A rapid analytical method is proposed for the determination of simazine, terbuthylazine, and their chloro dealkylated metabolites (simazine-desethyl, simazine-bisdesethyl, and terbuthylazine-desethyl) in soil. A sonication micromethod is presented for the extraction of s-triazine herbicides and their metabolites. Final determination is by gas chromatography (GC) with nitrogen–phosphorus detection. The identity of all compounds was confirmed by GC with mass selective detection in the selected-ion monitoring mode. All chromatograms were very clean, without interfering peaks, and no cleanup was needed. The limits of detection were 1 pg for simazine-bisdesethyl; 5 pg for simazine, terbuthylazine, and terbuthylazine-desethyl; and 10 pg for simazine-desethyl. The limits of quantitation were 1, 5, and 10 ppb, respectively. Mean recoveries from fortified soils ranged from 76% for simazine-bisdesethyl to 102% for simazine-desethyl, with relative standard deviations of 3–6%.

After application of a triazine herbicide to soil, only a fraction will be available for uptake by plant roots because losses occur via several processes in the top centimeter of soil. These processes include volatilization, adsorption by clay and/or organic matter in soils, leaching, and photodegradation. Monitoring of residue samples is maintained to determine the fate of the triazine herbicides and their toxic degradation products in plants, soils, water, and the general human environment (1). The application of herbicides to agricultural crops allows the triazines to be transported to surface waters by various mechanisms, such as nonpoint source run-off, groundwater discharge, or atmospheric deposition. To protect the quality of potable and surface water in Europe, a priority list of pesticides, also called the “black list,” has been compiled. The pesticides included in this list have the potential for probable or transient leaching. Atrazine, simazine, cyanazine, prometryn, terbuthylazine, and terbutryn are included in this list (2).

Since their introduction around 1960, triazine compounds have been widely used in agriculture as selective herbicides; consequently, they can give rise to residues in soil. Atrazine surely has been the most used triazine, although in recent years its use has decreased in various countries in favor of other s-triazine herbicides such as simazine and terbuthylazine, all of which are inhibitors of photosynthetic electron transport.

Simazine (6-chloro-N\textsuperscript{2},N\textsuperscript{4}-diethyl-1,3,5-triazine-2,4-diamine) is a selective systemic herbicide, adsorbed mainly through the roots, but also through the foliage, with translocation acropetally in the xylem, accumulating in the apical meristems and leaves. Terbuthylazine (N\textsuperscript{2}-tert-butyl-6-chloro-N\textsuperscript{4}-ethyl-1,3,5-triazine-2,4-diamine) is adsorbed mainly by the roots. Both herbicides are used for weed control in different crops and are relatively persistent in soils; the time required for 50% decomposition is the DT\textsubscript{50} value, and DT\textsubscript{50} = 27–102 and 88–106 days for simazine and terbuthylazine, respectively (3, 4). Like other chlorotriazines, simazine is converted in soil under various conditions to the hydroxy analogue and undergoes dealkylation to simazine-desethyl (6-chloro-N\textsuperscript{4}-ethyl-1,3,5-triazine-2-amine) and simazine-bisdesethyl (6-chloro-1,3,5-triazine-2,4-diamine). On the other hand, the principal metabolite of terbuthylazine detected in soil under laboratory conditions is terbutylazine-desethyl (N\textsuperscript{2}-tert-butyl-6-chloro-1,3,5-triazine-2,4-diamine; 3–5).

Methods for the determination of triazine herbicides and metabolites in soil are very important from an agricultural and environmental point of view. A wide variety of solvents and techniques (agitation, immunoassay, Soxhlet extraction, etc.) including cleanup procedures have been proposed; in many cases, the processes are tedious and time consuming, requiring excessive manipulation of the sample and large amounts of...
soil (6–11). The use of sonic energy for the extraction of organic compounds from soils is not new and was first reported by Johnsen and Starr (12). In a later publication (13), the sonication procedure was compared with blender, roller, and Soxhlet extraction of soil spiked with a variety of pesticides. Sonication was found to produce the highest recoveries of various pesticides. Currently, on-line microextraction procedures are very useful for extracting pesticide residues from vegetables and soils (14–16).

