wise, structure proof by tlc based on cochromatography with standards can be inaccurate and misleading if precautions are not taken to remove radioactive natural products resulting from extensive biodegradability.

Methoprene and all characterized metabolites are nontoxic to mammals (LD_{50} >5000 mg/kg, rat). In fact, hydroxycitronellic acid (7) is an air oxidation product of one of the most widely used perfume bases (hydroxycitronellal) and methoxycitronellal (5) itself is widely used as a floral fragrance (Arctander, 1969). Since methoprene IGR is rapidly biodegraded by both alfalfa and rice to innocuous metabolites, it should be considered an ecologically acceptable alternative for pest control.

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LITERATURE CITED

Arctander, S., "Perfume and Flavor Chemicals," Steffen Arctander, Elizabeth, N. J., 1969, pp 1728, 1870.
Bonnafous, J., Mani, J., Olivé, J., Mousseron-Canet, M., *Tetrahedron Lett.*, 1119 (1973).
Dark, W. A., Limpert, R. J., *J. Chromatogr. Sci.* **11**, 114 (1973).
Gückel, W., Synnatschke, G., Rittig, R., *Pestic. Sci.* **4**, 137 (1973).
Henrick, C. A., Staal, G. B., Siddall, J. B., *J. Agr. Food Chem.* **21**, 354 (1973).
Isler, O., Ed., "Carotenoids," Birkhäuser Verlag, Basel, 1971, p 117.
Menn, J. J., Beroza, M., Ed., "Insect Juvenile Hormones—Chemistry and Action," Academic Press, New York, N. Y., 1972, pp 3-341.
Miura, T., Takahashi, R. M., *J. Econ. Entomol.* **66**, 913 (1973).
Pokorny, S., Coupek, J., Luan, N. T., Pokorny, J., *J. Chromatogr.* **84**, 319 (1973).
Rosenblum, C., Trenner, N. R., Wolf, D. E., *J. Label. Compounds* **7**, 225 (1971).
Schaefer, C. H., Dupras, M. F., *J. Econ. Entomol.* **66**, 923 (1973).
Schaefer, C. H., Wilder, W. H., *J. Econ. Entomol.* **66**, 913 (1973).
Schmit, J. A., Williams, R. C., Henry, R. A., *J. Agr. Food Chem.* **21**, 551 (1973).
Schooley, D. A., Bergot, B. J., Creswell, K. M., Quistad, G. B., Staiger, L. E., Siddall, J. B., manuscript in preparation, 1974.
Stalling, D. L., Tindle, R. C., Johnson, J. L., *J. Ass. Offic. Anal. Chem.* **55**, 32 (1972).
Tschesche, R., Struckmeyer, K., Wulff, G., *Chem. Ber.* **104**, 3567 (1971).

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Fate of Neodecanoic Acid in Onion and Soil

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The metabolism of ^{14}C-carboxy-labeled neodecanoic acid (NDA) was studied in onions on which the compound is used for drying tops. It appeared that the compound was immobile in the plant and did not decompose appreciably. In muck soil about 10% of the added NDA decomposed with the release of $^{14}CO_2$ during the first 12 days after which $^{14}CO_2$ evolution was negligible. When onion foliage was added to muck soil containing labeled NDA, $^{14}CO_2$ was continuously evolved over a period of 30 days with little change in rate of evolution.

Neodecanoic acid (NDA) is a mixture of di-α-branched decanoic acids which have been shown to be useful for drying onion tops to facilitate harvesting (Pendergrass *et al.*, 1969; Isenberg and Abdel-Rahman, 1972). It is manufactured by the Enjay Chemical Co., Linden, N. J., and marketed by Agway, Inc., under the trade name Topper 5-E. Using thin-layer chromatography (Pendergrass *et al.*, 1969) harvest residues of NDA in onion bulbs treated at the rate of 55 kg/ha were estimated to be about 1 ppm. In the work reported, ^{14}C-carboxy-labeled NDA was used to determine its possible translocation from onion tops to bulbs and its stability in onions and soil.

