Bull. Environ. Contam. Toxicol. (1985) 35:291–295

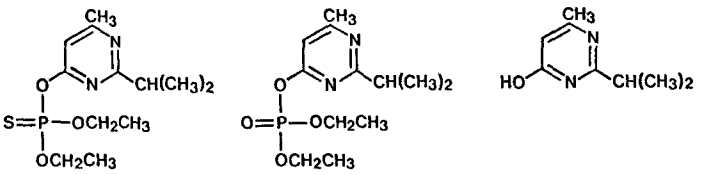
Archives of Environmental Contamination and Toxicology

Identification of Diazinon and its Metabolite in Spinach by Chemical Ionization Mass Spectrometry

Thomas Cairns, Emil G. Siegmund, and Jerry E. Froberg

Department of Health and Human Services, Food and Drug Administration, Office of Regulatory Affairs, 1521 West Pico Boulevard, Los Angeles, CA 90015

Diazinon [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] (**1**) is an organophosphorus insecticide registered for use on a wide variety of food and feed crops. Levels permitted in the U.S.A. on human food (Code of Federal Regulations 1983) range from 0.1 ug/g (ppm) in potatoes to 0.7 ppm in most leafy vegetables. One of the major metabolites is diazoxon (**2**), the oxygen analog of diazinon. Both of these phosphates have high cholinesterase inhibiting activity, while a second metabolite [2-isopropyl-4-methylpyrimidin-6-ol] (**3**) resulting from hydrolysis of diazoxon (Ralls et al. 1967) is of unknown mammalian toxicity.



(1) (2) (3)

During the course of pesticide surveillance of vegetables, an unknown analytical response (UAR) in spinach extract was encountered which was subsequently identified as (**3**). The presence of diazinon (**1**) in the sample was confirmed while no detectable levels of diazoxon (**2**) were found. This paper describes the analytical protocol adopted to confirm both diazinon (**1**) and its metabolite (**3**) in spinach at the ppm level by methane chemical ionization mass spectrometry and to elucidate the observed fragmentation pathway for diazinon.

MATERIALS AND METHODS

All spectra were obtained on a Finnigan Model 3300 quadrupole mass spectrometer equipped with a CI source and INCOS Data System; operating conditions: 45 cm x 2 mm i.d. glass column packed with

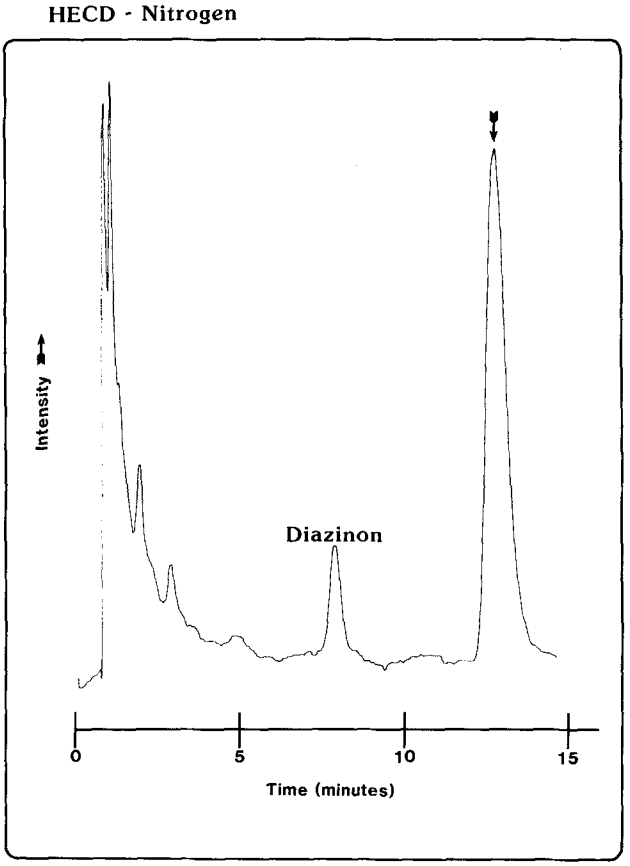


Figure 1. Gas chromatogram of spinach extract obtained using a HECD in the nitrogen mode; 2% DEGS at 150°C isothermal; peak eluting at 13.5 min represents the response for the encountered UAR.