This paper describes a simple method for the determination of simazine, terbuthylazine, and their major chloro dealkylated metabolites (Figure 1) in soil, using sonication extraction and determination by gas chromatography with nitrogen–phosphorus detection (GC–NPD) with confirmation of identity by gas chromatography with mass-selective detection (GC–MSD).

**Experimental**

**Apparatus**

(a) GC–NPD system.—Hewlett-Packard Model 6890 gas chromatograph equipped with a nitrogen-phosphorus detector, a Hewlett-Packard Model 6890 autosampler and a split-splitless injector connected to an HP ChemStation (Hewlett-Packard, Avondale, PA). The capillary column was an HP-5 (30 m × 0.25 mm id) with 5% diphenyl 95% dimethyl siloxane liquid phase (film thickness, 0.25 μm) (Hewlett-Packard). The injector and interface temperatures were 250 and 280°C, respectively. The operating conditions were as follows: acquisition mode, selected-ion monitoring (SIM); electron multiplier voltage, 1788 V; ionization foil temperature, 230°C; quadrupole temperature, 150°C; solvent delay, 8 min; scan mass range, 50–250 and SIM: simazine, m/z 173 and 201; simazine-desethyl, m/z 145, 158, and 173; simazine-bisdesethyl, m/z 68, 110, and 145; terbuthylazine, m/z 214 and 229; and terbuthylazine-desethyl, m/z 145, 186, and 201. The carrier gas was He at 1.2 mL/min. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90°C for 1 min, raised to 210°C (10°C/min), to 240°C (5°C/min), to 270°C (30°C/min), and held for 3 min.

(b) GC–MSD system.—Hewlett-Packard Model 6890 gas chromatograph equipped with a Hewlett-Packard Model HP5973 mass selective detector and a split-splitless injector, connected to an HP Vectra 500 integrator (Hewlett-Packard). An HP-5MS fused silica column (30 m × 0.25 mm id) was used, with 5% diphenyl 95% dimethyl siloxane liquid phase (film thickness, 0.25 μm) (Hewlett-Packard). The injector and interface temperatures were 250 and 280°C, respectively. The operating conditions were as follows: acquisition mode, selected-ion monitoring (SIM); electron multiplier voltage, 1788 V; ionization foil temperature, 230°C; quadrupole temperature, 150°C; solvent delay, 8 min; scan mass range, 50–250 and SIM: simazine, m/z 173 and 201; simazine-desethyl, m/z 145, 158, and 173; simazine-bisdesethyl, m/z 68, 110, and 145; terbuthylazine, m/z 214 and 229; and terbuthylazine-desethyl, m/z 145, 186, and 201. The carrier gas was He at 1.2 mL/min. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90°C for 1 min, raised to 210°C (10°C/min), to 240°C (5°C/min), to 270°C (30°C/min), and held for 3 min.

(c) Sonic dismembrator.—200 W generator equipped with standard titanium probe (Dr. Hielscher GmbH, Stahnsdorf, Germany).

(d) Centrifuge.—RC-5 superspeed, refrigerated (Sorvall Inc., Milan, Italy).

(e) Rotary vacuum evaporator.—Büchi Model 461 (Flawil, Switzerland), or equivalent, equipped with vacuum pump and water bath at 35°C.

**Reagents**

(a) Solvents.—Acetone, acetonitrile, and dichloromethane for pesticide residue analysis (J.T. Baker, Deventer, The Netherlands).

(b) Active ingredients.—Simazine and terbuthylazine were obtained from Novartis Agro (Barcelona, Spain); simazine-desethyl, simazine-bisdesethyl, and terbuthylazine-desethyl were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

(c) Standard stock and working solutions.—Weigh the necessary quantity of each active ingredient and dissolve in acetone to obtain a stock solution for each compound at an approximate concentration of 100 mg/L; prepare 2–4 dilutions (from 0.01 to 5 mg/L) of each stock solution to obtain working solutions for each compound.

