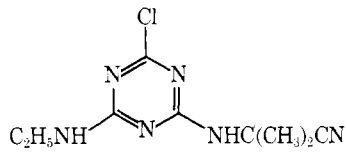
Fate of Triazine Herbicide Cyanazine in a Model Ecosystem

[ring-^{14}C]Cyanazine [2-(4-chloro-6-ethylamino-*s*-triazin-2-ylamino)-2-methylpropionitrile] was introduced into an aquatic model ecosystem. After 35 days in this ecosystem, the radioactivity in water was found to consist of 60% *N*-deethylcyanazine [2-(4-chloro-6-amino-*s*-triazin-2-ylamino)-2-methylpropionitrile], 18% cyanazine, 0.8% cyanazine amide [2-(4-chloro-6-ethylamino-*s*-triazin-2-ylamino)-2-methylpropionamide], 0.3% *N*-deethylcyanazine amide [2-(4-chloro-6-amino-*s*-triazin-2-ylamino)-2-methylpropionamide], 1.2% unknown polar metabolites, and 19% ether-unextractable metabolites. Radioactivity was persistent in water indicating degradation of the triazine ring to CO_2 proceeded slowly. However, cyanazine and its degradation products did not concentrate through the food chain. The total equivalent of radioactivity in the aquatic organisms ranged from 1.3 ppm in algae [*Oedogonium cardiacum* (Huss.)] to 0.05 ppm in fish (*Gambusia affinis* Baird and Girard) and 0.04 ppm in *Daphnia magna* Strauss. About 0.2 ppm of *N*-deethylcyanazine was found in the crabs (*Uca minax*), but no cyanazine was detected.

Cyanazine (I) is a selective triazine herbicide for the control of annual grasses and broad-leaved weeds in corn fields. Metabolism of cyanazine in plants and soil involved hydrolysis of the cyano group to give an amide and an acid, and hydrolysis of chlorine to hydroxycyanazine (Beynon et al., 1972a–c; Sirons et al., 1973). Cyanazine is generally considered to be less persistent than most other triazine herbicides. However, no information is available regarding the fate of cyanazine and its degradation products in the food chain. This paper reports results from a study of this compound using a model aquatic ecosystem developed by Metcalf et al. (1971).



I

MATERIALS AND METHODS

[ring-^{14}C]Cyanazine (specific activity 3.37 mCi/mmol; radiochemical purity 99.5%) and model metabolites were obtained from Shell Chemical Co. The model ecosystem procedures described by Metcalf et al. (1971) were followed with slight modification (Yu et al., 1974). Fifty microcuries (3.5 mg) of [^{14}C]cyanazine in 1.1 ml of chloroform was applied to the base of the sorghum plants (*Sorghum halopense*). This dosage is equal to 0.7 lb of active ingredient per acre of land.

At the end of the experiment water was extracted with diethyl ether and organisms were extracted with acetone (Yu et al., 1974). The extracts were then analyzed by thin-layer chromatography (TLC) using silica gel F-254 plate. The developing solvent consisted of methanol–acetone–chloroform (5:45:50, v/v).

RESULTS AND DISCUSSION

Radioactivity in water increased steadily during the experimental period of 35 days (Figure 1). This result indicated cyanazine and its degradation products remained in the water. Degradation of the triazine ring to CO_2 proceeded slowly.

Water was extracted with ether at the end of the experimental period. About 80% of the radioactivity could be readily extracted (Table I). According to TLC analysis, parent cyanazine consisted of 18% of the total radioactivity in water. However, over 60% of the radioactivity was found to be *N*-deethylcyanazine [2-(4-chloro-6-amino-*s*-triazin-2-ylamino)-2-methylpropionitrile]. Cyanazine amide [2-(4-chloro-6-ethylamino-*s*-triazin-2-ylamino)-2-methylpropionamide] and *N*-deethylcyanazine amide [2-(4-chloro-6-amino-*s*-triazin-2-ylamino)-2-methylpropionamide] consisted of 0.8 and 0.3%, respectively. A combined total of 0.3% of three unknown polar metabolites was also detected in the extract.

