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Chemical substances and chemical preparations

# Control of diuron and linuron herbicides

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# Data Sheet


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# Contents

# Summary

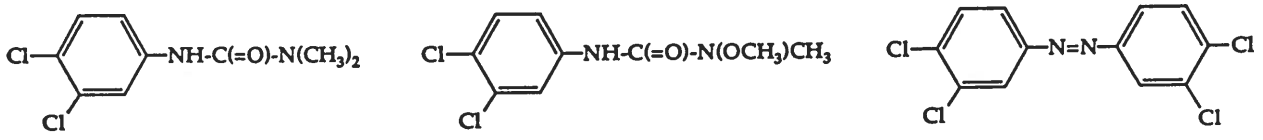
Contents of the active ingredient as well as content of an unwanted impurity 3,3',4,4'-tetrachloroazobenzene (TCAB) have been analysed in four pesticide samples from the Danish market containing diuron or linuron as the active ingredient.
The content of diuron and linuron in all four samples were within the tolerated limits specified in Danish regulations.
Levels of TCAB ranged between 0.04 ppm and 4.5 ppm. However, a satisfactory explanation for this considerable variation could not be deduced from the few results.

# 1 Introduction

Pesticides containing diuron or linuron as the active ingredient are used as herbicides for weed control on several crops such as potatoes, carots, asparagus and others as well as on a variety of seedlings grown in nurseries. These pesticides are, as all other pesticides, evaluated and approved for use in Denmark by the Danish Environmental Protection Agency (DEPA).

The active ingredients diuron and linuron have very similar chemical structure (Figure 1) and are both derivatives of 3,4-dichloroaniline, which is the starting material for the production of the technical grade active ingredient used in pesticides. 3,4-Dichloroaniline is known to contain 3,3',4,4'-tetrachloroazobenzene (TCAB, figure 1) as impurity and/or give rise to formation of TCAB during production of the pesticides *(Poland et al., 1976)*. TCAB is an unwanted contaminant in these pesticides because of its adverse biological effects, which include possible mutagenicity *(Hsia et al., 1977)* and induction of hepatic aryl hydroxylase *(Poland et al., 1976)*. TCAB has also been implicated in the outbreak of chloracne among chemical workers at a production site for 3,4-dichloroaniline *(Taylor et al., 1977)*.

The levels of TCAB occurring in diuron and linuron pesticides on the market have been investigated in several cases *(Sundström et al., 1978, Hill et al., 1981, Singh & Bingley, 1990, Hashimoto et al., 1993)*. These surveys showed great variations in the contents of TCAB ranging from below 0.001 ppm to 28 ppm. The diuron and linuron pesticides analysed were samples collected from the respective national markets (Sweden, USA, Canada and Japan). However, at the present time no such investigation has been carried out on the diuron and linuron pesticide products available on the Danish market.




I II III

*Figure 1.* Chemical structure of diuron (I), linuron (II) and TCAB (III).

Since no relation between type or origin of active ingredient and the level of TCAB can be deduced from the published investigations, they constitute an unsatisfactory basis for evaluating levels of TCAB in diuron/linuron pesticides in Denmark. It was therefore decided by DEPA and the National Environmental Research Institute (NERI) to carry out a similar survey on diuron and linuron pesticides from the Danish market as part of an on-going authority control program on pesticide formulations.
In addition to an investigation of the contents of TCAB impurity in diuron and linuron pesticides, it was decided also to carry out a determination of active ingredient content in the collected samples in order to control, whether the products complied with specifications with respect to active ingredient content. A description of the investigation and the results are reported in the following.

# 2 Experimental

## 2.1 Samples

At the time of sample collection for this investigation (April - May 1993) a total of two diuron and three linuron pesticides were approved for use in Denmark *(Miljøstyrelsen, 1993)*. Four of these five pesticides are represented (by one sample of each pesticide) in the present investigation. A list of the collected samples is shown in Table 1. The pesticide not included in the investigation was not available on the market during the period of sample collection.
All samples were analysed at NERI in the period October 1993 - January 1994.

*Table 1.* Diuron and linuron samples collected for analysis.

| NERI Reg. no. | Product | Active ingredient | Company |
|---|---|---|---|
| 3-000498 | Karmex 80 DF | Diuron | DuPont de Nemeours (Agro) |
| 3-000499 | Inter-Diuron | Diuron | Inter-Trade |
| 3-000500 | Afalon disp. | Linuron | Hoechst Danmark |
| 3-000501 | Afalon | Linuron | Hoechst Danmark |

## 2.2 Methods of analysis

*Determination of active ingredient.* Content of diuron or linuron was determined by high pressure liquid chromatography (HPLC) with UV detection using an "in house" developed method. Details of the analytical method is described in Appendix I.