EXPERIMENTAL SECTION

Plant Studies. A preliminary study was performed to determine the general pattern of NDA translocation in intact onion plants. A series of onions, cultivar Elba Globe, grown to maturity under greenhouse conditions of an 18-hr 27° day and 22° night was treated with 5-μl droplets of NDA containing 0.5 μCi of ^{14}C in xylene as the carrier. (Xylene is the solvent used in the commercial formulation of NDA.) This material was applied to bulb scales, softening neck tissue, green leaf, and the foliage at the point where the youngest leaf emerged through the pore in the neck. Plants were held intact for 15 days for translocation to occur and then dried and exposed for 1 week to Kodak Royal X-Omat medical X-ray film before development.

In subsequent greenhouse experiments, mature Elba Globe onion plants were sprayed with a radioactive NDA formulation in a plastic enclosure using spray conditions that were designed to simulate field application (Isenberg and Abdel-Rahman, 1972). Some bulbs were completely covered by the growing medium, while others were partially exposed. The sprayer was operated at 2.11 kg/cm^2 pressure and a formulation consisting of NDA (66.4%, 28.4 μCi of ^{14}C), xylene (28.6%), Atlox 3404 (1%), and Atlox 3403F (4%) was diluted with water equivalent to rates of 33.6 and 44.8 kg of NDA/ha. Agway Booster +E, marketed by Agway, Inc., Syracuse, N. Y., was used as a surfactant at a rate of 8.1 kg/ha. Following treatment, the plants were held in a greenhouse for 11 days without irrigation. Several control plants were treated in a similar fashion with NDA omitted. At harvest, each onion plant was subdivided into foliage, bulb, and outer two loose, dry bulb scales. All tissues were stored in a freezer at −20° up to, but not exceeding, 10 weeks.

In preparation for analysis of radioactivity, the tissues were freeze-dried and ground in a Wiley mill. The powdered samples were Soxhlet extracted with 150 ml of di-

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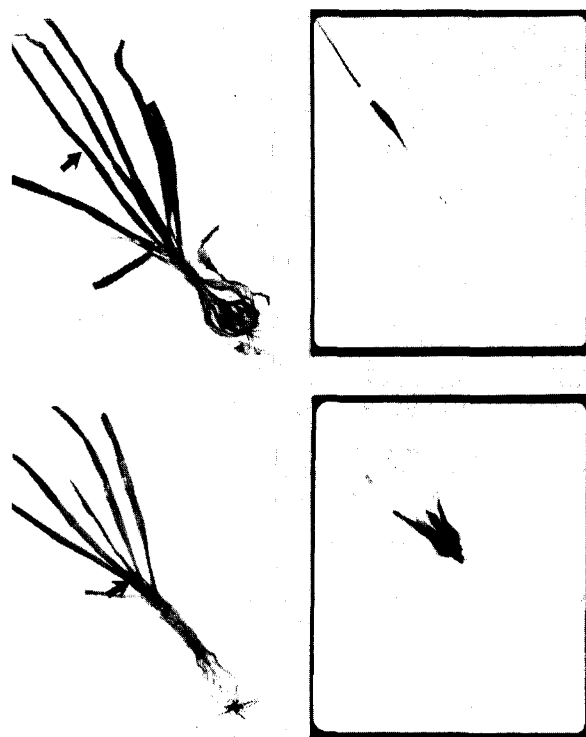

Figure 1. [^{14}C]NDA applied to green plant tissue (left) and the autoradiographs (right): (upper) application to green leaf; (lower) application to neck at the point where youngest leaf emerges.

