# Extraction of 3-Amino-1,2,4-triazole (Amitrole) and 2,6-Dichloro-4-nitroaniline (DCNA) from Soils

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New solvent mixtures were developed for the extraction of 3-amino-1,2,4-triazole (amitrole) and 2,6-dichloro-4-nitroaniline (DCNA) from soils. Concentrated ammonium hydroxide and glycol (1 + 4) gave much better recoveries of amitrole from soil than water extraction. Hydrochloric acid (1 *N*), acetone, and glycol (1 + 1 + 8) were much better than hexane for the extraction of DCNA from soils.

The failure to recover pesticides from soils by solvent extraction may be due to the actual disappearance of the chemical from the soil by leaching, evaporation, or by microbiological destruction. However, the pesticide may become tightly bound in the soil and resist extraction. Sund (1956) attempted to extract amitrole from several soils, and found the degree of recovery varied with different soils. The poorest recoveries were obtained from soils high in clay and organic matter content. He tried solutions of acids, bases, and salts, but found no better solvent than water. Ercegovich (1958) confirmed these findings, but used a saturated solution of barium hydroxide as solvent for the extraction of amitrole, since this solution gave a clear extract.

Both amitrole and DCNA can be destroyed by microbiological organisms in soils (Bondarenko, 1958; Groves and Chough, 1970).

## EXPERIMENTAL

**Amitrole.** In this investigation the search for better solvents for the extraction of amitrole from soils resulted in findings similar to those of Sund and Ercegovich with respect to solutions of acids, bases, and salts. However, we found that glycerol and ethylene glycol were somewhat better than water for extracting amitrole, and that a mixture of ammonium hydroxide (*ca.* 13 *N*) and glycol (1 + 4) would extract far more amitrole from soils than water. Several soils were used to investigate the extraction of amitrole, but of the soils tried, recovery was most difficult from Palouse silt loam. This soil had a clay content (vermiculite) of 25% and an organic matter content of 4%. After application of $^{14}C$-labeled amitrole to this soil, labeled carbon dioxide was evolved (Bondarenko, 1958), indicating probable microbiological action. No labeled carbon dioxide was evolved from the steam sterilized soil. The destruction of amitrole in soils could be chemical, since amitrole is destroyed in the presence of riboflavin and light (Hilton, 1961; Sutherland, 1961). We found that amitrole-$^{14}C$ in water solution with suspended riboflavin evolved labeled carbon dioxide in sunlight but not in the dark, and that all of the amitrole was decomposed if successive additions of riboflavin were made. Riboflavin is decomposed by light. This mechanism of decomposition is unlikely in soils, since only surface layers are exposed to light.

Samples of Palouse soil (10-g), sterilized and not sterilized, were placed in 150-ml Erlenmeyer flasks, and an aqueous solution of amitrole was added to make an herbicide content of 2 ppm and the water content of the soil about 22%. At sampling times the samples were dried in a vacuum desiccator over magnesium perchlorate, and then 5 ml of ammonium hydroxide, 20 ml of glycol, and some glass beads were added to one set of the flasks, 25 ml of water was added to another set, and the flasks were shaken on a wrist shaker for 70 min. The flask contents were then centrifuged at high speed until a clear supernatant formed. This supernatant was analyzed by the method of Storherr and Burke (1961), or, where the amitrole content of the soil was above 7 ppm, by the following modification of that method.

The supernatant (10 ml) was placed in a 150-ml beaker, 5 ml of water and 2.5 ml of concentrated $H_2SO_4$ were added, and the mixture was heated on a hot plate. One-tenth gram of Nuchar-190 *N* activated charcoal was added and the mixture was boiled 5 min. It was then filtered hot through a Whatman No. 42 filter paper. The filter was washed with hot water and the filtrate and washings were made to 25 ml.

A 5.0-ml aliquot of this solution, 0.5 ml of water, and 1.0 ml of $H_2SO_4$ were placed in a 150-ml beaker. Sodium nitrite solution (0.5%, 1.0 ml) was added and let stand 7 min with occasional swirling. Sulfamic acid solution (5%, 1.0 ml) was added and the beaker swirled vigorously. Finally, 1.0 ml of the dye solution [*N*-(1-naphthyl)ethylenediamine dihydrochloride, 1% aqueous solution] was added and the mixture let stand 5 min, then read on a spectrophotometer at 455 mμ. A standard curve was prepared by mixing in a 150-ml beaker 3.4 ml of water containing from 0 to 20 μg of aminotriazole, 1.6 ml of glycol, 1.5 ml of concentrated $H_2SO_4$, and then the sodium nitrite, sulfamic acid, and dye solutions as described for the sample. The sensitivity of this modification may be increased by using a larger aliquot of the supernatant and by making the filtrate and washings to a smaller volume, provided that the concentrations in the standard mixtures are correspondingly modified.

The recoveries of amitrole from the above described samples are shown in Table I. A reduction in recoveries with time is apparent for all samples, but is greater in the nonsterile soil extracts. The recovery reduction is partly attributable to microbiological decomposition in the case of the nonsterile soil samples, but the decreased recoveries from the sterile samples must have other causes, probably binding in the clay and organic matter of the soil. The decrease in recovery with time in this case may be due to the time required for the pesticide to diffuse to binding sites. The better extraction efficiency of the ammonium hydroxide–glycol mixture in comparison to water is obvious.

