HPLC Measurement of Chlorophenoxy Herbicides, Bromoxynil, and Ioxynil, in Biological Specimens to Aid Diagnosis of Acute Poisoning

R. J. Flanagan and M. Ruprah

A simple high-performance liquid chromatographic assay for eight chlorophenoxy (2,4-D and related compounds) and two benzonitrile (bromoxynil and ioxynil) herbicides has been developed to aid in the diagnosis of acute poisoning. Sample (whole blood, plasma/serum, urine, or tissue homogenate) or standard (100 μL) is vortex-mixed (ca. 5 s) with 20 μL of internal standard solution [1.00 g/L L 2,4,5-TP in 0.2 mol/L Tris buffer, pH 9.6: methanol (1:1)]. Dilute (0.2 mL/L) hydrochloric acid in methanol, 200 μL, is added and the mixture is again vortex-mixed (30 s). After centrifugation (9950 × g, 2 min) a 10–20 μL portion of the supernate is analyzed on a 250 × 5 mm (i.d.) Spherisorb S5 Phenyl column, with aqueous potassium dihydrogen orthophosphate (50 mmol/L, pH 3.5) and acetonitrile (3 to 1 by vol) at a flow-rate of 1.8 mL/min as eluent. The method is capable of resolving the chlorophenoxy/benzonitrile mixtures (2,4-D/MCPP, 2,4-D/DCPP, 2,4-D/ioxynil, 2,4-D/MCPD/DCPP, 2,4-D/2,4,5-T, and MCPD/ioxynil) encountered in the U.K. The limit of detection (at 240 nm) is 20 mg/L (10 mg/L for bromoxynil and ioxynil). Intra-assay and interassay CVs were <5% and <8%, respectively, for all analytes. Plasma/whole blood distribution ratios ranged from ca. 1.7 for 2,4-DB to ca. 2.0 for 2,4-D, emphasizing that results of whole-blood measurements must be multiplied by a factor of ca. 2 for comparison with plasma/serum data.

Additional Keyphrases: plasma/whole blood distribution ratios · chlorophenoxy herbicides—acute poisoning · benzonitrile herbicides—acute poisoning · urine · tissue analysis

Chlorophenoxy acid herbicides such as 2,4-D, 2,4,5-T, and MCPA (Table 1) are used to control broad-leaved weeds and, at higher application rates, for total vegetation control. They are formulated as esters, metal salts, or alkylamine salts, either alone or, more commonly, together with other (chlorophenoxy) pesticides, and are supplied in organic or aqueous solution or in powder form, some of which may contain up to half (by weight) of active ingredient. In humans, ingestion of the concentrates can cause irritation and burning of the buccal mucosa, nausea, vomiting, abdominal pain, pyrexia, confusion, hypotonia, hypotension, coma, metabolic acidosis, convulsions, and renal and muscle damage (1–5), and several fatalities have been reported (6–14). These compounds are strongly acidic (Table 1) and are largely excreted unchanged; alkaline diuresis can enhance the clearance of 2,4-D and possibly of other chlorophenoxy compounds (3).

Ioxynil (4-hydroxy-3,5-diodobenzoazinitrile) and bromoxynil (3,5-dibromo-4-hydroxybenzoazinitrile) have herbicidal properties similar to those of the chlorophenoxy. However, the principal action of the benzonitriles is to uncouple oxidative phosphorylation and thus the symptoms of acute poisoning are similar to those produced by other uncouplers such as pentachlorophenol and dinitro-o-cresol (DNOC): fatigue, excessive sweating, thirst, pyrexia, anxiety, tachycardia, and hyperventilation. Such symptoms, which resolved upon cessation of exposure, were noted in four patients employed in the manufacture of these compounds (15), and fatalities attributable to acute ioxynil poisoning have also been reported (16, 17).