*Determination of TCAB.* Content of TCAB impurity was determined by gas chromatography (GC) with electron capture detection following extraction with isooctane. Except for minor modifications the method was identical with the method reported by Singh & Bingley *(1990)*. Details of the analytical method is described in Appendix II.

# 3 Results and discussion

Results from the determination of content of active ingredient are shown in Table 2. Tolerated limits for variations in active ingredient content are also included in the table, and as it appears all four samples were found to comply with these limits.

*Table 2.* Content of active ingredient in diuron and linuron pesticide formulations.

| NERI reg.no. | Active ingredient | Content | | |
|---|---|---|---|---|
| | | Label claim | Analysis[1)] | Tolerance[2)] |
| 3-000498 | Diuron | 80 % | 80.1 ± 0.6 % | 77.5 - 82.5 % |
| 3-000499 | Diuron | 80 % | 79.5 ± 0.6 % | 77.5 - 82.5 % |
| 3-000500 | Linuron | 450 g/l, (~ 37.6 %) | 468 ± 2 g/l (~ 38.4 %) | 427 - 473 g/l |
| 3-000501 | Linuron | 50 % | 47.4 ± 0.1 % | 47.5 - 52.5 % |

1) Mean (minimum three determinations) ± 95 % confidence limits.
2) Tolerated limits for content of active ingredient according to Danish regulations in force *(Bekendtgørelse, 1993).*

Results from determination of content of TCAB are shown in Table 3. In spite of the fact that only four samples were analysed, the results indicate that levels of TCAB in diuron and linuron formulations from the Danish market are in the low ppm range or belov. These results are in good agreement with results reported by Singh & Bingley *(1990)* from analysing diuron and linuron formulations from the Canadian market. Thus, Singh & Bingley found that the content of TCAB was below 4 ppm in 24 out of 25 analysed samples, and in 22 of these samples the content of TCAB was in the range 0.7 - 4.0 ppm.

*Table 3.* Levels of TCAB found in samples of diuron and linuron herbicide formulations.

| NERI reg. no. | Active ingredient | Content of a.i. (label claim) | TCAB found[a)] µg/g |
|---|---|---|---|
| 3-000498 | Diuron | 80 % | 0.038 |
| 3-000499 | Diuron | 80 % | 0.98 |
| 3-000500 | Linuron | 450 g/l (~ 37.6 %) | 1.62 |
| 3-000501 | Linuron | 50 % | 4.42 |

a) Mean of duplicates.

The very low content (*ca.* 40 ppb) found in one of the two diuron formulations significantly differs from the three other results. A similar variation between levels of TCAB in different diuron samples was also found in a japanese investigation *(Hashimoto et al., 1993)*, where contents of TCAB found in three samples ranged between 0.7 ppb and 200 ppb. Whether these variations reflect differences in manufacturing conditions between companies or are within range of variations occuring between batches of production of pesticides cannot be deduced from the few results available.

# 4 Conclusions

Results from analysis of active ingredient content in diuron and linuron pesticide samples showed a good agreement between declared and actual content. All four analysed samples were found to comply with the tolerance limits specified in Danish regulations of pesticide products.

Determination of TCAB as an impurity in the four diuron and linuron samples showed that the content of TCAB ranged from 0.04 ppm to 4 ppm, and that one sample had a significantly lower content of TCAB than the three other samples. This difference could not be explained from the few results obtained in this investigation, but the results are in agreement with results reported in the litterature also showing contents of TCAB varying several orders of magnitude.

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# Appendix I

# Liquid chromatographic determination of diuron and linuron as active ingredients in pesticide formulations

## 1 Method

### 1.1 Principle

Diuron and linuron are determined by an "in-house" developed method using reverse phase high pressure liquid chromatography (RP-HPLC) with UV detection.

### 1.2 Apparatus

HPLC pump, autoinjector, column thermostat, UV detector and integrator or other data acquisition system.

### 1.3 Chromatographic conditions

Chromatographic column Nova-Pak $C_{18}$ (Waters), 3.9 mm x 150 mm, 4 µm; column temperature 25 °C; mobile phase acetonitrile/water, 50/50; flow rate 1.0 ml/min; injection volume 20 µl; detection wavelength 254 nm.

