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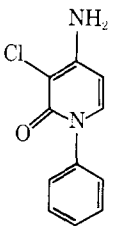
Fate of Pyrazon in a Model Ecosystem

Ching-Chieh Yu,*[1] Gary M. Booth,[2] Dale J. Hansen,[3] and Joseph R. Larsen[4]

Pyridazinone ring-^{14}C-labeled pyrazon [5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone] was slowly degraded in water. Thirty-two days after application of the compound to a model ecosystem, about 66% of the radioactivity in the water was found to be the parent compound. Very small amounts of 2-dephenylpyrazon [5-amino-4-chloro-3(2*H*)-pyridazinone] and five other unknown spots (combined total 1%) were detected only after acid hydrolysis. The remainder of the radioactivity was present as unextractable water-soluble products (33%). Combined parent compound and metabolites in organisms living in the ecosystem ranged from 0.06 ppm in fish to 0.6 ppm in crab. Analysis of the crab extracts revealed that no 2-dephenylpyrazon was present and that the parent pyrazon constituted about 76% of the total radioactivity in that organism. There was no evidence to indicate that pyrazon and its degradation products were magnified through the food chain.

Pyrazon, 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (I), is a selective herbicide used in red beet and sugar beet production (Fischer, 1962). Metabolism of this compound in plants and soil has been investigated (Frank and Switzer, 1969a,b; Ries *et al.*, 1968; Smith and Meggitt, 1970a,b; Stephenson and Ries, 1967, 1969). However, the fate of pyrazon in a food chain is not known. Recently, Metcalf *et al.* (1971) developed a model ecosystem to facilitate the study of the biodegradability and accumulation of pesticides in the environment. Several pesticides have been examined in this system (Yu *et al.*, 1974; Sanborn and Yu, 1973; Booth *et al.*, 1973). This study, which is part of a continuous effort to examine the fate and effects of pesticides in the environment, considers the fate of pyrazon in a model ecosystem.



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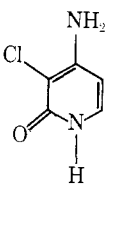

II

MATERIALS AND METHODS

Labeled Compound. Pyridazinone ring-^{14}C-labeled pyrazon (sp act., 4.7 mCi/mmol; radiochemical purity 98% by tlc and radioautography) was obtained from BASF Corporation.

Model Ecosystem. The procedures described by Metcalf *et al.* (1971) with some modifications (Yu *et al.*, 1974) were followed and the experiment with pyrazon was replicated two times simultaneously. Ring-^{14}C-labeled pyrazon (2.36 mg, 50 μCi) in 0.5 ml of acetone was applied to the base of the 7-day-old sorghum plants.

Sample Preparation. The work-up procedures were described previously (Yu *et al.*, 1974).

Illinois Natural History Survey and Illinois Agricultural Experiment Station, Urbana, Illinois 61801.
[1] Present address: Department of Entomology, New York State Agricultural Experiment Station, Geneva, N.Y. 14456.
[2] Present address: Department of Zoology, Brigham Young University, Provo, Utah 84601.
[3] Present address: Agricultural Division, Monsanto Co., St. Louis, Mo. 63166.
[4] Present address: Department of Entomology, University of Illinois, Urbana, Ill. 61801.

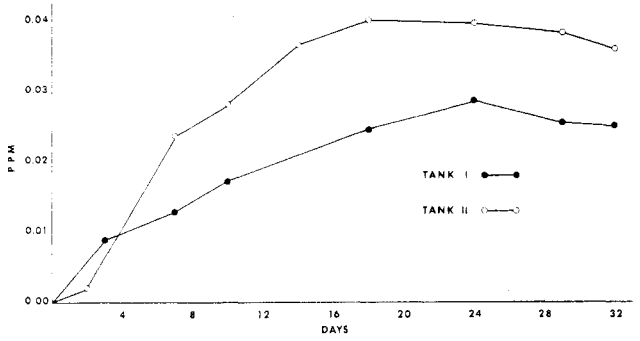

Figure 1. Concentration of pyrazon and metabolites in water.

Thin-Layer Chromatography (Tlc). Silica gel F-254 plates (Brinkman), 20 × 20 cm (0.25 mm thickness), developed in benzene–ethanol (60:40, v/v) were used to separate the metabolites in solvent extracts from water and organisms (Stephenson and Ries, 1969).

RESULTS AND DISCUSSION

Radioactivity in the water was monitored throughout the experimental period (Figure 1). The concentration of pyrazon and degradation products increased steadily until a peak at the 24th day was reached and then decreased very slowly.