It is important to use simple, selective methods to measure chlorophenoxy herbicides and related compounds in biological samples, because they are often formulated with each other or with other pesticides. In addition, emergency assays can be useful in management, because alkaline diuresis may be used in treatment. Some methods for chlorophenoxy herbicides have involved solvent extraction followed by ultraviolet spectrophotometry, directly (7) or after thin-layer chromatographic separation (10), or gas-liquid chromatography (GLC) after methylation (3, 18–20) or ethylation (21). However, ultraviolet spectrophotometric assays are nonselective, whereas GLC methods, although giving excellent sensitivity for residue analysis, are lengthy and may present problems, because additional products may be formed during derivatization (22).

HPLC clearly offers advantages in analysis for polar compounds such as the chlorophenoxy and benzonitrile herbicides, and various methods for measurement of these compounds have been described (22–26). However, these procedures were designed primarily for residue analysis, and there remains a need for simple methodology for use in the diagnosis of acute poisoning. The HPLC method described here offers advantages of speed, simplicity, small sample requirement, and sensitivity adequate to detect these compounds at the concentrations in plasma attained.

Table 1. Simplified Nomenclature and pK₂ Values of the Chlorophenoxy and Benzonitrile Herbicides Studied

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical name</th>
<th>pK₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromoxynil</td>
<td>3,5-Dibromo-4-hydroxybenzonitrile</td>
<td>4.1</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>2.6</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>4-(2,4-Dichlorophenoxy)butyric acid</td>
<td>4.8</td>
</tr>
<tr>
<td>DCPP (2,4-DP; Dichlorprop)</td>
<td>2-(2,4-Dichlorophenoxy)propionic acid</td>
<td>3.0</td>
</tr>
<tr>
<td>2,4,5-TP (Fenoprop)</td>
<td>2-(2,4,5-Trichlorophenoxy)propionic acid</td>
<td>2.8</td>
</tr>
<tr>
<td>Ioxynil</td>
<td>4-Hydroxy-3,5-diodobenzoazinitrile</td>
<td>4.0</td>
</tr>
<tr>
<td>MCPA</td>
<td>4-Chloro-2-methylphenoxyacetic acid</td>
<td>3.1</td>
</tr>
<tr>
<td>MCPB</td>
<td>4-(4-Chloro-2-methylphenoxy)butyric acid</td>
<td>4.8</td>
</tr>
<tr>
<td>MCPY (Mecoprop)</td>
<td>2-(4-Chloro-2-methylphenoxy)propionic acid</td>
<td>3.8</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>2,4,5-Trichlorophenoxyacetic acid</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Footnotes:
1. Chemical names are spelled out in Table 1.
2. Received March 20, 1989; accepted March 22, 1989.
after oral ingestion of the concentrates. Use of a phenyl-
silyl-modified silica column with an aqueous buffer:acetoni-
trile (3 + 1) eluent can resolve most commonly encoun-
tered mixtures of these compounds within 10 min.

Materials and Methods

Materials and Reagents

The chlorophenoxy herbicides we studied (Table 1) were
obtained as the free acids from FBC (Haughton, U.K.) and
bromoxynil and ioxynil were from May & Baker (Dage-
ham, U.K.). Methanol and acetonitrile were “HPLC”
grade (Rathburn, Walkerburn, U.K.) and potassium dihy-
donate orthophosphate, disodium hydrogen orthophos-
phate, tri(hydroxymethyl)methylamine (Tris), and con-
centrated hydrochloric acid were analytical reagent grade
(BDH, Poole, U.K.). Subtilisin A was from Novo Enzyme
Systems (Farnham, U.K.). Buffers used were aqueous po-
tassium dihydrogen orthophosphate/disodium hydrogen or-
throposphate (67 mmol/L, pH 7.4; the “phosphate buffer”) and
aqueous Tris (20 mmol/L, pH 9.6; the “Tris buffer”).
2,4,5-TP (Table 1) was normally used as the internal
standard as a 1.00 g/L solution in equilibrium Tris buffer:
methanol.