### 1.4 Preparation of standard

Stock solutions of standards (0.10, 0.25 and 0.50 mg/ml) are prepared by dissolving diuron reference standard (+99 %) or linuron reference standard (99.5 %) in acetonitril. Calibration standards are prepared by diluting 5.0 ml stock solution to 50 ml with HPLC mobile phase.

### 1.5 Preparation of sample

*WP formulation:* An amount of formulation sample corresponding to 25 mg diuron or linuron is weighed into a 100 ml volumetric flask. Acetonitrile is added to volume and the mixture is sonicated for 5 min. 5.0 ml is diluted to 50 ml with HPLC mobile phase and the solution is filtrated before chromatographic analysis.
*SC formulation:* An amount of formulation sample corresponding to 25 mg linuron is weighed and suspended in 5.0 ml water followed by addition of acetonitrile to 100 ml. The mixture is sonicated for 5 min. Finally, 5.0 ml is diluted to 50 ml with HPLC mobile phase and the solution is filtrated before chromatographic analysis.

### 1.6 Determination

Determination is performed by analysing the sample (duplicate injections) in series with three concentrations of calibration standard; standard injections bracketing the sample. A calibration curve is constructed for each series by plotting peak areas against standard concentrations. Identification is done by comparing retention time of sample and standard (external standard). Typical chromatograms of standards and samples are shown in figure 1.

## 2 Evaluation of method performance

### 2.1 Linearity

Linearity of the HPLC analysis has been examined by injecting standards (6 different concentrations between 0.5 µg/ml and 50 µg/ml) and plotting peak areas against concentrations. A satisfactory linearity ($r = 0.9997$) was found.

### 2.2 Precision

Precision of the method (calculated as relative standard deviation of six determinations on the same sample) was found to 0.1 % (diuron, WP), 0.2 % (linuron, WP) and 0.5 % (linuron, SC), respectively.