Analyses of the water at the end of the experiment (32nd day) are presented in Table I. About 35.4% of the radioactivity in the unhydrolyzed water was found to be the parent pyrazon. Upon acid hydrolysis (0.025 *N* HCl at 70° for 20 hr), an additional 30.1% was released as the parent compound. Thus, pyrazon constituted 65.5% of the total radioactivity in the water. Very small amounts of 2-dephenylpyrazon [5-amino-4-chloro-3(2*H*)-pyridazinone (II)] (0.2%), and five unknown spots (0.8%) were detected only after acid hydrolysis. These products may be produced by the action of HCl with parent compound, or they may be in the conjugated forms but released upon acid hydrolysis. The unextractable residue after acid hydrolysis constituted 33.3% of the total radioactivity. It appears that pyrazon was slowly degraded in water with minor release of the phenyl moiety.

The total equivalent of radioactivity in the ecosystem organisms ranged from 0.06 ppm in the fish to 0.6 ppm in the crab (Table II). Probably the crab moving more freely between terrestrial and aquatic phases had the greatest opportunities for contact with the pyrazon sources.

Table II also shows the distribution of radioactivity in the solvent extracts and unextractable residues for each organism. The acetone-extractable fractions constituted 35% of the total in the algae to 78% in the crab. Since most of the extracts contained less than 500 cpm, only the crab extracts were analyzed by tlc and radioautography. Parent pyrazon consisted of 76% of the total radioactivity, or about 0.5 ppm in actual concentration, and no 2-dephenylpyrazon was detected. It appears that the crab metabolized pyrazon very slowly.

There was no evidence to indicate that pyrazon and its degradation products were magnified in the food chain. For example, the total radioactivity in a food chain from algae → mosquito → fish decreased from 0.21 to 0.06 ppm.

Table I. Concentration (ppm) of Pyrazon, Metabolites, and Degradation Products in Unhydrolyzed and Hydrolyzed Water

		Tank I			Tank II		
		UHy-H_2O[b]	Hy-H_2O[c]	Total (%)	UHy-H_2O[b]	Hy-H_2O[c]	Total (%)
Pyrazon	0.66[a]	0.00922	0.00690	0.01612 (61.6)	0.01306	0.01250	0.02556 (69.3)
2-Dephenylpyrazon	0.49	0.0	0.00006	0.00006 (0.2)	0.0	0.00008	0.00008 (0.2)
Unknown I	0.42	0.0	0.0	0.0	0.0	0.00008	0.00008 (0.2)
Unknown II	0.23	0.0	0.0	0.0	0.0	0.00005	0.00005 (0.1)
Unknown III	0.16	0.0	0.00005	0.00005 (0.2)	0.0	0.00004	0.00004 (0.1)
Unknown IV	0.10	0.0	0.00005	0.00005 (0.2)	0.0	0.00010	0.00010 (0.3)
Unknown V	0.00	0.00003	0.00012	0.00015 (0.1)	0.00003	0.00009	0.00012 (0.3)
Unextractable		0.01690	0.00973	0.00973 (37.2)	0.02380	0.01085	0.01085 (29.4)
Total ^{14}C				0.02615			0.03690

[a] R_f value; silica gel F-254, benzene–ethanol (60:40). [b] UHy-H_2O = water extracted with diethyl ether. [c] Hy-H_2O = water treated with 0.025 *N* HCl at 70° for 20 hr and then extracted with diethyl ether.

Table II. Concentration (ppm) of Pyrazon, Metabolites, and Degradation Products in the Organisms

	Organisms[a]							
Metabolites	Algae	Clam	Crab	Daphnia	Elodea	Fish	Mosquito	Snail
Total ^{14}C	0.21 (0.27, 0.15)	0.07 (0.07, 0.06)	0.63 (0.90, 0.36)	0.10 (0.12, 0.08)	0.16 (0.19, 0.13)	0.06 (0.06, 0.06)	0.32 (0.35, 0.29)	0.19 (0.23, 0.14)
Pyrazon 0.66[b]			0.48 (0.69, 0.27)					
2-Dephenyl-pyrazon 0.49			0.0 (0, 0)					
Unknown 0.00			0.02 (0.04, 0.01)					
Unextractable	0.13 (0.16, 0.10)	0.02 (0.02, 0.02)	0.13 (0.17, 0.09)	0.05 (0.06, 0.05)	0.11 (0.13, 0.08)	0.02 (0.02, 0.02)	0.15 (0.15, 0.15)	0.06 (0.07, 0.05)

[a] Where no values are listed the extract was not chromatographed because it was <500 cpm. [b] R_f value; silica gel F-254, benzene–ethanol (60:40). Values in parentheses are for tanks I and II, respectively.