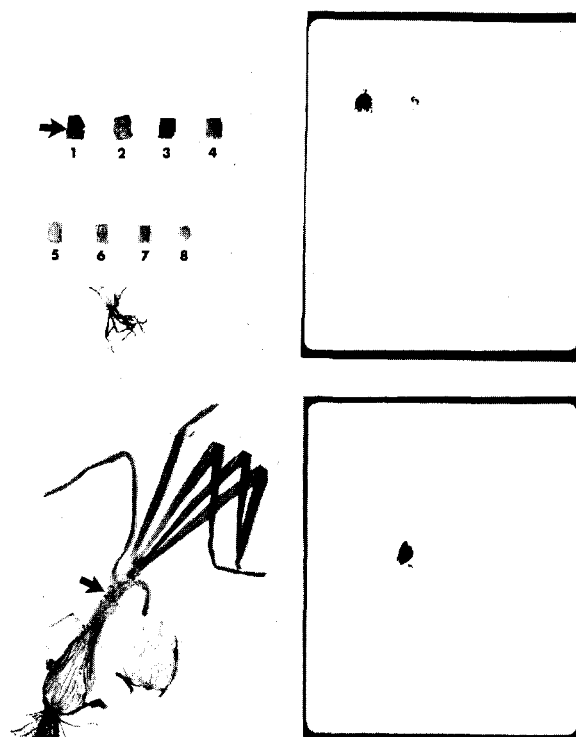

Figure 2. [^{14}C]NDA applied to dry scale tissue (left) and the autoradiographs (right): (upper) application to surface of bulb; successive layers of scales are shown (number 1 is outermost scale, number 8 is innermost); the first 4 were dry, remainder were fleshy; (lower) application to surface of soft neck.

ethyl ether for 8 hr. The ether extracts were evaporated to 30–50 ml and partitioned against three 30-ml portions of 1% w/v NaOH. The aqueous alkali solutions were combined, acidified with 4 ml of 6 *N* HCl, and extracted with three 30-ml volumes of ether. The combined ether solutions were evaporated to 10 ml and two 1-ml aliquots of each sample were assayed for radioactivity using a Model 3310 Packard liquid scintillation counter. The scintillation solutions contained 2,5-diphenyloxazole (PPO) (0.6%), 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) (0.005%), and 10 ml of toluene. The counting efficiency of each sample ranged in value from 65 to 87% and was determined by the internal standardization method using [^{14}C]toluene. The measured sample radioactivity was appropriately corrected for counting interferences.

The possible presence of metabolites in ether extracts of [^{14}C]NDA treated onion foliage was investigated with the aid of thin-layer chromatography (tlc) and autoradiography. The foliar extract (50–100 μl) and [^{14}C]NDA standards were spotted on silica gel H and developed either in isooctane–acetone–acetic acid (83:15:2, v/v) (Mizany, 1967) or in petroleum ether–diethyl ether–acetic acid (70:30:1, v/v). The plates were then exposed to Kodak medical X-ray film (Royal X-Omat) for 10 weeks prior to development.

Soil Studies. The fate of NDA in soil was examined in a muck (peat) soil with a previous history of onion growth in Elba, N. Y. The soil was treated with 30 ppm of NDA (2.77 μCi of ^{14}C) and 20% water. This soil mixture was placed in a tightly sealed bell jar through which air was pumped at a rate of 50–150 cm^3/min. Radioactive materials were collected in a solution consisting of 27 ml of phenylethylamine, 27 ml of methanol, and 46 ml of toluene contained in a gas washing bottle (Woeller, 1961). The wash solution was replaced at intervals of 1–2 days and its radioactivity was generally measured with a counting efficiency of 59% after adding PPO (0.5%) and POPOP (0.01%).

Because onion foliage is detached from the bulb and left in the field during the harvesting operation, the influence of this tissue on the fate of NDA in soil was studied in Elba muck soil containing water (20%) and onion tops (2.2% fresh weight basis) previously treated with 0.071 μCi of [^{14}C]NDA. The radioactive onion tissue was ground to a coarse consistency in a Wiley mill with the aid of Dry Ice before addition to the soil. Collection and measurement of radioactivity were performed as previously described.

RESULTS AND DISCUSSION

Whole-plant studies on translocation, employing autoradiographic techniques, provided evidence that NDA remains in the area of application. On green foliage tissue, some ^{14}C-labeled material spread distal to the treatment site (Figure 1). However, even when applied to the top of the neck, NDA did not appear to percolate down between layers of leaf tissue toward the bulb. On dry scales covering the neck and bulb, the NDA was essentially immobile (Figure 2). When applied to the bulb surface, the radioactive residue was traced only to the first two of four dry outer scales, all of which would be removed and discarded prior to consumption of the bulb. At the time of physiological maturity, the stage of these plants when treated, there is swelling of the bulb as sugars synthesized in the foliage are translocated for storage. However, ^{14}C-labeled material applied to the neck surface was not picked up in the translocation stream but remained *in situ*. In these treatments, no radioactive exposure was found associated with edible bulb tissue which suggests that NDA is not very mobile in the plant.