High-Performance Liquid Chromatography

A constant-flow reciprocating pump (Applied Chroma-
tography Systems, Model 750/04) was used with a syringe-
loading valve (Rhodyne, Model 7125, 20-μL loop). A stain-
less-steel column (250 × 5 mm i.d.) packed with Spherisorb
S5 Phenyl (Hichrom, Reading, U.K.) was used at ambient
temperature with aqueous potassium dihydrogen ortho-
ophosphate (0.05 mol/L, pH 3.5):acetonitrile (3 + 1) at a
flow-rate of 1.8 mL/min as eluent. The column effluent was
monitored by ultraviolet absorption at 240 nm (Applied
Chromatography Systems, Model 750/11). The eluent was
degassed daily with helium before use and recirculated into
the eluent reservoir.

Sample Preparation

Sample (whole blood, plasma/serum, urine, or tissue
homogenate) or standard (100 μL) was vortex-mixed (ca.
5 s) with internal standard solution (20 μL) in a 60 × 5 mm
(i.d.) glass test tube (Drewey tube; Samco, Old Woking,
U.K.). Methanolic hydrochloric acid (2 mL of concd. HCl
per liter, 23 mmol/L), 200 μL, was added and the mixture
again vortex-mixed for 30 s. After centrifugation (9950 × g,
2 min), a 10–20 μL portion of the supernate was taken for
analysis.

Hamilton gas-tight luer-fitting syringes (1.0- and 10.0-
ml volume) fitted with stainless-steel needles were used to
add the internal standard and hydrochloric acid solutions,
respectively. The syringe used to add the hydrochloric acid
was rinsed with methanol after use. Samples were
analyzed in duplicate. Tissue specimens (duplicate ca.
100-mg portions) were analyzed after incubation (60 °C,
4 h) with subtilisin A (ca. 50 mg) and phosphate buffer (100
μL).

Assay Calibration and Analytical-Recovery Studies

Assay calibration was by analysis of standard solutions
containing 50, 100, 200, and 400 mg of each compound per
liter (25, 50, 100, and 250 mg/L for bromoxynil and ioxynil)
prepared in Tris buffer or (bromoxynil, ioxynil) equine
serum (Gibco, Paisley, U.K.). These solutions were pre-
pared by dilution from 5.00 g/L stock solutions of each
compound in methanol. Blood samples containing analyte
concentrations outside the calibration range were diluted
with equine serum and re-analyzed. Dilution with de-
ionized water was similarly used with urine or tissue
homogenates when necessary. Standard solutions of the
chlorophenoxy herbicides prepared in equine serum in the
range 0.10–0.40 g/L by dilution from the 5.00 g/L metha-
nolic stock solutions were used as quality-assurance spec-
mens and in analytical-recovery studies.

Plasma/Whole Blood Ratios

Solutions of each compound (Table 1) were prepared in
heparinized whole blood (hematocrit ca. 50%) freshly ob-
tained from a healthy volunteer. Analyte concentrations of
0.10 to 0.40 g/L (bromoxynil and ioxynil: 0.05 and 0.10 g/L)
were prepared by accurately measuring the required vol-
ume of 5.00 g/L methanolic stock solution into a 10-mL
volumetric flask, evaporating the methanol under a stream
of compressed air, and adding the required volume of blood.
After stirring (ca. 2.5 h, ambient temperature) on a roller
mixer, a 5-mL portion of each specimen was taken, and the
plasma was separated by centrifugation. The remaining
portion of whole blood was stored at −5 to −20 °C for 16 h
to ensure complete hemolysis. The plasma and whole blood
analyte concentrations were measured subsequently as
described above.

Clinical Specimens

Samples of blood (10 mL, heparinized), urine (20 mL),
and liver, kidney, or brain (50–100 g) were requested from
patients referred during 1984–87 to the Poisons Unit,
Guy's Hospital, who were thought to have ingested chloro-
phenoxy or benzonitrile herbicides. Specimens from ad-
ditional patients were analyzed if poisoning with these com-
ounds was thought to be a possibility. In each case, clinical
information was obtained from discharge summaries,
postmortem reports, or from the inpatient notes.

