Herbicide Residues in Tobacco Leaves and their Transfer into the Smoke

Urea Herbicides Patoran® and Molipan®*


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1.0 INTRODUCTION

In many countries the shortage of man-power calls for simplification of the most time-consuming cultural practices. The expensive weed control after the transplanting of the tobacco seedlings is one of the procedures which can be reduced by the use of herbicides, as is now done for many other crops. Among the several substances tested recently the urea derivatives gave encouraging results in Belgium (9), Austria (23, 24) and West Germany (16, 25) in controlling the most important weeds without damaging the tobacco plant. On the basis of successful agronomic tests, it was decided to study — in a collaborative experiment — the transfer of the herbicides Patoran and Molipan from the soil into the leaves, and from the latter into the smoke.

Table 1  Rate of application and formulae of the Herbicides Patoran® and Molipan®

<table>
<thead>
<tr>
<th>Trade names</th>
<th>Rate of application (commercial product)</th>
<th>% of active matter</th>
<th>Formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patoran</td>
<td>4 kg per ha (0.4 g per m²)</td>
<td>50% metabromuron</td>
<td>N-(4-Bromophenyl)-N'-methoxy-N'-methyl-urea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Br-OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-NH-C-N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CH₃</td>
</tr>
<tr>
<td>Molipan =</td>
<td>2.5 kg per ha (0.25 g per m²)</td>
<td>30% linuron</td>
<td>N-(3,4-Dichlorophenyl)-N'-methoxy-N'-methyl-urea</td>
</tr>
<tr>
<td>Afalon s</td>
<td></td>
<td></td>
<td>Cl-OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-NH-C-N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20% monolinuron</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-(4-Chlorophenyl)-N'-methoxy-N'-methyl-urea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl-OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-NH-C-N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CH₃</td>
</tr>
</tbody>
</table>

* Presented at the CORESTA Symposium on Products for Tobacco Treatment, Stockholm, September 1968.

2.0 EFFECTS OF HERBICIDE APPLICATION

2.10 Experimental methods

The two herbicides Patoran and Molipan (Table 1) were compared in preplanting application. The experiments were conducted with a Burley type called Sota 27 (7). Plot area 50 m²; 3 replicates. Soil characteristics: Sandy clay loam to clay loam; pH 7.1 to 7.4. Herbicides were applied 8 hours before planting. No hoeing was carried out after transplanting. Leaves were harvested conventionally, i.e. three primings: 5–6 lower leaves, 9 middle leaves and 3 upper leaves. A sample of the green middle leaves was dried in an oven at 80°C; other leaves were air cured.

2.20 The agronomical effect

2.22 Weed control  The spectra of the weeds controlled by Patoran and Molipan are large and identical.
Table 2 Importance of the weeds, 45 days after herbicide treatment

<table>
<thead>
<tr>
<th>Herbicide Treatment</th>
<th>Green weight kg per m²</th>
<th>Poa annua</th>
<th>Chenopodium album</th>
<th>Stellaria media</th>
<th>Sinapis arvensis</th>
<th>Mercurialis annua</th>
<th>Veronica persica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patoran</td>
<td>0.318 (29.2%)</td>
<td>0.1</td>
<td>0.4</td>
<td>1.9</td>
<td>1.4</td>
<td>6.1</td>
<td>51.1</td>
</tr>
<tr>
<td>Molipan</td>
<td>0.247 (22.7%)</td>
<td>0.2</td>
<td>0.3</td>
<td>2.4</td>
<td>0.6</td>
<td>4.7</td>
<td>40.0</td>
</tr>
<tr>
<td>Untreated</td>
<td>1.089 (100%)</td>
<td>15.0</td>
<td>9.7</td>
<td>20.3</td>
<td>7.0</td>
<td>32.0</td>
<td>49.0</td>
</tr>
</tbody>
</table>

The most common weeds in tobacco fields are affected:
- Poa annua
- Chenopodium album
- Stellaria media
- Capsella bursa pastoris
- Sinapis arvensis
- Anagallis arvensis
- Matricaria chamomilla

The following weeds were less affected:
- Plantago major
- Plantago media
- Mercurialis annua

No control was achieved over:
- Veronica persica

The two last mentioned weeds grow more than usual because there is no competition. This explains why, in spite of a strong decrease in number and species, the green weight of the weeds is still as high as 20% of that of the untreated plots (Table 2). It may be concluded, therefore, that there is a risk of „selecting“ weeds by the indiscriminate use of one specific herbicide.