In subsequent greenhouse studies, where onion plants were exposed to [^{14}C]NDA under field-simulated spray conditions, the various tissues from these treated plants were analyzed for radioactivity. Application of either 33.6 or 44.8 kg of NDA/ha resulted in marked foliar damage similar to that observed for these application rates under field conditions (Isenberg and Abdel-Rahman, 1972). As

Table I. Residues of Equivalent Neodecanoic Acid[a] in Various Onion Tissues at Harvest

Onion tissue	Residue,[b] ppm			
	33.6 kg of NDA/ha		44.8 kg of NDA/ha	
	Covered[c]	Exposed[d]	Covered	Exposed
Bulbs (outer two scales removed)	0.39 ± 0.33	0.31 ± 0.32	3.32 ± 2.85	4.13 ± 2.77
Outer two bulb scales	19.2 ± 15.0	5.4 ± 5.0	70 ± 34	22 ± 6
Onion tops	231 ± 198	280 ± 219	340 ± 208	270 ± 76

[a] Based on measurements of total radioactivity. [b] Mean of five replicates ± standard deviation. [c] Treated onion plants with soil-covered bulbs. [d] Treated onion plants with partially exposed bulbs.

shown in Table I, radioactivity was detected in all parts of the treated onions including tops, bulbs, and outer two bulb scales. Large deviations in residue levels were observed in these tissues and this may reflect variations in amount of foilage per plant. As expected, the highest residue levels were generally observed in the foliage receiving the higher rate of NDA. On the other hand, the bulbs of plants treated at the 44.8 kg/ha rate contained nearly 10 times as much residue as in the bulb tissues of plants treated at the lower rate. The reason for this large difference in bulb residue levels at the two application rates is not readily apparent. The recoveries of 1, 0.1, or 0.01 ppm of NDA added to three replicate plants were 95 ± 6, 86 ± 7, and 62 ± 34%, respectively. Radioactivity in the bulb extracts was 3 to 30 times greater than the "background" radioactivity in extracts from the control plants.

The soil coverage of onion bulbs had a significant effect on residue levels in certain bulb tissues. For instance, plants with soil-covered bulbs contained three times more residue in the outer two bulb scales than that in analogous scales of onion plants with exposed bulbs (Table I). The cause of this apparently anomolous condition is not known; however, it may indicate that the soil acted as an NDA storage reservoir and aided the entry of NDA into these tissues. In spite of this significant difference in outer-scale residue levels, the amount of soil coverage had little apparent effect on residues in the remaining onion bulb tissues. For example, plants with soil-covered and partially exposed bulbs receiving the same NDA treatment had similar bulb residue levels (Table I).

The low harvest residue levels in onion bulbs reported in this investigation and field experiments (Isenberg and Abdel-Rahman, 1972) suggest that NDA remains primarily in the tops and outer dry bulb scales that are generally removed from onions prior to marketing. Indeed, the residue levels in outer bulb scale layers and foliage were significantly greater than that in the bulb after removal of loose scales as demonstrated by the 65- to 903- and 5- to 49-fold higher levels in the tops and scales, respectively (Table I).

The chemical nature of the residue in treated onion plants was examined with the aid of tlc and autoradiography. Because the minute quantity of residue in the bulbs precluded successful analysis by the methods employed, the residue of this tissue was not analyzed. Development of foliar extracts from treated plants in either of the two solvent systems and examination by autoradiography revealed a single spot with the same R_f as that of authentic [^{14}C]NDA. This indicated that NDA was not metabolized in the onion foliage during the 11-day treatment period which is about as long as field-sprayed onions would remain before harvest. Further evidence that NDA is apparently resistant to breakdown in onions was obtained by measuring the release of radioactivity from three onion plants contained in a bell jar, each treated with 0.33 μCi of [^{14}C]NDA applied to the foliage as a single spot. Only trace quantities of radioactivity from the plants were detected during a 3-week period (Figure 3).