(d) Filter paper.—1PS, 150 mm dia. (Whatman Inter., Ltd., Maidstone, UK).

**Soil Used in Extraction Procedure**

The soil samples were taken in Campo de Cartagena, Murcia (southeastern Spain). Field samples were sifted to a size of 2 mm, homogenized, and extracted with their naturally occurring water content (10% moisture). The values of pH, electrical conductivity (dS/m), and organic matter (g/kg) were 8.18, 0.12, and 10.3, respectively.
Figure 2. Chromatograms (NPD) obtained for (a) a standard solution (b) a spiked soil sample (0.5–0.1 mg/kg), and (c) a control soil sample. Peak identity: 1, simazine-bisdesethyl; 2, simazine-desethyl; 3, terbuthylazine-desethyl; 4, simazine; and 5, terbuthylazine.
Extraction Procedure

Weigh 5 g homogenized soil and transfer to 100 mL beaker. Add 9.5 mL distilled water and 20 mL acetonitrile, and immerse titanium probe to depth of 1 cm from bottom of beaker. Sonicate sample for 15 min at 0.5 cycles and 60% amplitude. Add 20 mL dichloromethane and centrifuge for 10 min at 1900 \( g \). Quantitatively filter solution through glass funnel containing a filter paper. After filtration, evaporate organic phase to dryness through rotary vacuum evaporation (35°C), redissolve residue in 10 mL acetone, and inject solution into each GC system under conditions described in Apparatus section.

Recovery Assays

Soil samples without pesticide residues were homogenized and spiked with simazine and terbuthylazine at 2 levels, 5 and 0.5 mg/kg, and with their metabolites at 2 levels, 0.5 and 0.1 mg/kg. After evaporation of the spiking solvent, the samples were allowed to equilibrate for 120 min before extraction and were processed according to the procedure in the previous section. Five replicates were analyzed in each case.

Experiment with Aged, Spiked Soil Samples

Triplicate containers (500 g) of soil were spiked with a solution of simazine and terbuthylazine in water–acetone (80 + 20, v/v) to obtain an approximate concentration of 5 mg/kg for each herbicide. Moisture content was set at 60% of the field capacity, and lost water was restored every 15 days. Soil samples were thoroughly mixed and incubated at 20°C for 150 days. Soil samples were analyzed on days 0, 5, 13, 34, 80, and 140 according to the procedure described above.

Results and Discussion

Figure 2 shows chromatograms of a standard solution, a soil sample spiked with the 5 compounds, and a control analyzed for the purpose of checking the absence of triazine residues in the soil. All chromatograms were very clean without interfering peaks in the chromatographic area of interest. A chromatogram obtained in the SIM mode is shown in Figure 3. The NPD and MSD showed high sensitivity and selectivity.

Several standard solutions, with concentrations of 0.01–5 ng/µL, were injected (in the NPD mode) to obtain the linearity of detector response and the detection limits of the 5 compounds studied. The values obtained by linear regression show a good correlation between concentration and peak area (\( r > 0.995 \)) for the 5 compounds studied. The limits of detection (LOD; obtained at a signal-to-noise ratio of 3) were 1 pg for simazine-bisdesethyl; 5 pg for simazine, terbuthylazine, and terbuthylazine-desethyl; and 10 pg for simazine-desethyl.

Table 1 shows the recoveries obtained for the 2 herbicides and 3 metabolites at 2 concentration levels. Three solvents (acetonitrile, acetone, and ethyl acetate) were tested as extractants, and the best results were obtained with acetonitrile for all compounds. In all cases, recoveries were >89% with the exception of simazine-bisdesethyl (74%). Recovery values were higher than those obtained for soils by other researchers using different extraction methods (7, 14, 17). The relative standard deviation (RSD) was <6% in the most unfavorable case. The limits of quantitation (LOQ; bearing in mind the LOD for each compound, weight of sample, volume of extract, and volume injected) for the NPD were 1 ng/kg (ppb) for simazine-bisdesethyl; 5 ng/kg for simazine, terbuthylazine, and terbuthylazine-desethyl; and 10 ng/kg for simazine-desethyl. In the case of the MSD (in the SIM mode), the LOQ were equivalent.