Results and Discussion

Choice of Chromatographic and Detection Conditions

Initially, we found that many of the compounds of inter-
est were resolved on an octadecylsilyl-modified silica col-
umn (125 × 5 mm i.d., Zorbax ODS; Du Pont) with an
aqueous sodium acetate (5 g/L)glacial acetic acid (0.5 mL/
L):methanol (1 + 1) eluent. However, 2,4-D and MCPP
were not resolved from ioxynil and DCPP, respectively.
These pairs of compounds could be resolved by adjusting
the pH of the aqueous portion of the eluent from 6 to 4, but
resolution was lost in other areas, and 2,4-DB and MCPP
were still retained relatively strongly. In contrast, with the
phenylisilyl-modified column we could measure ioxynil in
the presence of the commonly occurring chlorophenoxy
(Figure 1) and both 2,4-DB and MCPP eluted at reasonable
retention times (Table 2). However, 2,4-D and 2,4-DB were
not separated from MCPA and MCPP, respectively, and
2,4,5-T was not resolved from DCPP and MCPP. Additional
mobile phase and (or) column combinations (22–25) would
thus be needed should these compounds be present to-
gether.

The chlorophenoxy herbicides have ultraviolet absorp-
tion maxima at ca. 280 nm and minima at ca. 252 nm, but
show good absorption at ca. 240 nm and below (Figure 2).
In contrast, the benzonitriles have relatively weak maxima at
c.a. 285 nm, moderate absorption at ca. 254 nm but, again,
good absorption below 254 nm (ioxynil) and 240 nm (bromoxynil) is evident (Figure 2). The HPLC detection wavelength used routinely, 240 nm, was thus aimed at maximizing the response for the compounds studied while maintaining a moderate degree of selectivity. However, a detection wavelength of ca. 280 nm clearly could be used if required in the analysis for the chlorophenoxy compounds, with little loss of sensitivity and possibly a gain in selectivity. Bromoxynil and ioxynil are also amenable to electrochemical oxidation detection, because they possess a phenolic hydroxyl function, and this property has been exploited to give good sensitivity in residue analysis (28).

No interference from endogenous compounds or from drugs or other pesticides has been observed in the samples analyzed during four years (Figure 3); sample analyses performed without adding the internal standard (2,4,5-TP) have not revealed the presence of co-eluting compounds. However, if 2,4,5-TP should be present in specimens to be analyzed then an alternative chlorophenoxy compound such as MCPA could be used as the internal standard. Although retention data for a number of pesticides, drugs, and analogs of 2,4-D have been generated (Table 2), interference from many of these sources is unlikely, either because they absorb poorly or not at all at 240 or 280 nm or because the concentrations in plasma that are associated with serious toxicity are relatively low.

The column was found to be relatively stable, and flushing it with pure acetonitrile or methanol after use helped to maintain performance. Acidified methanol was more effective than methanol alone in precipitating plasma protein, and this probably helped prolong column life, although cleaning and re-packing of the inlet frit and the top of the column bed, respectively, were occasionally required. Acidified acetonitrile could also be used to precipitate plasma protein, but this has not been investigated as yet. A 125 × 5 mm (i.d.) column packed with Lichroprep 60 Si (E. Merck) was used in series between the pump and the injection valve, with the aim of pre-saturating the eluent with silica and thus minimizing the risk of dissolution of the analytical column, but simple recirculation of the column effluent appeared to be just as effective. Similarly, a 50 × 2 mm (i.d.) column packed with Lichroprep 60 Si was positioned between the injection valve and the analytical column initially, but it did not appear to prolong greatly the life of the analytical column.

**Assay Calibration and Reproducibility**

On analysis of the calibration solutions, the ratio of the peak height of each analyte to the peak height of the internal standard, when plotted against analyte concentration, was linear over the range studied (Table 3). Equine serum was used in preparing standards for bromoxynil and ioxynil, because these compounds are not sufficiently soluble in water. Results obtained on analysis of such equine-
Standards

Gradient

Fig. 2. Ultraviolet absorption spectra of 2,4-D, MCPP, bromoxynil, and ioxynil in HPLC eluent [aqueous potassium dihydrogen orthophosphate (50 mM/L, pH 3.5)/acetonitrile (3 + 1)]

Fig. 3. Chromatograms obtained by using the chlorophenoxy and benzonitrile herbicide assay. Sample preparation: plasma (100 μL) vortex-mixed (ca. 30 s) with internal standard solution (1.00 g/L 2,4,5-TP, 20 μL) + methanolic hydrochloric acid (2 mL/L, 200 μL) and centrifuged (9950 × g, 2 min). Injection: 20 μL supernate. Chromatographic conditions: as in Fig. 1.