The evidence that NDA residues are present primarily

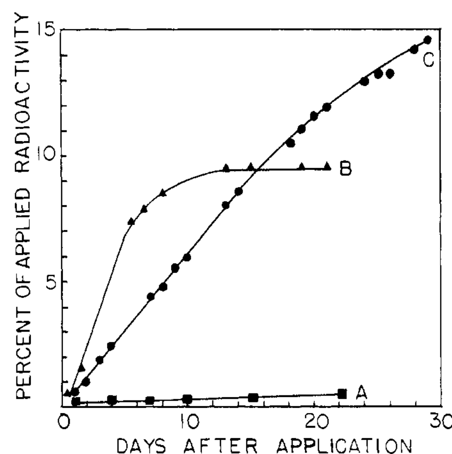

Figure 3. Release of radioactive materials from (A) [^{14}C]NDA treated onion plants, (B) treated soil, and (C) soil plus radioactive onion debris.

in onion foliage at the time of harvest and that they are relatively resistant to breakdown in the intact plant led to the study of the fate of NDA residues in soil. Figure 3 shows that radioactive materials are slowly released either from soil spiked directly with [^{14}C]NDA or from soil containing radioactive onion foliage. In the soil containing [^{14}C]NDA but no onion tissue, a plateau level of released radioactivity corresponding to about 10% of the applied dose was observed after 10 days (Figure 3). This apparent termination of NDA breakdown in the soil suggests either that the microbiological activity was limiting in the organic soil mixture or that only one of the several neodecanoic acid isomers in the NDA formulation was degraded during the course of the study. On the other hand, radioactive materials continued to be released from the soil containing [^{14}C]NDA onion debris over a 4-week period, thus suggesting the eventual release of a substantial portion of the radioactivity from the soil mixture. The rather persistent nature of NDA in soil and plant material may in part be due to the neo configuration on the carbon α to the carboxyl which would prevent degradation by β oxidation, a metabolic pathway followed in the decomposition of phenoxyalkanoic acid herbicides in plants (Gutenmann and Lisk, 1963) and soils (Gutenmann *et al.*, 1964). Since it is the carboxyl carbon which is labeled the measurement of evolved radioactive carbon dioxide could only result from a decarboxylation reaction. It is possible, however, that degradation of the molecule at positions other than the carboxyl may proceed simultaneously and therefore would not be measured by the procedure.

The results of this investigation support the field observations (Pendergrass *et al.*, 1969) that NDA acts in a contact rather than a systemic manner. The material is virtually all retained at the surface of application leaving the edible bulb tissue nearly uncontaminated. At a spray application level of NDA which brings about acceptable foli-

age desiccation under field conditions, 33.6 kg/ha (Isenberg and Abdel-Rahman, 1972), the bulb residue is less than 0.4 ppm. The problem of minimum detectable levels of NDA encountered before (Pendergrass *et al.*, 1969) was alleviated by using ^{14}C-labeled material.

LITERATURE CITED

Gutenmann, W. H., Lisk, D. J., *J. Agr. Food Chem.* **11**, 304 (1963).
Gutenmann, W. H., Loos, M. A., Alexander, M., Lisk, D. J., *Soil Sci. Soc. Amer. Proc.* **28**, 205 (1964).
Isenberg, F. M., Abdel-Rahman, M., *HortScience* **7**, 471 (1972).
Mizany, A. I., *J. Chromatogr.* **31**, 96 (1967).
Pendergrass, A., Isenberg, F. M., St. John, L. E., Jr., and Lisk, D. J., *HortScience* **4**, 294 (1969).
Woeller, F. H., *Anal. Biochem.* **2**, 508 (1961).

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Persistence and Metabolism of Thiophanate-methyl in Soil

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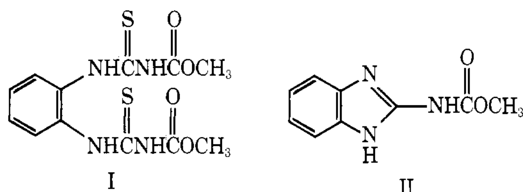
The fungicide thiophanate-methyl, 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene, underwent rapid conversion in soil to methyl 2-benzimidazolecarbamate (MBC). The rate of conversion was more than four times faster in soil at pH 7.4 than in soil at pH 5.6. The rate of thiophanate-methyl conversion to MBC was also reduced by steam treatment of the soil. Soil incubated 51 days with MBC-2-^{14}C (ring) and MBC-*methyl*-^{14}C released less than 1 and 16%, respectively, of the applied radioactivity as $^{14}CO_2$. The recovery of ^{14}C in acetone extracts of MBC-2-^{14}C treated soils was 79-91% in samples extracted immediately after treatment, and 53-78% 43 days after treatment, depending on soil and a rate of application of 10 or 100 ppm. Nearly all of the extractable ^{14}C chromatographed as MBC.