## DCNA

When $^{14}C$-labeled DCNA was applied to samples of Walla Walla silt loam from fields where DCNA had been applied for several years for the control of white rot (*Sclerotium cepivorum*) in onions, labeled carbon dioxide was evolved. Incubating these soils with added DCNA increased, over a

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period of several weeks, rate of destruction of applied DCNA. The Walla Walla soil had a clay content of about 12% and an organic matter content of 3%. No DCNA had been previously applied to the Palouse soil and it evolved no labeled carbon dioxide. The ammonia–glycol mixture used for the extraction of amitrole was useless for extraction of DCNA. However, a mixture of 1 *N* hydrochloric acid, acetone, and glycol (1 + 1 + 8) gave higher recoveries than hexane extraction. Many chlorinated pesticides can be extracted from soils successfully by hexane. The recoveries from sterile and nonsterile laboratory-treated Walla Walla soil samples are shown in Table II. The recoveries from sterile Walla Walla soil samples were higher than from the nonsterile samples.

A field plot of applied DCNA, for the study of persistence of the pesticide, was prepared by removing the top 6 in. of soil from the plot and mixing it in a cement mixer with the calculated amount of formulated (8%) DCNA to make the pesticide content 15.1 ppm. Samples taken from this field plot at various times were extracted with hexane and with the 1 + 1 + 8 mixture. The latter samples were dried in a vacuum desiccator over magnesium perchlorate prior to extraction. Aliquots of the 1 + 1 + 8 mixture extracts were diluted with water, 2 to 3 volumes, extracted with benzene, and the benzene extracts were analyzed by gas chromatography (Cheng and Kilgore, 1966).

The samples extracted with hexane were not previously dried and were similarly analyzed. The results are shown in Table III. Here the DCNA was applied in formulated form and such applications were easier to recover by extraction than laboratory applied pesticide. In the laboratory samples the application was in the form of water–10% acetone solution, and the distribution was more nearly homogeneous. If the application was made as an acetone solution, crystals were formed after evaporation of the solvent, and a long time was required for the DCNA to form a homogeneous distribution by diffusion. Extraction from such samples resulted in high recoveries.

The superiority of the 1 + 1 + 8 mixture for the extraction of these field-applied DCNA samples is apparent. Probably less effective recoveries would have been obtained if the soil had contained more moisture, or if the DCNA had been applied in aqueous solution.

Another solvent mixture was developed for DCNA extraction that gave results nearly as good as those obtained with the 1 + 1 + 8 mixture, and the manipulations were easier and more rapid. Extraction of the vacuum-dried sample was carried out by the addition of concentrated hydrochloric acid and benzene, 1 + 5, and shaking. The benzene layer usually separated quickly, often without centrifuging, and could be injected into a gas chromatograph without further purification.

**Table I. Extraction of Amitrole from Soils with Water and Ammonium Hydroxide–Glycol (5 + 20) Mixture**

| Time after application (days) | Nonsterile soil | | Sterile soil | |
|---|---|---|---|---|
| | Water (%) | (5 + 20) (%) | Water (%) | (5 + 20) (%) |
| 0 | 48.1 | | | |
| 1 | 36.7 | 97.6 | 49.6 | 97.3 |
| 12 | 21.3 | 55.9 | 36.1 | 77.9 |
| 17 | 3.2 | 15.2 | 36.9 | 67.7 |

**Table II. Recovery of DCNA from Sterile and Nonsterile Soils with Mixture 1 + 1 + 8**

| Time after application (days) | Recovery, % | |
|---|---|---|
| | Sterile soil | Nonsterile soil |
| 1 | 76.2 | 62.3 |
| 16 | 71.6 | 36.0 |
| 29 | 67.0 | 30.4 |
| 66 | 68.2 | 24.0 |

**Table III. Extraction of DCNA from Treated Field Plot Soil with Hexane and 1 + 1 + 8**

| Time after application (days) | Recovery % | |
|---|---|---|
| | Solvent:hexane | 1 + 1 + 8 |
| 0 | 90 | |
| 13 | 73 | |
| 35 | 48 | |
| 66 | 38 | |
| 72 | 35 | |
| 98 | 26 | 106 |
| 98 | 28 | 101 |
| 700 | | 106 |
| 700 | | 104 |

## DISCUSSION AND CONCLUSIONS

If the failure to recover these pesticides from soils was due to their elimination from the soils by such processes as volatilization, leaching, or biological or chemical destruction, then no improvement in extraction effectiveness would be possible. While these elimination processes may occur in the practical use of the pesticides, it is possible to avoid most of these effects in laboratory experiments by soil sterilization and the use of closed systems. Where the extraction effectiveness can be changed, some sort of reversible binding is involved. Some of the mechanisms suggested are that polar compounds may be bound in the clay–organic matter ion exchange systems similar to the binding of nutrient ions; that entrapment between atom-layers of expandable lattice clays may occur; that insoluble compounds or complexes may be formed; and that adsorption may occur due to electrostatic or residual valency forces. Sugar and amino acid derivatives of amitrole have been reported in plants but not soils.

Solvent mixtures have been described for the recovery of amitrole and DCNA from soils that were a conspicuous improvement over previously used solvents. These solvents will not give perfect results for all soils and conditions, but the examples given were soils that have presented difficulties in the extraction of these pesticides. Doubtless better solvent mixtures will be found in the future.

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