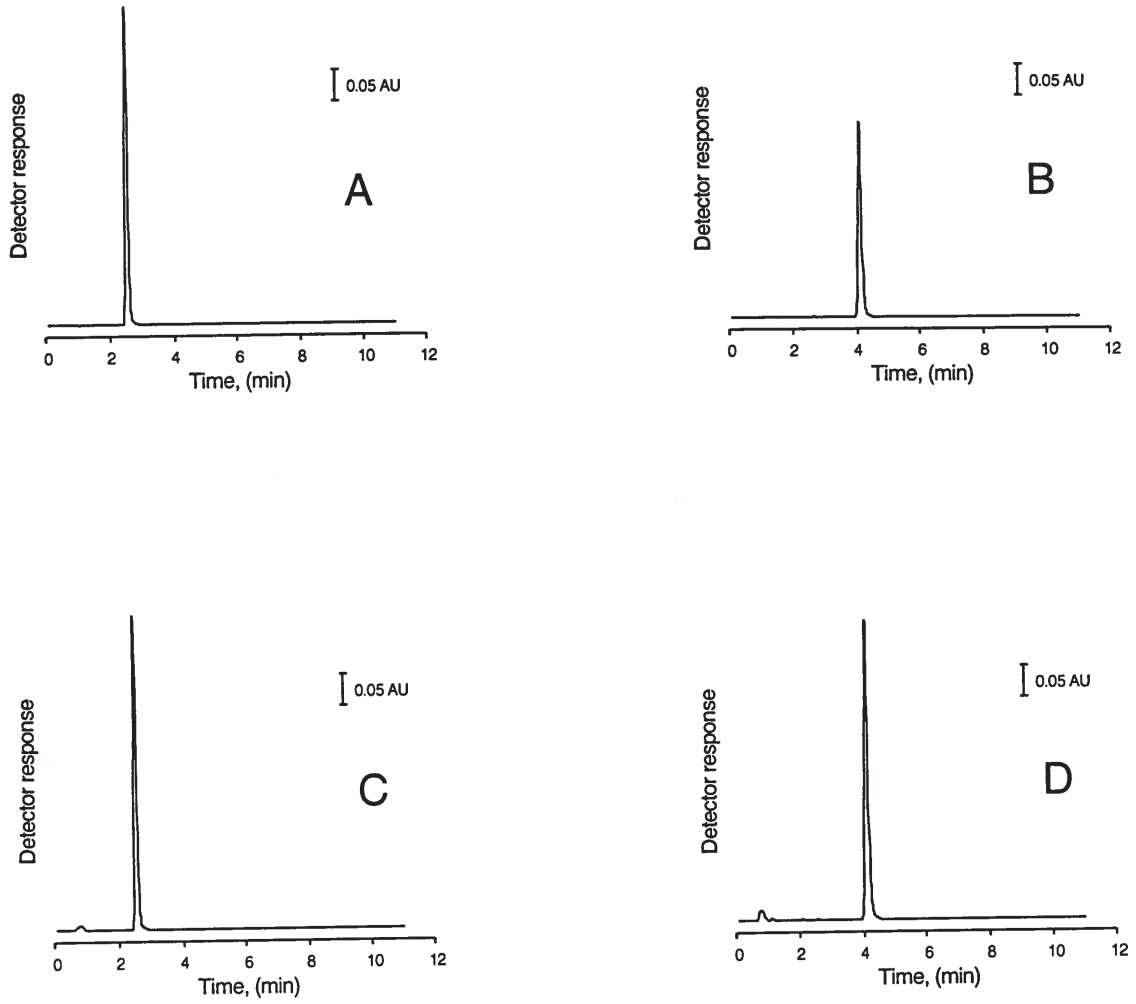


*Figure 1.* Typical chromatograms from analysis of diuron and linuron in pesticide formulations. A = diuron standard (0.25 mg/ml), B = linuron standard (0.25 mg/ml), C = diuron sample (WP formulation) and D = linuron sample (SC formulation).

# Appendix II.

# Gas chromatographic determination of 3,3',4,4'-tetrachloroazobenzene (TCAB) as impurity in diuron and linuron pesticide formulations

## 1 Method

### 1.1 Principle

The method is essentially identical to the method described by Singh & Bingley *(1984)*. The method includes liquid extraction of TCAB from an aqueous suspension of formulation sample using isooctane as extraction solvent. Following silica gel column clean-up the sample is analysed by capillary column gas chromatography (GC) with electron capture detection.

### 1.2 Apparatus

Gas chromatograph equipped with $^{63}Ni$ electron capture detector and integrator or other data acquisition system.

### 1.3 Chromatographic conditions

Chromatographic column 25 m x 0.32 mm id CP-Sil 8CB (Chrompack) fused silica capillary column, film thickness 0.25 µm. Operating conditions: injector, 250 °C; detector, 350 °C; column head pressure, 16 psi; column temperature program, 150 °C to 280 °C at 10 °C/min, 280 °C for 2 mins. Injection: 1 µl, split, split ratio *ca.* 1:20.

### 1.4 Preparation of standard

Calibration solutions of standards (4 µg/l and 40 µg/l) are prepared by diluting a TCAB standard solution, 10 ng/µl in cyclohexane (Riedel-de Haën) with cyclohexane.

### 1.5 Preparation of sample

1 g formulation sample is dissolved/suspended in 100 ml mixture (methanol-water-hydrochloric acid, 90/9/1) in a 125 ml separating funnel and extracted twice with 25 ml isooctane. The combined isooctane extract is washed with 25 ml water, dryed (anhydrous sodium sulfate) and transferred quantitatively to a round bottom flask. The extract is concentrated on a rotary evaporator to 1 - 2 ml.

A column (25 cm x 1.4 cm id) is slurry packed with 15 g silica gel (activated at 125 °C overnight) using isooctane and topped with a layer (*ca.* 1.5 cm) of anhydrous sodium sulfate.

The concentrated extract is quantitatively transferred onto the silica gel column using two 1 ml portions of 10 % ethyl acetate in

isooctane and the column eluted with 100 ml 10 % ethyl acetate in isooctane. The eluate is concentrated to *ca.* 1 ml on rotary evaporator and quantitatively transferred to 10 ml volumetric flask and diluted to volume with cyclohexane.

A single blind sample is analysed in series with each sample following the described sample preparation except that sample addition is omitted.

### 1.6 Determination

Determination is performed by analysing the sample (duplicate injections) in series with the two concentrations of calibration standard; standard injections bracketing the sample. Peak area measurements are used for calculations.
Identification is done by comparing retention time of sample and standard (external standard). Verification of peak identification is performed by injecting both samples and standard solutions on a confirmatory column (60 m x 0.25 mm id DB-1701 fused silica capillary column, film thickness 0.15 µm).

Typical chromatograms of blind, standard and sample from the analysis are shown in figure 1.