2.22 Effect on the tobacco plant  The toxicity of the products tested was weak in preplanting application. The number of aborted plants was 3.2 per cent for Patoran and 14.3 per cent for Molipan. This weaker toxicity of Patoran has been confirmed in other experiments. The influence on the yield is subject to considerable controversy, as it is strongly related to the influence of hoeing. Under our conditions, however, we observed, in 1967, an obvious decrease in green weight yield in the treated unhoed plots, as compared with the untreated hoed plots, but, almost no difference was observed in 1968. This, of course, depends on the soil characteristics, the amount and frequency of rainfall, and the particular behaviour of the variety. Although the first results contradicted those reported in West Germany (16), we cannot reach definite conclusion before carrying out more experiments. On the hand, the herbicidal treatment has no influence on the physical aspect of the cured tobacco, as demonstrated by the prices obtained:

Average price of 1 kg of cured tobacco:
- Plot treated with Patoran: 6.45 Swiss francs
- Plot treated with Molipan: 6.42 Swiss francs
- Untreated plot: 6.40 Swiss francs

2.30 Herbicide residues in the leaves

2.31 Patoran  The results, reported in Table 3, show that the Patoran residues are spread uniformly over the lower, middle and upper leaves, with a maximum content of 1.2 p.p.m. These data are of a similar order of magnitude, but smaller, than those published by Schmid (25). There is no obvious difference between unfermented and fermented leaves. The residues are much higher (1.1-1.2 p.p.m.) in the laminae than in the midribs (0.1-0.3 p.p.m.).

2.32 Molipan  Unlike the Patoran residues, the Molipan residues decrease from the lower leaves to the upper leaves (Table 4). More residues were found in unfermented leaves than in fermented tobacco (Tables 4 and 5, see below). This result was not confirmed by the gaschromatography determination, probably due to sampling problems. For all stalk positions before and after fermentation, the laminae of the leaves contain more residues than the midribs (Table 5).
Table 5 Residues of Molipan in the laminae and the midribs of tobacco leaves
p. p. m. of linuron + monolinuron based on tobacco dry weight (Colorimetric method 4.33)

<table>
<thead>
<tr>
<th>Primings</th>
<th>Unfermented</th>
<th>Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper leaves</td>
<td>laminae</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>midribs</td>
<td>0.34</td>
</tr>
<tr>
<td>Middle leaves</td>
<td>laminae</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>midribs</td>
<td>0.58</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>laminae</td>
<td>2.16</td>
</tr>
</tbody>
</table>

2.3 Comparison with other crops There are very few data published on residues of urea derivatives in plants. In potatoes, where Patoran is applied at the same rate as for tobacco, the residue is about 0.01 p.p.m. (6). On sugar cane where N-(4-chlorophenyl)-N',N'-dimethylurea was applied as pre-emergence treatment 7 months prior harvest, 0.008 p.p.m. were found in the stalk and less than 0.02 p.p.m. in the sugar syrups (3).

3.0 TRANSFER OF HERBICIDE RESIDUES FROM THE TOBACCO INTO THE CIGARETTE MAINSTREAM SMOKE

From the smoker's point of view, the only relevant figure is the herbicide concentration in the mainstream smoke. It was with this consideration in mind that the transfer and filtration rates were investigated.

3.1 Transfer rates

It was presumed that a part of the herbicides was to be delivered into the smoke as such, while another part was destroyed pyrolytically. Among the products of the pyrolysis, 4-bromoaniline (formed through the degradation of metobromuron and 4-bromoaniline and 3,4-dichloroaniline (formed through the degradation of monolinuron and linuron) were supposed to be present. The analytical method (4.54, Table 10) was therefore devised to take this situation into account, and the results (Tables 7 and 9) were presented accordingly (See Fig. 1). The cigarettes were smoked according to the DIN 10240 standard; the general results are shown in Table 6. The unfermented cigarette filler delivered more moist smoke condensate. Significantly less than 1 µg of each of the herbicide was present in the tobacco to be burnt in a single cigarette.

3.1.1 Metobromuron and 4-bromoaniline Using cigarettes made with both unfermented and fermented tobacco, about 14 percent of the herbicide was found in the mainstream smoke, but as much as two-thirds of this yield was present as 4-bromoaniline. This transfer rate is slightly inferior to the transfer rate of nicotine (15 to 20 per cent) (Table 7).

3.1.2 Monolinuron and 4-chloroaniline No reliable results were obtained with unfermented tobacco. In the cigarettes made with fermented tobacco, the transfer rates were comparable with those for metobromuron and 4-bromoaniline (Table 7).

3.1.3 Linuron and 3,4-dichloroaniline No reliable results were obtained with unfermented tobacco. In the cigarettes made with fermented tobacco, transfer rates twice as high as for monolinuron and 4-chloroaniline were found, i.e. 6.7 per cent of the herbicide as linuron and 20.6 per cent as 3,4-dichloroaniline (Table 7).