2% DEGS on 80-100 mesh Chromosorb W; carrier gas and reagent gas for chemical ionization, 30 mL methane/min; column inlet, 180°C; column temperature, 170°C, isothermal.

For analysis by GC/MS, 20-g portions from homogenized 10 kg sampled lots of spinch were extracted by the Luke procedure (Luke et al. 1981) and cleaned up by using a carbon column (Luke & Doose 1983). The extract was then concentrated to 0.1 mL with a stream of dry nitrogen and 2 uL was injected onto the GC/MS.

RESULTS AND DISCUSSIONS

The extract was originally examined using the Hall Electrolytic Conductivity Detector (HECD) in the nitrogen mode (Figure 1).

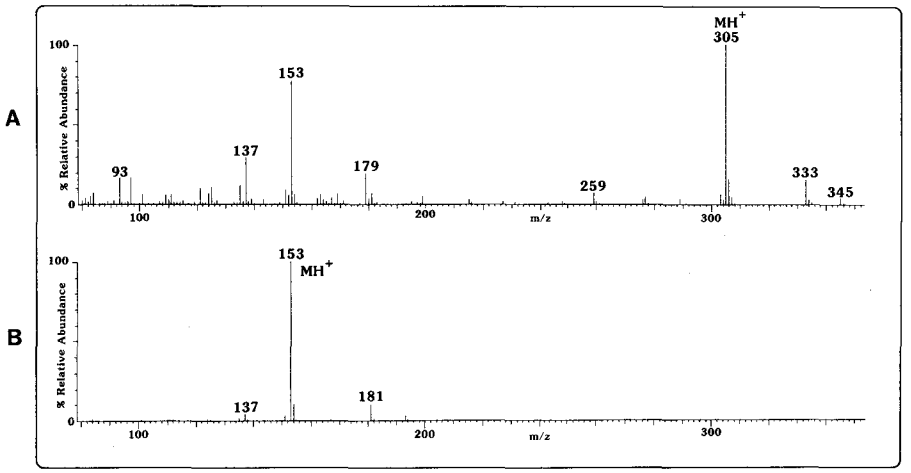
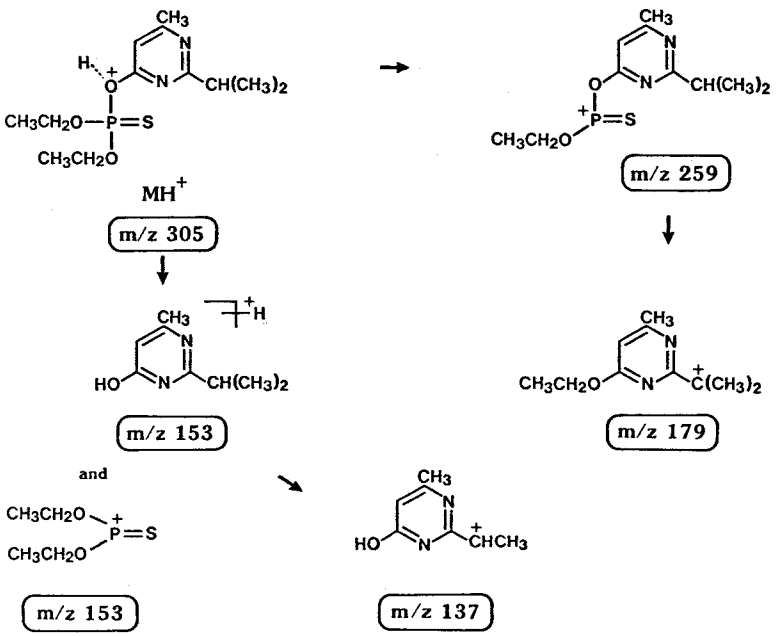


Figure 2. Methane chemical ionization spectra for, (A) diazinon; and (B) 2-isopropyl-4-methylpyrimidin-6-ol.

Clearly, a number of nitrogen-containing compounds were present, but the presence of the major component at R_t = 13.5 min constituted a UAR response due to the lack of comparative GC retention data from our internal data base.