## 2 Evaluation of method performance

### 2.1 Linearity

Linearity of the GC analysis has been examined by injecting standards (6 different concentrations between 0.004 µg/ml and 2.0 µg/ml) and plotting peak areas against concentrations. Acceptabel linearity could not be achieved throughout the entire range due to a curved relationship between concentration and peak area. Therefore, quantitation was performed as single point calibration using appropriate dilution of sample to give peak area approximately equal to one of the calibrations standards.

### 2.2 Precision

Precision of the method was calculated as the mean relative standard deviation obtained by duplicate analysis on four different samples in this investigation. A mean relative standard deviation of 4.0 % was found.

### 2.3 Recovery

Determination of recovery was performed on each sample (duplicate measurement) by comparing results obtained without and with addition of a known amount of TCAB to the sample. A mean recovery (four different samples) of 89.5 % was found, which was considered acceptable for this analysis.

## 2.4 Detection limit

Method detection limit (S/N = 3) was calculated from triplicate analysis of a diluted standard solution treated as a sample. A detection limit of 0.4 pg TCAB (injected amount) corresponding to a content of 4 ng/g sample was found.

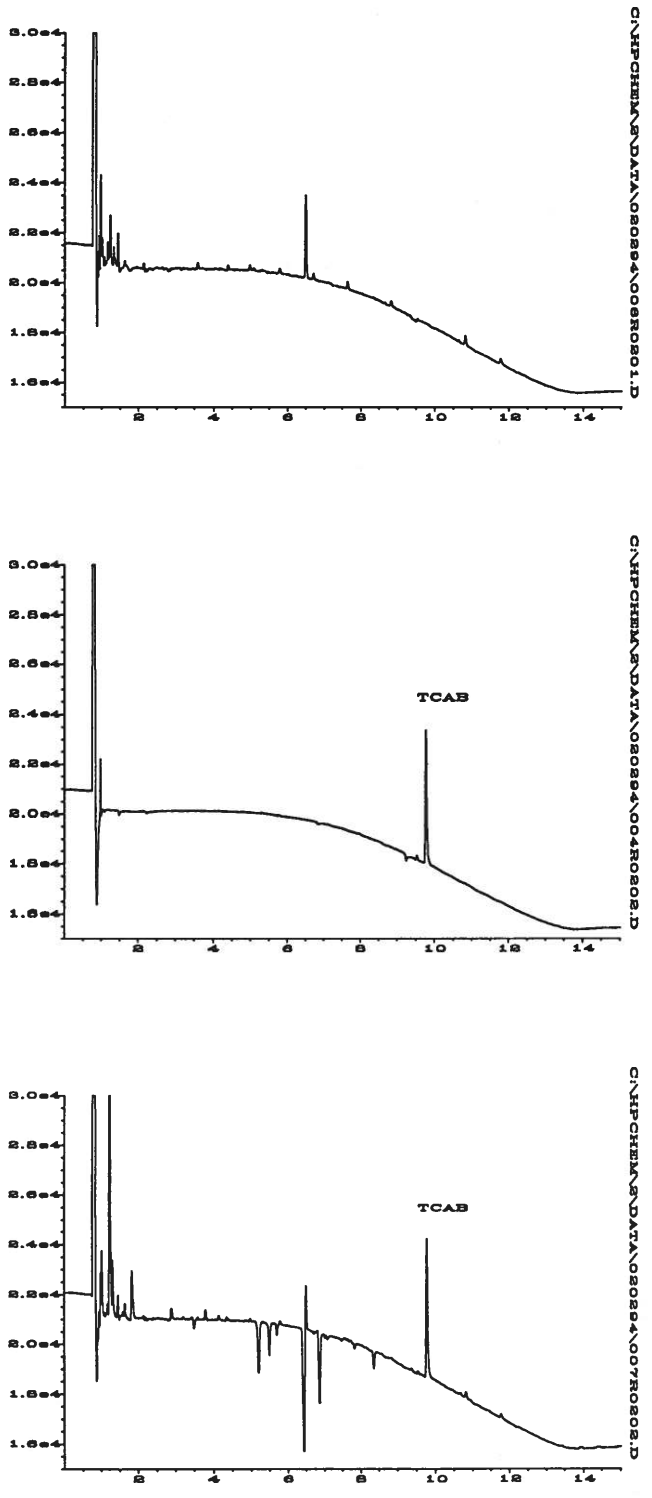


*Figure 1.* Typical chromatograms from analysis of TCAB. A = blind, B = standard (40 µg/l) and C = sample (TCAB content 0.98 µg/g).

# National Environmental Research Institute

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