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Application of a Thermionic Detector in the Analysis of *s*-Triazine Herbicides

Shahamat U. Khan* and Ranajit Purkayastha[1]

The response characteristics and retention times of seven *s*-triazine herbicides have been studied using a thermionic detector fitted with a cesium bromide (CsBr) tip and various gas chromatographic columns such as OV-17, SE-30, Reoplex-400, and Carbowax 20M. Thermionic detection using the Carbowax 20M column gave sensitivities comparable to those by Coulson electrolytic conductivity detector. Recoveries of atrazine added to water at 0.02, 0.05, and 2.0 ppm were between 92 and 122%; for corn at 0.1, 0.2, and 1.0 ppm, between 101 and 110%; and for soil at 0.23, 0.46, and 1.1 ppm, between 88 and 101%. The detector has also been applied to an evaluation of some extraction procedures and extracting solvents for atrazine residue in a field treated soil.

A variety of *s*-triazine herbicides are used for weed control in crops and methods are needed for their selective detection and determination at the residue level. Since the *s*-triazines contain nitrogen in their molecular structure, detectors responding to nitrogen would be most useful for their residue analysis. The recent availability of thermionic detectors has prompted some workers to investigate their utility for the residue analyses of *s*-triazines (Ebing, 1968; Tindle *et al.*, 1968; Schultz, 1970; McKone *et al.*, 1972). Tindle *et al.* (1968) used a $RbSO_4$ thermionic detector for the determination of atrazine, simazine, propazine, and prometryne residues in water, soil, and corn. They reported minimum detectable limits better than 0.5 ng of *s*-triazine, with a very favorable selectivity of response for organo-nitrogen compounds compared to carbonaceous materials. Schultz (1970) used a CsBr phosphorus detector for the determination of some *s*-triazine herbicides in crops. The minimal detectable amounts based on 3% full scale deflection were about 0.5 ng of atrazine and 1.0 ng of Bladex (SD15418).

The aim of the present work was to investigate the gas chromatographic characteristics of *s*-triazines using a thermionic detector with a CsBr tip. Since atrazine is the most widely used herbicide among the *s*-triazines, the use of a thermionic detector for the residue analysis of this herbicide in water, soil, and corn samples was also investigated.

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade and used as received. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], atratone [2-(ethylamino)-4-(isopropylamino)-6-methoxy-*s*-triazine], ametryne [2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-*s*-triazine], prometone [2,4-bis(isopropylamino)-6-methoxy-*s*-triazine], prometryne [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine], propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine], and simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] were analytical reference grade samples obtained from CIBA-Geigy. Solutions of these herbicides were prepared in methanol.

Determination of Residues in Water, Soil, and Corn Samples Fortified with Atrazine. (a) *Water.* The water samples (100–500 ml) were fortified with a standard solution of atrazine in methanol at the 0.02-, 0.05-, and 2.0-ppm levels. The fortified sample was transferred to a separatory funnel and extracted with three 70-ml portions of methylene chloride. The extracts were combined, dried with anhydrous Na_2SO_4, and concentrated to a small volume by rotary vacuum evaporation at about 35°. The material was quantitatively transferred on the top of a deactivated (13% H_2O) alumina column (0.8 in. diameter, 25 g of aluminum oxide W200 basic, Woelm, previously washed with 100 ml of CCl_4) topped with 0.5 in. of anhydrous Na_2SO_4. The column was eluted with 100 ml of 2% diethyl ether in carbon tetrachloride. No atrazine was eluted by this eluate. Following this the column was eluted with 200 ml of 6% diethyl ether in carbon tetrachloride and the collected eluent was evaporated to dryness in a rotary evaporator. The residue was dissolved in hexane and analyzed by gas chromatography.

(b) *Corn.* The corn samples were chopped and finely ground. Subsamples (5 g) were fortified with atrazine in methanol at the 0.1-, 0.2-, and 1.0-ppm levels. The fortified sample was extracted with acetonitrile (100 ml) with mechanical shaking for 90 min. The mixture was filtered under suction through a Hyflo Super-Cel bed and the filter bed was washed with 100 ml of 35% aqueous acetonitrile. The combined filtrate was partitioned with hexane and the aqueous phase was collected. The hexane phase was repartitioned with aqueous acetonitrile. The combined aqueous phase was extracted with three 50-ml por-

Chemistry and Biology Research Institute, Research Branch, Canada Agriculture, Ottawa, Ontario K1A OC6, Canada.
[1] Deceased.