Table 1. Recovery of herbicides and metabolites from spiked soil samples

<table>
<thead>
<tr>
<th>Herbicide/metabolite</th>
<th>Fortification level, µg/g</th>
<th>Mean recovery ± RSD(^a), % (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simazine</td>
<td>5.00</td>
<td>98.9 ± 2.6</td>
</tr>
<tr>
<td>Simazine-desethyl</td>
<td>0.5</td>
<td>91.8 ± 4.0</td>
</tr>
<tr>
<td>Simazine-bisdesethyl</td>
<td>0.1</td>
<td>101.9 ± 5.0</td>
</tr>
<tr>
<td>Terbuthylazine</td>
<td>5.00</td>
<td>91.2 ± 3.0</td>
</tr>
<tr>
<td>Terbuthylazine-desethyl</td>
<td>0.5</td>
<td>89.2 ± 4.8</td>
</tr>
<tr>
<td>Terbuthylazine-desethyl</td>
<td>0.1</td>
<td>97.9 ± 3.4</td>
</tr>
</tbody>
</table>

\(^a\) \( n = 5 \).

\(^b\) RSD = relative standard deviation.
In regard to the experiment with aged, spiked soil samples, the mean concentrations of simazine and terbutylazine found 3 h after spiking were 4.77 and 4.92 mg/kg, respectively. Eighty days later, the residual levels were 0.12 mg/kg for simazine and 1.48 mg/kg for terbutylazine. At the end of the experiment (140 days), the mean concentrations in the soils were 0.09 mg/kg for simazine and 0.34 mg/kg for terbutylazine, which were 2 and 7% of the initial concentrations, respectively. Simazine-desethyl, simazine-bisdesethyl, and terbutylazine-desethyl were detected as metabolites. Their concentrations at the end of the experiment were 0.42, 0.21, and 0.56 mg/kg, respectively.

The dissipation rate was found to be greater for simazine than for terbutylazine, although it is necessary to keep in mind the mineralization and formation of bound residues in both cases. After 8 months of soil incubation (pH 7.3, with 22% clay, 73% silt, and 1.08% organic C) under laboratory conditions, Barriuso et al. (10), working with simazine 14C-labeled on the aromatic ring, found that the total mineralized, total extractable, and total nonextractable radioactivity percentages of initial 14C were 63.6, 1.4, and 20.3, respectively. These findings indicate rapid mineralization of simazine under the conditions of the experiment. In another study (4) performed with 14C-terbutylazine, uniformly labeled with 14C on the s-triazine ring, in 3 soils during a 45 day incubation period after application of the active ingredient at 1 kg/ha, degradation was slow and led to a very limited mineralization, 0.9–1.2%, whereas a large proportion of the compound, 33.5–43.1%, became nonextractable. In addition, the decrease in terbutylazine concentration was accompanied by an increase in the terbutylazine-desethyl concentration. Various hypotheses are proposed to explain the formation of bound residues, including chemical binding to soil organic compounds, trapping in the internal voids of soil organic matter, incorporation into phenolic polymers, and bioincorporation in cellular structures through the activity of soil microorganisms (18, 19). These nonextractable residues, although more difficult to leach, increase the persistence of the herbicides in the top soil layers and are a latent source of pollution because of various factors that can cause them to be remobilized in soil solutions (20, 21).

Conclusions

Although other techniques such as Soxhlet or solid-phase extraction are commonly used to extract different pesticides in sediments and soils, the sonication procedure reported here extracted equal, and in some cases higher, quantities of extractable residues of simazine and terbutylazine as well as their dealkylated chlorotriazine metabolites in soils, compared with other extraction methods. The proposed method yields recoveries in the range of 74–102%, with acceptable repeatability (RSD < 5%) and LOQ that ranged from 1 to 10 ng/kg. Another advantage of the sonication technique is the decrease in time and expense required for the analysis of large numbers of samples that must be processed in many field investigations.

References