3.1.4 Reliability of the results These transfer rates must be considered as the upper limits under actual smoking conditions. In fact, the gas-chromatographic procedure applied to the determination of monolinuron and linuron was less sensitive than the corresponding procedure for metobromuron. The presence of interfering substances could, therefore, not be completely

Table 6 Analytical results of smoking tests
Patoran-treated tobacco

<table>
<thead>
<tr>
<th>Upper leaves</th>
<th>Number of Cigarettes per analysis</th>
<th>Burnt Tobacco/Cig. mg</th>
<th>Crude condensate mg/g of burnt tobacco</th>
<th>Metabolism in burnt tobacco µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfermented</td>
<td>10</td>
<td>610</td>
<td>75</td>
<td>0.53*</td>
</tr>
<tr>
<td>Fermented</td>
<td>10</td>
<td>700</td>
<td>63</td>
<td>0.96*</td>
</tr>
</tbody>
</table>

Molipan-treated tobacco

<table>
<thead>
<tr>
<th>Upper leaves</th>
<th>Number of Cigarettes per analysis</th>
<th>Burnt Tobacco/Cig. mg</th>
<th>Crude Condensat mg/g of burnt tobacco</th>
<th>Residue in burnt tobacco (µg/g) Monolinuron</th>
<th>Linuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfermented</td>
<td>40</td>
<td>600</td>
<td>80</td>
<td>0.77**</td>
<td>0.45**</td>
</tr>
<tr>
<td>Fermented</td>
<td>20</td>
<td>700</td>
<td>54</td>
<td>0.57</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* Average of 4 gas-chromatographic measurements
** Not ascertained values
Table 7 Transfer of herbicides and corresponding anilines into the particulate phase of cigarette smoke

<table>
<thead>
<tr>
<th>Patoran- treated tobacco</th>
<th>Metobromuron</th>
<th>Monolinuron</th>
<th>Linuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>µg/g of burnt tobacco</td>
<td>Percent-transfer</td>
<td>µg/g of burnt tobacco</td>
</tr>
<tr>
<td>unfermented</td>
<td>0.021</td>
<td>4.0</td>
<td>0.046</td>
</tr>
<tr>
<td>fermented</td>
<td>0.035</td>
<td>3.7</td>
<td>0.096</td>
</tr>
</tbody>
</table>

* No result
** Not ascertained value

Table 8 Retention of herbicides and anilines by paper and cellulose acetate filters (measured in fermented upper leaves)

<table>
<thead>
<tr>
<th>Type of filter</th>
<th>Metobromuron µg per g burnt tobacco</th>
<th>4-bromoaniline in µg of metobromuron per g burnt tobacco</th>
<th>Sum of monolinuron and 4-chloroaniline in µg of monolinuron per g burnt tobacco</th>
<th>Sum of linuron and 3,4-dichloroaniline calculated as linuron per g burnt tobacco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper filter</td>
<td>0.021*</td>
<td>0.045*</td>
<td>0.030</td>
<td>0.123</td>
</tr>
<tr>
<td>Cellulose filter</td>
<td>0.023*</td>
<td>0.042*</td>
<td>0.024</td>
<td>0.069</td>
</tr>
<tr>
<td>Acetate filter</td>
<td>0.035**</td>
<td>0.096**</td>
<td>0.073</td>
<td>0.180</td>
</tr>
</tbody>
</table>

* Average of 2 results
** Average of 5 results

3.20 Retention by cigarette filters

For Patoran-treated tobacco, the retention of both metobromuron and 4-bromoaniline by conventional cellulose (paper) and a cellulose-acetate filter was determined. The filters were selected for the same efficiency towards crude smoke condensate. In the case of the Molipan-treated tobacco, the same filters were used, but no distinction was made between linuron and 3,4-dichloroaniline and between monolinuron and 4-chloroaniline. The results are listed in Table 8.

3.21 Molipan-treated tobacco

The retention values for linuron/3,4-dichloroaniline and monolinuron/4-chloroaniline were of the same order of magnitude as those for nicotine, except in the cellulose-acetate filter, where both were higher.

3.22 Patoran-treated tobacco

This result was not confirmed with metobromuron, which was retained in the same proportion as nicotine by both the cellulose and the cellulose-acetate filter. 4-Bromoaniline, on the other hand, was retained to a significantly higher extent by both the cellulose and the cellulose-acetate filters. This observation would be in accordance with the higher volatility of the 4-bromoaniline and its specific affinity for the filter material.

3.23 Material balance

The accuracy of the retention figures found for the cigarettes containing Patoran tobacco was checked by combining the results of the direct and indirect methods of determination of the retention values. The figures listed in Table 9 show clearly that the sum of the products extracted from the filter, and contained in the smoke having left the filter, equals the amounts of the products being delivered by the all-tobacco cigarette.