Thiophanate-methyl (TM) or 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene (I) is a broad-spectrum systemic fungicide. The metabolism of TM has been studied in mice and the bean plant (Noguchi, 1971). A TM metabolite common to all the organisms studied is methyl 2-benzimidazolecarbamate (MBC) (II).



Reported here are studies on the persistence and metabolism of TM and its metabolite MBC in North Dakota soils.

MATERIALS AND METHODS

The soil types used in these experiments and their pH were Barnes sandy loam, 7.4; Fargo silty clay, 7.7; and Towner loamy fine sand, 7.2. The organic contents of the soils were 4.2, 3.4, and 1.1%, respectively. The soils were air dried and passed through 20 mesh screen before use. In certain experiments the pH of the soil was adjusted with 1 *N* HCl with sufficient time for equilibration.

Extraction of TM and MBC. The procedures of Pennwalt Corporation (1972) were adapted for this study. Each soil sample (25 g, dry weight) was mixed with 125 ml of acetone and 20 g of Na_2SO_4, and refluxed for 1 hr. The mixture was filtered immediately after refluxing by suction filtration through M-sintered glass, the soil residue was washed three times with 25-ml portions of acetone, and the filtrate and washings were concentrated to 5-10 ml at 30° under reduced pressure. When TM was to be determined, 50 ml of freshly prepared 1% $AgNO_3$ in 95% ethanol was added to the concentrate and the mixture was heated 20 min on the steam bath in order to convert TM to MBC quantitatively. To assay MBC, the treatment with alcoholic $AgNO_3$ was omitted and the 1 *N* HNO_3 added directly to the concentrated acetone extract. TM was determined by difference.

The dilute HNO_3 solution was extracted twice by shaking for 1 min periods with two 50-ml portions of chloroform and the chloroform extracts were discarded. The aqueous phase was adjusted to pH 6.5 with 15 *N* NH_4OH. The neutralized solution was extracted by shaking for 1 min periods with three 25-ml portions of chloroform. The chloroform extracts were concentrated at 30° under reduced pressure to a 5-10-ml volume and rinsed with chloroform into a 20-ml, screw-cap vial. The remaining solvent was removed with a stream of air and the residue assayed colorimetrically for MBC.

Determination of MBC. To the vial containing the MBC residue were added 2 ml of chloroform and 3 ml of color reagent (100 mg of Bromocresol Purple, 20 g of Na_2SO_4, and 4 ml of acetic acid diluted to 1 l. with water). The vial was sealed, shaken 1 min, and allowed to stand 20 min. An additional 3 ml of chloroform was added and the mixture was shaken 1 min and allowed to stand 20 min. The chloroform phase was filtered through filter paper into a cuvet and the absorbance read at 410 nm against a blank obtained from untreated soil.

This colorimetric assay is not specific for MBC (Sutherland, 1964; Stansbury, 1964). Therefore, some soil extracts were examined for the presence of compounds other than MBC. The soil samples were extracted as described above and were streaked on silica gel G thin-layer plates containing an inorganic fluorescent indicator. The plates were developed with either ethyl acetate-*n*-hexane-acetic acid (30:70:2), or ethyl acetate. Only areas of the plates corresponding to the R_f of authentic MBC gave a positive colorimetric test when extracted with methylene chloride. At the same R_f, the methylene chloride extracts gave an ultraviolet absorption spectra identical with that of authentic MBC.

Decomposition of TM in Soil. One milliliter of TM dissolved in acetonitrile (250 μg/ml) was added to each soil sample (25 g in a 125-ml flask). After evaporation of the solvent, the soil was stirred 10 min, water was added

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