Samples: (A) analyte-free human plasma, (B) 1 + 1 dilution with analyte-free human plasma of a plasma sample from a patient who had ingested 2,4-D & DCPP (plasma concentrations 0.23 and 0.64 g/L, respectively), and (C) plasma from a patient who had ingested MCPP and ioxynil (plasma concentrations 0.15 and 0.088 g/L, respectively). Peaks: (1) 2,4-D, (2) DCPP, (3) MCPP, (4) 2,4,5-TP, (5) ioxynil

serum-matrix chlorophenoxy standards were similar to those given by the Tris buffer calibration solutions (Table 3).

The intra- and interassay CVs obtained on replicate analysis of standard solutions prepared in equine serum were less than 5% and less than 8%, respectively, for the analytes studied (Table 4). The limits of detection were 20 mg/L for the chlorophenoxy compounds and 10 mg/L for bromoxynil and ioxynil.

Stability Studies

Standard solutions of the chlorophenoxy compounds prepared in de-ionized water or in water:methanol (1 + 1) showed some decomposition after five to six weeks at room temperature. This problem was overcome by preparing solutions of these compounds in Tris buffer:methanol (1 + 1), the internal standard solution then being stable for at least a year at ambient temperature, and the calibration solutions stable for at least six months at 2 to 8 °C. The standard equine-serum-matrix chlorophenoxy, bromoxynil, and ioxynil solutions were stable for at least a year at −5 to −20 °C.

Plasma:Whole Blood Ratios

An initial experiment with 2,4-D standards in hemolyzed whole blood and plasma (prepared as described under Materials and Methods) gave a plasma:whole blood ratio of 2.27 at 0.10 g/L, decreasing to ca. 1.9 at higher concentra-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration, g/L</th>
<th>Intra-assay CV, %</th>
<th>Interassay CV, %</th>
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<tr>
<td>2,4-D</td>
<td>0.05</td>
<td>3.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>3.2</td>
<td>5.2</td>
</tr>
<tr>
<td>DCPP</td>
<td>0.05</td>
<td>2.9</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>4.2</td>
<td>8.3</td>
</tr>
<tr>
<td>Ioxynil</td>
<td>0.05</td>
<td>2.9</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>MCPP</td>
<td>0.05</td>
<td>3.6</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>3.3</td>
<td>4.5</td>
</tr>
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<td></td>
<td>0.40</td>
<td>2.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* n = 10 in each case.

Table 3. HPLC of Chlorophenoxy and Benzonitrile Herbicides: Assay Calibration and Recovery Data

Table 4. HPLC of Chlorophenoxy Herbicides and Ioxynil: Assay Reproducibility

Table 5. HPLC of Chlorophenoxy Herbicides: Assay Reproducibility

* MCPP (1.00 g/L) in Tris buffer:methanol (1 + 1) used as the internal standard solution. All calibration points are mean of duplicates.
Table 5. Plasma:Whole Blood Ratios of Chlorophenoxy and Benzonitrile Herbicides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration measured, g/L*</th>
<th>Plasma:whole blood ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Whole blood</td>
</tr>
<tr>
<td></td>
<td>g/L</td>
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</tr>
<tr>
<td>Bromoxynil</td>
<td>0.050</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>0.102</td>
</tr>
<tr>
<td>2,4-D (I)</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.19</td>
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<td>0.30</td>
<td>0.32</td>
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<td></td>
<td>0.40</td>
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<td>2,4-D (II)</td>
<td>0.10</td>
<td>0.09</td>
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<td>0.11</td>
</tr>
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<td>0.40</td>
<td>0.40</td>
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<tr>
<td>Ioxynil</td>
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<tr>
<td></td>
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<td>0.095</td>
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<tr>
<td></td>
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<td>0.39</td>
</tr>
<tr>
<td>MCPP</td>
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<td></td>
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<tr>
<td>2,4,5-T</td>
<td>0.10</td>
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</tr>
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<td></td>
<td>0.40</td>
<td>0.39</td>
</tr>
<tr>
<td>2,4,5-TP</td>
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<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* Values are mean of duplicates.