3.30 Conclusions

1. The transfer rate of the herbicide residue into the mainstream smoke is generally less than 5 per cent.
2. Twice as much of the herbicide residue is transferred into the mainstream smoke as the corresponding aniline.
3. The total transfer rate (1 + 2) approaches that of nicotine.
4. The retention of the herbicides by conventional cellulose and cellulose-acetate filters is comparable to the retention of nicotine by paper.
Table 9  Filtration material balance for Patoran
μg of metobromuron per g of burnt tobacco

<table>
<thead>
<tr>
<th>Type of filter</th>
<th>In condensate</th>
<th>In filter</th>
<th>Total A+B+C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Paper filter</td>
<td>0.017</td>
<td>0.047</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>0.026</td>
<td>0.043</td>
<td>0.062</td>
</tr>
<tr>
<td>Cellulose acetate filter</td>
<td>0.017</td>
<td>0.043</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>0.030</td>
<td>0.041</td>
<td>0.071</td>
</tr>
<tr>
<td>Without filter</td>
<td>0.035</td>
<td>0.096</td>
<td>-</td>
</tr>
</tbody>
</table>

1) Length of filter 20 mm, of tobacco rod 70 mm; cigarettes smoked to a tobacco butt end of 23 mm (= total butt length of 43 mm).
2) Length of cigarette (= length of tobacco rod) 70 mm; cigarettes smoked to a butt length of 23 mm.

Table 10  Analytical steps

10–20 g of ground tobacco (4. 33. 1)

1. Smoke condensate from 10–20 cigarettes + internal standard

2. Partition between hydrochloric acid and isooctane

3. Acid hydrolysis

4. Alkaline steam distillation and extraction into solvent

5. Extraction of anilines into hydrochloric acid

6. Diazotisation of the anilines

7. Coupling

8. Azo dye

9. Column chromatography

10. Purified azo dye

11. Spectrophotometric measurement

12. Iodination

13. Iodinated halogeno-benzenes

14. Extraction into hexane

15. Purification with potassium permanganate

16. Purified iodinated halogeno-benzenes

17. ECD Gas chromatography
with the retention of nicotine by the same filters. The anilines, however, appear to be retained to a higher extent than the original herbicides.

4.0 ANALYTICAL METHODS AND PROCEDURES

4.10 Analytical possibilities

Several methods have been proposed for the determination of halogen-substituted ureas. A non exhaustive list of references is given here:

4.11 Bio-assay There specific properties as herbicides are used for this test (5).

4.12 Column absorption chromatography Separation on aluminium oxide followed by UV spectrophotometry (13).

4.13 Paper chromatography (21).

4.14 Thin-layer chromatography (15a, 19, 12).

4.15 Spectrophotometry of a colored derivative After the observation that herbicides of type which is considered here, cannot be extracted quantitatively from vegetable material with current solvents, and that a hydrolysis step is necessary for the total recovery of the aniline moiety of the herbicide molecule (22), all the quoted methods concentrate on the determination of the halogenated anilines (2, 4, 8, 11, 17, 20, 26, 27).

4.16 Gaschromatography of the trimethylsilyl derivatives of the herbicides (10), of the halogenated anilines after hydrolysis of the herbicides (15b, 19), of the brominated anilines (14) and of the halogenated benzenes obtained from the halogenated anilines whose amino group was replaced by iodine (1).

4.17 Discussion For the determination of herbicide residues in tobacco, the spectrophotometric methods (4.15) promised to be sufficiently sensitive. The procedure described below is an adaptation to tobacco of the method by Schredt and Geissbühler (26). Among the GC methods only the procedure of I. Baunok and H. Geissbühler (1) promised to be sensitive and specific enough for the determination of the herbicide residues and their degradation products in cigarette smoke condensate. Therefore, this method was used for the determination of the herbicides and their anilines degradation products in the cigarette mainstream smoke. The partition step 4.34.2 was newly introduced.

4.20 Analytical pathway

The principle of the chemical reactions involved are shown in Fig. 1. The details of the analytical steps are given in Table 10.

4.30 Analytical procedures for tobacco and tobacco smoke

4.31 Apparatus Smoking machine with electrostatic smoke trap.
Potassium permanganate solution, 1% in 1 N sodium hydroxide
Internal standard solutions:
3,4-Dichloroaniline (0.7 μg) and linuron (1.1 μg active substance) in 25 ml isooctane.
4-Bromoaniline (0.7 μg) and metrobromuron (1.1 μg active substance) in 25 ml isooctane.

4.33.0 Procedure for application on tobacco
4.33.1 Preparation of sample The original tobacco sample (at least 25 g) is dried at 40° C for 24 hours and ground to less than 0.5 mm particle size. About 10 g are accurately weighed into a 1/2 litre round-bottom flask.

4.33.2 Acid hydrolysis After the addition of 120 ml of water and 60 ml of 1 N hydrochloric acid and, if necessary, some drops of antifoam emulsion, the mixture is heated under reflux during 30 minutes and then left to cool for 15 minutes.

4.33.3 Steam distillation and extraction Through the reflux condenser, 30 ml of 10 N sodium hydroxide are added and the reflux condenser is replaced by the Bleidner extractor (2). A 250 ml round-bottom flask containing 100 ml of isooctane is attached at the solvent side (upper arm) of the Bleidner apparatus. The distillation/extraction is started and maintained for two hours in such a way as to condense equal volumes of water and isooctane.

4.33.4 Extraction of anilines into hydrochloric acid The cooled isooctane solution is transferred to a 250 ml separating funnel and extracted twice, each time with 5 ml of 1 N hydrochloric acid.