Upon acid hydrolysis (0.025 *N* HCl, 70°, 20 hr), no further release of cyanazine and the three other known products was found. However, an additional 0.9% of the three unknown degradation products was released. About 19% of

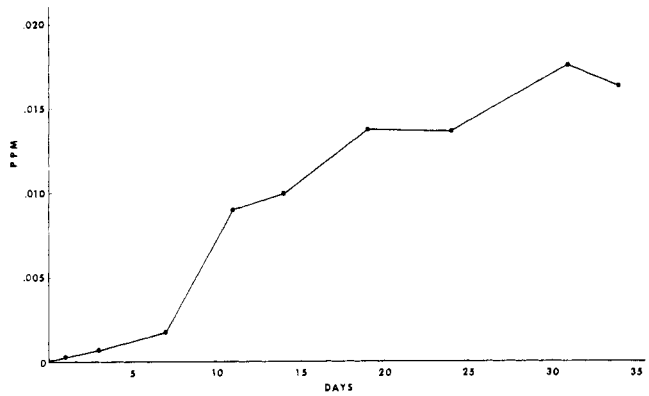


Figure 1. Radioactivity in tank water monitored through experimental period.

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

		Concentration, equivalent ppm		
		UHy-H_2O	Hy-H_2O	Total (%)
Cyanazine	0.55[a]	0.00321	0.0	0.00321 (18.0)
N-Deethylcyanazine	0.47	0.01075	0.0	0.01075 (60.4)
Cyanazine amide	0.37	0.00014	0.0	0.00014 (0.8)
N-Deethylcyanazine amide	0.26	0.00006	0.0	0.00006 (0.3)
Unknown A	0.16	0.00001	0.00006	0.00007 (0.4)
Unknown B	0.07	0.00001	0.00007	0.00008 (0.5)
Unknown C	0.00	0.00003	0.00003	0.00006 (0.3)
Unextractable		0.00359	0.00343	0.00343 (19.3)
Total ^{14}C				0.01779

[a] R_f value, silica gel F-254, methanol–acetone–chloroform (5:45:50).

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

		Concentration, equivalent ppm						
		Algae	Crab	Daphnia	Elodea	Fish	Mosquito	Snail
Cyanazine	0.55[a]	–	0.0	–	0.621	–	–	–
N-Deethylcyanazine	0.47	–	0.172	–	0.0	–	–	–
Cyanazine amide	0.37	–	0.0	–	0.0	–	–	–
N-Deethylcyanazine amide	0.26	–	0.0	–	0.0	–	–	–
Unknown A	0.16	–	0.0	–	0.0	–	–	–
Unknown B	0.07	–	0.058	–	0.0	–	–	–
Unknown C	0.00	–	0.081	–	0.008	–	–	–
Unextractable		0.127	0.209	0.020	0.025	0.016	0.075	0.062
Total ^{14}C		1.256	0.521	0.040	0.654	0.051	0.103	0.108

[a] R_f value, silica gel F-254, methanol–acetone–chloroform (5:45:50). –, extract not chromatographed because less than 500 dpm.

the radioactivity in water could not be extracted even after acid hydrolysis.

Concentration of cyanazine and degradation products in the organisms was low (Table II). The total equivalent of radioactivity in the aquatic organisms ranged from 1.3 ppm in algae [*Oedogonium cardiacum* (Huss.)] to 0.05 ppm in fish (*Gambusia affinis* Baird and Girard) and 0.04 ppm in *Daphnia magna* Strauss. The extracts of crab (*Uca minax*) and *Elodea canadensis* which contained radioactivity greater than 500 dpm were analyzed by TLC. The results showed that *Elodea* contained 0.6 ppm of cyanazine (Table II). No other degradation products were detected in *Elodea* except for a small amount of very polar metabolites. On the other hand, no cyanazine was found in the crab extracts. About 0.2 ppm of *N*-deethylcyanazine and a small amount of polar metabolites were detected in the crabs. There was no evidence to indicate that cyanazine and its degradation products concentrated through the food chain. For example, in a food chain of algae → mosquitoes (*Culex pipiens quinquefasciatus* Say) → fish, total radioactivity decreased from 1.3 ppm in algae to 0.05 ppm in fish.

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Structure of the Tremor-Producing Indole, TR-2

The structure of a tremorgenic indole, TR-2 ($C_{22}H_{27}N_3O_6$), has been established by spectroscopic studies as II. The structure consists of 6-*O*-methyltryptophan, proline, and isoprene moieties. The two amino acids are combined to form a diketopiperazine compound. TR-2 was chemically derived from verruculogen (TR-1), shown to be I. TR-1 and TR-2 are structurally similar to fumitremorgens A and B from *Aspergillus fumigatus*.

The biological, physical, and chemical properties of the tremorgenic mycotoxin, verruculogen (TR-1), isolated from *Penicillum verruculosum* (ATCC no. 24640; NRRL no. 5881), have been reported (Cole et al., 1972; Cole and Kirksey, 1973). A subsequent X-ray study of TR-1 showed the structure to be I (Fayos et al., 1974). When TR-1 in ethanol solution was hydrogenated in the presence of palladium on carbon (5%) at room temperature, two products, isovaleral-