The sample was then re-examined by methane chemical ionization (CI) to determine the molecular weights of the two major components (Figure 2). The peak eluting at about 7.5 min was determined to be diazinon at a concentration level of 0.1 ppm. The base peak at m/z 305 represented the protonated molecular ion $[MH]^+$ while adduct ions at m/z 333 and 345 corresponding to $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$ respectively provided confirmation of this ion assignment. However, the UAR eluting at 13.5 min (Figure 1) was determined to have the much lower molecular weight of 152 (Figure 2B). By deduction of the fragmentation pathway for diazinon (Scheme 1), the UAR was strongly suspected to be 2-isopropyl-4-methylpyrimidin-6-ol (**3**). This preliminary identification was later confirmed by use of a reference standard and quantified at the incurred concentration level of 1 ppm. No trace of diazoxon (**2**), however, was found in the spinach extract.

Under methane CI conditions the fragmentation of diazinon (**1**) exhibited some unique features for an organophorphorus compound. While the base peak is the protonated molecular ion at m/z 305, the appearance of a strong ion at m/z 153 offered two possible ion structures resulting from bond cleavage at the P-O site with the charge remaining on either side of that bond cleavage. Under electron impact (EI) conditions (Damico 1966) it had been determined by exact mass measurements that the ion at m/z 152 had the



Scheme 1. Proposed fragmentation pathway for diazinon under methane chemical ionization conditions.

ion structure containing the nitrogen ring. Under methane CI, however, the appearance of ions at m/z 153, 121 and 93 are highly indicative of the phosphorothiate moiety via initial cleavage of the ring substituent followed by expulsion of S and then an ethyl grouping. The definitive argument for protonation at the oxygen atom between the phosphorus and the ring is then established whatever the ensuing fragmentation mechanism. Appearance of an ion at m/z 259 due to loss of an ethyl group reflects the soft ionization mechanism employed. This ion structure was not found under EI conditions. The ion at m/z 179 represented a cleavage of the P-O bond with concomitant rearrangement or migration of an ethyl group to the aromatic bonded oxygen atom. Such an ion was previously detected in the EI spectrum of diazinon (Damico 1966). More interestly, however, is the appearance of the ion at m/z 137 which under EI by exact mass measurement had the formula $C_6H_9N_2O$. Orginally the mechanism postulated for production of this ion was from m/z 179 via loss of propylene. In additional metastable studies, however, the m/z 137 was determined to have resulted from the ion at m/z 152 by loss of a methyl grouping (Damico 1972).

The similarities observed in the fragmentation of diazinon using EI and CI conditions are unusual in that such cases are unique rather than typical. Perhaps an obvious explanation for the

observed comparisons is that the site of protonation under CI is the oxgyen atom between the phosphorus and the aromatic ring as is the site for electron abstraction under EI. Such a prevailing situation would then direct the fragmentation processes down quasi-similar routes. Under CI the site of initial protonation is often not the same site for electron removal and hence different fragmentation pathways develop. The case of diazinon is a good example of parallel fragmentation pathways primarily due to the same selection for initial bond cleavage.

The mass spectrum for the metabolite (**3**) was easily interpreted in that it exhibited the protonated molecular ion at m/z 153 with an adduct ion at m/z 181 with a minor fragment ion at m/z 137 already elucidated above for diazinon.

The presence of this metabolite of diazinon at the 1-ppm level represented an order of magnitude greater than that found for diazinon. Persistence of this metabolite (**3**) in spinach long after spraying with diazinon should be of concern, especially at residue levels much higher than that permitted for diazinon.

REFERENCES

Code of Federal Regulations (1983) 40 CFR 180.153

Damico JN (1966) The mass spectra of some organophosphorus pesticide compounds. J Assoc Off Anal Chem **49**: 1027-1045

Damico JN (1972) Pesticides. In: Waller GR (ed) Biochemical applications of mass spectrometry. Wiley-Interscience, NY, p 623-653

Luke MA, Froberg JE, Doose GM, Masumoto HT (1981) Improved multiresidue gas chromatographic determination of organophosphorus, organonitrogen, and organohalogen pesticides in produce using flame photometric and electrolytic conductivity detectors. J Assoc Off Anal Chem **64**: 1187-1195

Luke MA, Doose GM (1983) A modification of the Luke multiresidue procedure for low moisture non-fatty products. Bull Environ Contam Toxicol **30**: 110-116

Ralls JW, Gilmore DR, Cortes A, Schutt SM, Mercer WA (1967) Residue level of diazinon and its transformation products on tomatoes, spinach and beans. Food technology **21**: 1030-1032

Received October 28, 1984; accepted October 30, 1984.