4.33.5 Diazotisation of the anilines The combined acid extracts are transferred to a glass or Teflon-stoppered 50 ml Erlenmeyer flask and cooled to 0° C in an ice bath. After adding 2 ml of 2% aqueous sodium nitrite, the mixture is allowed to stand at 0° C for 25 minutes. Excess nitrite is then destroyed by adding 2 ml of 10% aqueous sulfamic acid. The flask is vigorously shaken until the nitrogen generation stops (5-10 minutes).

4.33.6 Coupling The solution is heated to room temperature, 2.0 ml of 2% N-(1-naphthyl)-ethylenediamine hydrochloride solution are pipetted in the flask and the mixture is set aside for 20 minutes.

4.33.7 Chromatographic separation of the azo dye A suspension of 50 ml (25 g) of cellulose powder in 100 ml of 1 N hydrochloric acid is kept for a short time under vacuum to eliminate air bubbles and is then poured into a chromatography column (40 × 2 cm). Air pressure is applied to obtain a final height of 10 cm, and the top of the cellulose powder is covered with a cotton plug.

The dye solution is quantitatively transferred to the column by carefully delivering it from a pipette, and drained into the column by applying slight air pressure. The dye is retained in the upper third of the column. The interfering dyes are eluted with 200-250 ml of a solvent mixture 1 N hydrochloric acid + glacial acetic acid (9 vol. + 1 vol.). The azo dyes derived from the herbicide anilines are located as a 2-3 cm band halfway up the column; they are eluted with 30-40 ml of a solvent mixture 1 N hydrochloric acid + glacial acetic acid (1 vol. + 2 vol.). The eluent is collected in a 50 ml volumetric flask and made up to the mark with the 1 + 2 elution mixture. The dye is stable for at least three hours.

4.33.8 Spectrophotometric measurements The absorption spectrum of the dye is a symmetrical peak between 450 and 650 nm, with the maximum peak height at 550 nm. The absorbance is measured at 550 nm against distilled water as a reference, by using 2 cm or, if the absorbance is too weak, 4 cm cells. The absorbance value is correlated with the total amount of herbicides, with the aid of the calibration chart (Example: Fig. 2).

4.33.9 Calibration The procedure is applicable for 4-bromoaniline, 4-chloroaniline and 3,4-dichloroaniline (called "aniline" in the following description). 2 mg of the aniline are dissolved in 500 ml 1 N hydrochloric acid, giving a concentration of 4 μg per ml. 0.5, 1, 2, 5 and 10-ml aliquotes of this solution (2-40 μg of the aniline) are pipetted into glass or Teflon-stoppered 50-ml volumetric flasks and diluted to 10 ml with 1 N hydrochloric acid. The diazotisation and coupling
operations are performed according to the procedures 4.33.5 and 4.33.6, and the dye solution is filled up to the mark with 1 N hydrochloric acid. The absorbance at 550 nm is measured as described under 4.33.8, and the absorbances are plotted against the total amounts of the original herbicides (Fig. 2).

4.34 Procedure for application on smoke condensate
4.34.1 Preparation of the smoke condensate 10 or 20 cigarettes are smoked according to the CORESTA or DIN 10240 specifications. The smoke condensate, collected in an appropriate (e.g. electrostatic) trap, is extracted with 25 ml of iso-octane containing the following internal standards: 0.7 μg of 3,4-dichloroaniline and 1.1 μg of linuron (active substance) when the sample is tobacco containing metobromuron, or, 0.7 μg of 4-bromoaniline and 1.1 μg of metobromuron (active substance) when the sample is tobacco containing linuron and monolinuron.

4.34.2 Partition between iso-octane and hydrochloric acid 25 ml of 1 N hydrochloric acid are added to the 25 ml iso-octane extract in the smoke trap, and the iso-octane layer decanted into a 1½ litre round-bottom flask. The acid layer is extracted with two further 25 ml portions of iso-octane. The combined iso-octane extracts (75 ml, containing the original herbicides) and the hydrochloric acid extract (2.5 ml, containing the halogenated anilines) are submitted separately to the following sequence of operations:

- Acid hydrolysis Isooctane extracts: After the addition of 50 ml of 1 N hydrochloric acid and 75 ml of water, the mixture is hydrolysed as indicated under 4.33.2. Hydrochloric acid extract: The 25 ml of the extract, plus another 25 ml of 1 N hydrochloric acid used for rinsing the smoke trap and separating funnel and 75 ml of water are submitted to the treatment indicated under 4.33.2.

- Steam distillation and extraction Isooctane extracts: The procedure 4.33.3 is followed, except that the 250 ml round-bottom flask on the solvent side of the Bleidner apparatus receives only 25 (instead of 100) ml of iso-octane. Hydrochloric acid extract: The procedure 4.33.3 is followed.