Ratios of 2.11 at 0.10 g/L and 1.80 at 0.40 g/L were obtained in a second experiment with 2,4-D (Table 5). The results obtained with the other chlorophenoxy compounds were similar in that the ratio was generally higher at lower analyte concentrations (Table 5), the lowest absolute values (1.82 and 1.60 at 0.10 and 0.40 g/L, respectively) being obtained with 2,4-DB. Ioxynil and bromoxynil gave similar results, although lower analyte concentrations (50 to 100 mg/L) were studied.

Only slight hemolysis occurred when we prepared the standards in whole blood, although this was more noticeable at higher concentrations. Nevertheless, these data, obtained by using blood with a hematocrit of ca. 50%, emphasize that the compounds under study are largely distributed in blood plasma. Thus, the results of whole-blood analyses must be multiplied by a factor of ca. 2 to facilitate comparison with plasma/serum data, and vice versa.

Results of Analyses of Patients' Samples

Of samples from 41 patients analyzed during 1984-87, more than one of the compounds under study was present in 35 cases, although only one product had been ingested by each patient; 2,4-D (34 cases), MCPB (16), DCPP (12), ioxynil (11), 2,4,5-T (six), and MCPA (two) were the compounds detected. The method proved capable of resolving all of the mixtures encountered, namely 2,4-D/MCPP (seven cases), 2,4-D/DCPP (seven), 2,4-D/ioxynil (seven), 2,4-D/MCPP/DCPP (five), 2,4-D/2,4,5-T (six), and MCPA/ioxynil (three). Total blood chlorophenoxy and ioxynil concentrations ranged from 0.05 to 1.49 and 0.01 to 0.32 g/L, respectively. Thirty-three patients were severely poisoned; seven died before or on admission, and a further six died in the hospital. Ioxynil was encountered in seven fatalities, three of whom died in the hospital.

In one patient, who had ingested a preparation containing 2,4-D and ioxynil, plasma 2,4-D concentrations were measured locally by ultraviolet spectrophotometry (7). A forced diuresis was commenced and continued for four days on the basis of these results, although the patient made a full clinical recovery within 36 h. Subsequent HPLC analysis of the specimens showed that 2,4-D had been cleared rapidly (plasma elimination half-life, 9.6 h) but that ioxynil was cleared much more slowly (half-life, ca. 112 h). The plasma concentrations of this latter compound corresponded with the "2,4-D" results obtained by the spectrophotometric method (Figure 4).

It is a feature of the use of chlorophenoxy and benzonitrile herbicides that they are often formulated together with other (chlorophenoxy) herbicides or other pesticides, as emphasized by the data discussed above. Clearly, the results of toxicological analyses reported in the literature and elsewhere that were generated by nonselective ultraviolet spectrophotometric methods must be regarded as dubious unless it is clear that only one chlorophenoxy or benzonitrile herbicide was present in each case.

In conclusion, acute poisoning with chlorophenoxy and benzonitrile herbicides is uncommon, despite their easy availability. However, if the concentrates are ingested deliberately, then the outcome is usually serious and may well be fatal. The components of most of the chlorophenoxy herbicide mixtures encountered in the U.K. can be analyzed selectively by the present method, and this procedure can be used to assess the severity of poisoning quickly and to monitor therapy.

Ultraviolet spectrophotometric methods are clearly inappropriate for toxicological analysis for chlorophenoxy and benzonitrile herbicides. The fact that these compounds have a plasma:whole blood ratio of ca. 2 must also be remembered when results are interpreted.

We thank FBC Ltd. and May & Baker for their gifts of pure compounds and Mr. J. D. Ramey, Toxicology Unit, St. George's Hospital, and Mr. P. Streete, Mr. M. O'Connell, and Dr. G. N. Volans, Poisons Unit, for help and encouragement.
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