as well as teratogenic, mutagenic and carcinogenic, effects (2, 7, 8, 11).
formulation used there, a 1:1 mixture of butyl esters of 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-T (Herbicide Orange), was found to contain 0.1—47 μg/g of 2,3,7,8-tetra-CDD (3). Smaller amounts of other less toxic PCDDs and PCDFs have been reported by Rappe et al. (10). As a result of governmental regulations, efforts were made during the 1970s to minimize the formation of 2,3,7,8-tetra-CDD, and nowadays most producers of 2,4,5-T claim that their products contain less than 0.1 μg/g.

Concerning the situation prior to the late 1960s, Rappe et al. (10) have reported the results of analyses of five European 2,4,5-T ester formulations with production dates between 1962 and 1967. These formulations were used in Finland. The expected 2,3,7,8-tetra-CDD was by far the most abundant PCDD isomer, and it was present at levels of 0.1—0.9 μg/g; in one of the samples it was under the detection limit (0.01—0.05 μg/g).

Several incidents have been reported where workers were more or less seriously affected due to occupational exposure to PCDDs and PCDFs during the industrial preparation or handling of chlorophenols, phenoxy acids, and PCBs (7,8). The most typical symptoms are chloracne and liver damage. The heaviest exposures are associated with explosions and other accidents in plants producing 2,4,5-trichlorophenol, an intermediate in the production of 2,4,5-T. Data recorded from one of these accidents suggest an increase of carcinomas in exposed workers (12).

The phenoxy esters have been widely used in Scandinavia for spraying forests to control unwanted hardwood; this practice goes back to the end of the 1940s. In addition the amine salts of phenoxy acids have also been used for notching (1).

In case-control studies (4,5,6) Hardell found an increased risk for both malignant mesenchymal sarcomas and malignant lymphomas among Swedish workers exposed to phenoxy herbicides during the 1950s and 60s. It was not possible to determine whether this carcinogenic effect was exerted by these compounds per se or by PCDDs or PCDFs that can be found as impurities in the commercial formulations.

The purpose of this investigation was to study this problem. Some of the 2,4,5-T and 2,4-D formulations used in Sweden in the 1950s and early 60s have been analyzed for 2,3,7,8-tetra-CDD and other PCDDs and PCDFs.

METHODS

Sample preparation

One-half a gram of each sample was added, in n-hexane, to a silica column (150 × 11 mm, silica gel, Merck) and eluted with 30 ml of 5 % methylene chloride in n-hexane. The eluate was concentrated on a Kuderna-Danish evaporator, and any aromatic solvent possibly present was blown off with a stream of nitrogen. The residue was rechromatographed on an alumina microcolumn (1.0 g basic alumina, Woelm, in a 150 x 5 mm disposable Pasteur pipette) with 2 % and 50 % methylene chloride in n-hexane. The last fraction was concentrated to 100 μl, and an aliquot was used for the analysis. Recoveries of known quantities (0.125 ppm) of a tetra-CDD and a penta-CDF were in all cases > 80 % when they were added to some samples.

Gas chromatography/mass spectrometry

A 50-m Silar 10 c glass capillary column (inner diameter 0.35 mm) was coupled to a Finnigan 4000 Quadrupole mass spectrometer. The detailed experimental conditions have been described elsewhere (2). Splitless injection of the samples was carried out with the vaporizer at 275°C. The column temperature was kept at 100°C for 2 min and then programmed with 20°C/min to 180°C and with 5°C/min to 240°C. Complete electron impact mass spectra were recorded with a Finnigan 6111 data system (m/e 35—300, 1.4 s/scan). After data acquisition, we searched the spectra for PCDDs and PCDFs by running specific mass chromatograms and estimated the
Table 1. Amounts of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in the 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 2,4-dichlorophenoxy acetic acid (2,4-D) formulations analyzed (μg/g formulation). [— = not analyzed, n.d. = < 0.05 μg/g (detection limits = 0.01—0.05 μg/g)]

| Sample and production date | PCDDs |    |    | PCDFs |    |    | Other compounds detected |
|----------------------------|-------|----|----|-------|----|----|-------------------------|
|                            | Di-   | Tri | Tetra | Penta | Hexa | Di- | Tri | Tetra | Penta | Hexa | detected  |
| 1  Unknown before 1964     | n.d.  | n.d. | n.d.  | n.d.  | —   | n.d. | n.d. | n.d.  | n.d.  | n.d.  | DDE     |
| 2  2,4-D-ester before 1964 | n.d.  | n.d. | n.d.  | n.d.  | —   | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 3  2,4,5-T-acid, 1952      | n.d.  | n.d. | 1.10* | n.d.  | —   | 0.06 | 0.10 | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 4  2,4,5-T, unknown        | n.d.  | n.d. | 0.50* | n.d.  | n.d. | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 5  2,4,5-T, 99 %           | n.d.  | n.d. | n.d.  | n.d.  | —   | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 6  2,4-D-ester before 1960| n.d.  | n.d. | n.d.  | n.d.  | —   | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 7  2,4-D, 1964             | n.d.  | n.d. | 0.40* | n.d.  | n.d. | 0.06 | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 8  2,4,5-T, 1960           | n.d.  | n.d. | 0.05  | n.d.  | n.d. | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 9  2,4-D, 1965             | n.d.  | n.d. | 6.1   | n.d.  | n.d. | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |
| 10 2,4,5-T, United States  | 0.05  | 6.1*| n.d.  | n.d.  | n.d. | n.d. | n.d. | n.d.  | n.d.  | n.d.  | Cl₂-CDPE |

a 2,3,7,8- isomer.
b 2,3,7,8- (6.0 μg/g) plus 1,3,7,8- (0.1 μg/g) isomers.
c CDPE = chlorinated diphenyl ether.

amounts of these compounds by comparing with runs of known quantities of isomeric pure compounds used as standards. Detection limits under these conditions were 0.01—0.05 ppm for the di- to hexachloro isomers.

RESULTS AND DISCUSSION

Table 1 presents the amounts of PCDDs and PCDFs found in the 10 samples analyzed. In one case (sample 9) it was not possible to analyze for lower chlorinated isomers due to disturbing interference from solvent residues. As no tetrachloro or higher chlorinated isomers were found in this sample, no further attempts were made in additional clean-up. The most contaminated sample (number 10), from the United States, was possibly prepared by the high temperature method for the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene (9). The amount of contaminants found in this and other samples (4, 5 and 8) was of the same order of magnitude as was earlier found by Rappe et al. in European samples (10).

The observation of traces of DDE (a DDT metabolite) was unexpected. They were observed in samples collected after having been used in forestry, and they could have been unintentionally contaminated during handling in the forests. In the evaluation of the previously mentioned epidemiologic studies of Hardell et al. (4, 5, 6), it was not possible to determine whether the carcinogenic effect was exerted by the phenoxy acids per se or by the PCDDs or PCDFs that can be found as impurities. Although this report is by no means a complete study of the older phenoxy acids used in Sweden, it is still an indication that the phenoxy acids per se are the reason for the increased levels of sarcomas and lymphomas among sprayers heavily exposed to these herbicides.

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