- Extraction of anilines into hydrochloric acid The procedure 4.33.4 is applied. (Starting with this step, the iso-octane-extract products and the hydrochloric-acid extract products are worked up identically.)

- Diazotisation of the anilines The procedure 4.33.5 is followed.

4.34.3 Iodination 0.5 ml of potassium iodide/iodine solution is added to the ice cold diazonium salt solution, and the mixture allowed to stand at room temperature for 25 minutes. It is then transferred to a water bath at room temperature which is heated to boiling within 15 minutes, by periodically loosening the stopper to prevent excess pressure. Excess iodine is then reduced by adding approximately 200 mg of sodium sulfite powder.

4.34.4 Extraction into hexane The solution is made alkaline with 1.1 ml of 10 N sodium hydroxide solution and extracted with 20 ml of hexane. After vigorous shaking, about 10 ml of the organic phase are transferred to a 20 ml screw-capped vial with a pipette.

4.34.5 Purification with potassium permanganate The solution is shaken with an equal volume of 1% potassium permanganate in 1 N sodium hydroxide solution. The mixture is allowed to stand for 1 to 2 hours before agitating it again and transferring the hexane to a clean 10 ml vial.

4.34.6 Gas chromatography A 4 μl aliquot is injected into the column per single determination. The instrument is operated according to the specifications summarized in
Table 11  Operating conditions for gas chromatography

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Method used for metobromuron and derived aniline (Recommended procedure 4.34.6)</th>
<th>Method used for linuron, monolinuron and derived anilines (Not recommended procedure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>F &amp; M 810</td>
<td>Aerograph 1520</td>
</tr>
<tr>
<td>Support material</td>
<td>Glass, 120 cm x 6 mm</td>
<td>Glass, 150 mm x 3 mm</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>Chromosorb P 80, 100 mesh</td>
<td>Gas-chrom Q, 60/80 mesh</td>
</tr>
<tr>
<td>Gas</td>
<td>3% SE-30</td>
<td>6% Silicone QF-1</td>
</tr>
<tr>
<td>Gas flow-rate</td>
<td>20 ml/min</td>
<td>20 ml/min</td>
</tr>
<tr>
<td>Injector port temp.</td>
<td>145°C</td>
<td>130°C</td>
</tr>
<tr>
<td>Column temp.</td>
<td>120°C</td>
<td>100°C</td>
</tr>
<tr>
<td>Detector temp.</td>
<td>200°C</td>
<td>250°C</td>
</tr>
<tr>
<td>Type of detector</td>
<td>Pulsed (150 μs per pulsation)</td>
<td>Conventional ECD</td>
</tr>
<tr>
<td>Attenuation</td>
<td>10 x 8</td>
<td>1 x 16 or 1 x 8</td>
</tr>
<tr>
<td>Volume of liquid</td>
<td>2 to 4 μl</td>
<td>4 μl</td>
</tr>
</tbody>
</table>

Table 11. The substances are eluted in the order shown in the chromatogram of Fig. 3. Examples of chromatograms obtained without the use of an internal standard are shown in Figures 5a, 5b, 6a and 6b. The total amount of herbicide (or halogenated aniline when the acid extract 4.34.2 is analysed) per total amount of smoke condensate in one smoke trap is derived from the peak-height ratio of unknown: internal standard, with the aid of the calibration charts (Example: Fig. 4).

4.34.7  Calibration  For linuron, monolinuron and the corresponding 3,4-dichloro- and 4-chloroanilines: Dissolve 0.14, 0.28, 0.56 and 1.12 μg of 3,4-dichloroaniline and 4-chloroaniline and 0.22, 0.44, 0.88 and 1.76 μg of linuron and monolinuron in 25 ml of isooctane containing 1.1 μg of metobromuron and 0.7 μg of 4-bromoaniline (internal standards).

For metobromuron and the corresponding 4-bromoaniline: Dissolve 0.14, 0.28, 0.56 and 1.12 μg of 4-bromoaniline and 0.22, 0.44, 0.88 and 1.76 μg of 3,4-dichloroaniline.

The analytical procedure, starting with 4.34.2 (partition between hydrochloric acid and isooctane) is then followed as described above. On the chromatogram the peak heights are measured, and the ratios

\[ R = \frac{\text{peak height of unknown}}{\text{peak height of internal standard}} \]

calculated.

Remark: The gas chromatograph used for the procedure related to Patoran was equipped with a pulsed EC detector of high sensitivity. About 20 times less sensitivity was obtained with the standard EC detector used for the procedure related to Molipan. In the latter case, the calibration curve had to be established with correspondingly more substance, or the final hexane solution had to be evaporated to a small volume before it was injected into the column. When smoke samples are analysed this way, however, the interference due to background contamination may become a severe handicap.

4.40  Discussion of the methods

4.41  Spectrophotometric method (4.33.1–4.33.9)

Specificity: The specificity of the method is fair. The chromatographic step 4.33.7 eliminates all interfering colour except a low background level corresponding to approximatively 0.2 p.p.m. herbicide content in the tobacco leaf. This background colouration being fairly constant, all results may be corrected by subtracting 0.2 p.p.m. However, the method is unspecific when several herbicides are present in the same sample. Linuron and monolinuron which are present in the herbicide Molipan, cannot be determined separately by this method.

Sensitivity: Due to the background interference corresponding to about 0.2 p.p.m. herbicide, residue levels of less than 0.5 p.p.m. cannot be measured accurately.

Accuracy: From 1 to 4 p.p.m. of herbicide added to the tobacco are recovered quantitatively (recovery from 94...
Figure 5  Gas Chromatograms of halogenated iodobenzes
extracted from smoke condensate (isoctane fraction) which was obtained from:
Fig. 5a: Metobromuron containing tobacco
Fig. 5b: Linuron and Monolinuron containing tobacco
(1) 4-Chloro-iodobenzene from monolinuron (actually not present in chromatogram Fig. 5a);
(2) 4-Bromo-iodobenzene from metobromuron (actually not present in chromatogram Fig. 5b);
(3) 3,4-Dichloro-iodobenzene from linuron (actually not present in chromatogram Fig. 5a)

Figure 6  Gas Chromatograms of halogenated iodobenzes
extracted from smoke condensate (hydrochloric acid fraction) which was obtained from:
Fig. 6a: Metobromuron containing tobacco
Fig. 6b: Linuron and Monolinuron containing tobacco
(1) 4-Chloro-iodobenzene from 4-chloroaniline (actually not present in chromatogram Fig. 6a);
(2) 4-Bromo-iodobenzene from 4-bromoaniline (actually not present in chromatogram Fig. 6b);
(3) 3,4-Dichloro-iodobenzene from 3,4-dichloroaniline (actually not present in chromatogram Fig. 6a)
to 104%). On the other hand, higher than actual contents are obtained due to unspecified background absorption corresponding to about 0.2 p.p.m. herbicide.

Repeatability: In the range of 1 to 4 p.p.m., the standard deviation is about 0.04 p.p.m.

Conclusion: The method is at the limit of usability for the determination of herbicide residues in tobacco, which were found to be of 2 p.p.m. and less in the samples investigated.

4.42 Gas-chromatographic method (4.34.1–4.34.6) Specificity: Smoke obtained from linuron and monolinuron-free tobacco shows small background peaks in the gaschromatogram in both the herbicide and the aniline fractions (Fig. 5a and 5b). The background peak is more evident in the metobromuron-free tobacco (Fig. 6a and 6b).

A gross estimation indicates about 0.01 p.p.m. (parts apparent herbicide per million parts of burnt tobacco) interference, i.e. the measured herbicide amounts are about 0.1 µg higher than the actual amounts.

Sensitivity: About 20–50 µg per injection, or 0.1 µg of herbicide per analysis. When the final hexane solution is evaporated to a small volume, as little as 0.005 µg of herbicide per analysis might be determined. In practice, however, the sensitivity is limited by background interference causing a loss of accuracy.

Accuracy: From the discussion of the specificity and sensitivity, it follows that the accuracy of the method depends strongly on the purity of the sample. If very small concentrations of herbicides are to be determined (less than 0.2 µg per 10 grams of tobacco to be smoked), the interference through background contamination is such that the apparent herbicide content may amount to 150% of the actual content.

Repeatability: The standard deviation through all steps of the analysis (from smoking the cigarette to the gaschromatography) can be grossly estimated as 0.005 p.p.m. (parts herbicide per million parts of tobacco to be smoked) or about 0.015 µg on the total amount per analysis.

Conclusion: The sensitivity, accuracy and repeatability of the method are sufficient to determine herbicide residues, and the anilines pyrolytically derived from these herbicides, in tobacco smoke.

SUMMARY

The herbicides Patoran (= Metobromuron) and Molipan (= Monolinuron + Linuron), when applied to the soil previous to planting in concentrations of 4 and 2.5 kgs/ha, respectively, were not toxic to the tobacco plant, but controlled most of the weeds except Veronica persica and Mercurialis annua.

In the air-cured tobacco, the Patoran residue was about 1.2 p.p.m.; it was independent of the leaf position and unchanged after fermentation, but higher in the laminae (1.2 p.p.m.) than in the midrib (0.2 p.p.m.). The Molipan residues were higher in the lower (2 p.p.m.) than in the upper leaves (1 p.p.m.) and decreased after fermentation by approximately 40%; the laminae contained more residues than the midrib.

When the tobacco was smoked as cigarettes about 4% of the Patoran residue was transferred into the mainstream smoke; another 10% was found in the smoke condensate after pyrolytical degradation to 4-bromoaniline. The transfer rates for the Molipan residues were 2.5% for monolinuron (plus 10% recovered as 4-chloroaniline) and 6% for linuron (plus 20% recovered as 3,4-dichloroaniline). The retention of these residues from the mainstream smoke by cellulose and cellulose acetate cigarette filters was of the same order of magnitude as the retention of nicotine.

Different analytical principles were applied for the residue determinations in tobacco (as azo dyes by spectrophotometry) and in smoke condensate (as bromo-iodo- and chloro-iodo-benzenes by pulsed ECD gaschromatography). The analytical procedures are given in detail.

ZUSAMMENFASSUNG

Der Herbizide Patoran (= Metobromuron) und Molipan (= Monolinuron + Linuron), vor dem Pflanzen der Setzlinge mit einer Dosis von 4 bzw. 2,5 kg/ha appliziert, wirken nicht toxisch auf die Tabakpflanze und vernichten die meisten Unkrautpflanzen mit der Ausnahme von Veronica persica und Mercurialis annua. Der Patoran-Rückstand im luftgetrockneten Tabak beträgt etwa 1 p.p.m.; er ist unabhängig von der Blatthöhe und ändert sich kaum infolge der Fermentation, ist aber bedeutend größer in der Blattspreite (1,2 p.p.m.) als in der Mittelrippe (0,2 p.p.m.). Die Molipan-Rückstände hingegen sind größer in tiefen (2 p.p.m.) als in hohen (1 p.p.m.) Blättern und erniedrigen sich durch die Fermentation um ca. 40%. Wie beim Patoran ist der Rückstand im Blattgut viel größer als in der Mittelrippe.

Beim Rauchen des Tabaks in Form von Cigaretten geht etwa 40% des Patorans in den Hauptstromrauch über; etwa 10% wird zusätzlich als 4-Bromanilin (durch pyrolytische Zersetzung aus Metobromuron entstanden) im Rauchkondensat wieder gefunden. Aus Molipan-haltigem Tabak gehen analog etwa 2,5% Monolinuron und 6% Linuron in den Hauptrauch über, während weitere 10% 4-Chloranilin und 20% 3,4-Dichloranilin als Molipan-Zersetzungsprodukte im Rauchkondensat gefunden werden.

Durch Cellulose- und Celluloseacetatfilter werden diese Stoffe dem Rauch in etwa gleichem Verhältnis wie Nikotin entzogen.

Verfahren der analytischen Bestimmung sind eingehend beschrieben.

Im Tabak wurden die Herbizidrückstände nach Überführung in Azofarbstoffe spektrophotometrisch, im Rauchkondensat nach Überführung in Brom-jod- bzw.
Chlor-jod-benzole gaschromatisc mit einem pulsierenden Elektroneneinfangdetektor bestimmt.

Résumé

Les herbicides Patoran (= métobromuron) et Molipan (= monolinuron + linuron) appliqués avant plantation, ne sont pas toxiques pour le tabac aux doses respectives de 2,5 kg/ha et 4 kg/ha; la plupart des mauvaises herbes sont détruites à l’exception de Veronica persica et Mercurialis annua.

Pour le Patoran, les résidus trouvés dans les feuilles de tabac séchées à l’air sont de l’ordre de 1,2 p.p.m., ne varient guère selon l’étage de cueillette et ne sont que peu influencés par la fermentation. La teneur du limbe en métobromuron et ses dérivés est beaucoup plus élevée que celle trouvée dans les côtes.

Pour le Molipan, les résidus trouvés dans les feuilles de tabac séchées à l’air sont plus élevés avant fermentation qu’après et décroissent des feuilles basses aux feuilles hautes. On a trouvé après fermentation: feuilles basses 2 p.p.m., feuilles médianes et hautes 1 p.p.m. Le limbe contient 2–3 fois plus de résidus que les côtes. En fumant ces tabacs sous forme de cigarettes, 4 % du Patoran sont transférés dans la fumée principale, et 10 % additionnels s’y retrouvent après dégradation pyrolytique en 4-bromaniline. Le transfert du monolinuron est de 2,5 % (plus 10 % sous forme de 4-chloraniline) et celui du linuron de 6 % (plus 20 % sous forme de 3,4-dichloraniline). Toutes ces substances sont retenues de la fumée par des filtres en cellulose et acé­tate de cellulose dans la même proportion que la nicotine.

Deux principes analytiques ont été appliqués: pour la détermination dans le tabac, la transformation en colorants azoïques suivie de spectrophotométrie; pour la détermination dans le condensat de fumée la transformation en bromiodo- et chloriodobenzènes suivie de chromatographie en phase gazeuse avec un détecteur de capture d’électrons pulsé. Les procédés y relatifs sont décrits.

* REFERENCES

6. Ciba Technical Information, Patoran 50 WP, Ref. 34.8 T 536 f.

Acknowledgment: The authors were assisted by the following co-workers to whom they express their gratitude:

F. Gilliéron (agronomic part), Miss M. Chavanne, A. Cossey and C. Salomon (Patoran residue analysis in tobacco), Mrs. L. Durand (Molipan residue analysis in tobacco), Miss. L. Flury, Miss M. Schmucki and F. Moser (Molipan transfer into smoke).

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