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Identification and Confirmation of Atrazine in Pond Water

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Investigation of a fish kill in a farm pond resulted in a sample of water from the pond being brought to the laboratory for analysis. Recent applications to the farmland surrounding the pond had included Counter (an organophosphate), Sutan (a thiocarbamate), and Bladex (a triazine). Although Counter could not be identified in the pond water, the fish could have died as a result of exposure to this highly toxic insecticide. Attempts to identify Sutan residues were unsuccessful and analysis centered on confirmation of Bladex (cyanazine) in the water.

EXPERIMENTAL

Apparatus. A gas chromatograph equipped with a ^{3}H electron capture detection operated in the d.c. mode was used with 1.8 m x 4 mm i.d. glass column packed with 4.0% SE-30/6% OV-210 coated on 80/100 mesh Gas Chrom Q. Column temperature was 189°C; carrier gas flow was 50 mL/min.

A Finnigan 4023 gas chromatograph/mass spectrometer/data system (4023 GC/MS/DS) equipped with a 1.8 x 4 mm i.d. glass column packed with 4.0% SE-30/6% OV-210 coated on 80/100 mesh Gas Chrom Q was utilized for the confirmation of atrazine. Inlet, column, separator, transfer line, and ionizer temperatures were 220, 190, 250, 245, and 250°C, respectively; helium carrier gas flow was 40 mL/min; electron energy was 70 eV; filament emission was 0.3 ma; electron multiplier voltage was 1800 V.

Procedure. Extracts of pond water were made using methylene chloride and gas chromatographed using electron capture detection. Extracts of the samples were transferred to 1 mL concentration tubes and evaporated to 100 µL under a stream of nitrogen. Ten µL were injected into the GC/MS.

RESULTS AND DISCUSSION

The specific interest in this investigation came from the fact that Bladex was applied to surrounding farmland, but on examination, only atrazine was found in the farm pond. The origin of the atrazine is one of speculation.

A peak was found on a QF1-SE-30 column which did not have the same retention time as Bladex, but which did have good electron capturing characteristics. Retention times found were: pond

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water - 2.85 cm; cyanazine - 3.55 cm. Examination on an SE-30 - OV-210 column gave the following results: pond water - 3.3 cm; atrazine - 3.3 cm. These data suggested very strongly that the triazine in the pond water was atrazine and not cyanazine. Figure 1 depicts both the electron capture gas chromatogram and the reconstructed ion chromatogram obtained from the GC/MS of the pond water extract. The unknown peak under investigation nas a RRT=0.58 relative to aldrin.

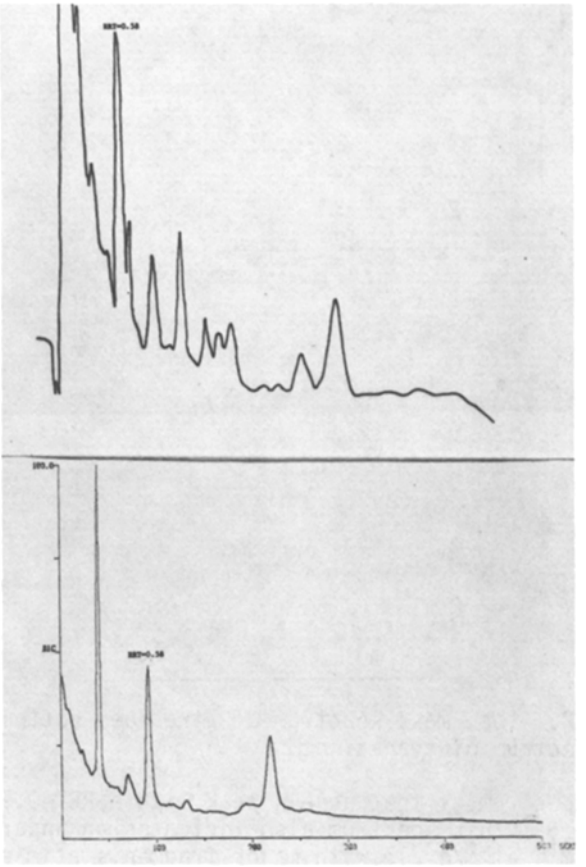

Figure 1. Top, electron capture gas chromatogram of the pond water extract; bottom, the reconstructed ion chromatogram obtained from the GC/MS of the pond water extract.

Standards of both atrazine and cyanazine were prepared and injected into the mass spectrometer. The mass spectra were obtained and entered into the laboratory's pesticide library for future comparison with the mass spectrum of the unknown peak found in the pond water. Figure 2 represents the mass spectra of atrazine and cyanazine. The mass spectrum of atrazine

indicates an apparent molecular ion, M^+, at m/e 215 with major fragments at m/e 200, 173, 93, 68, and 58.

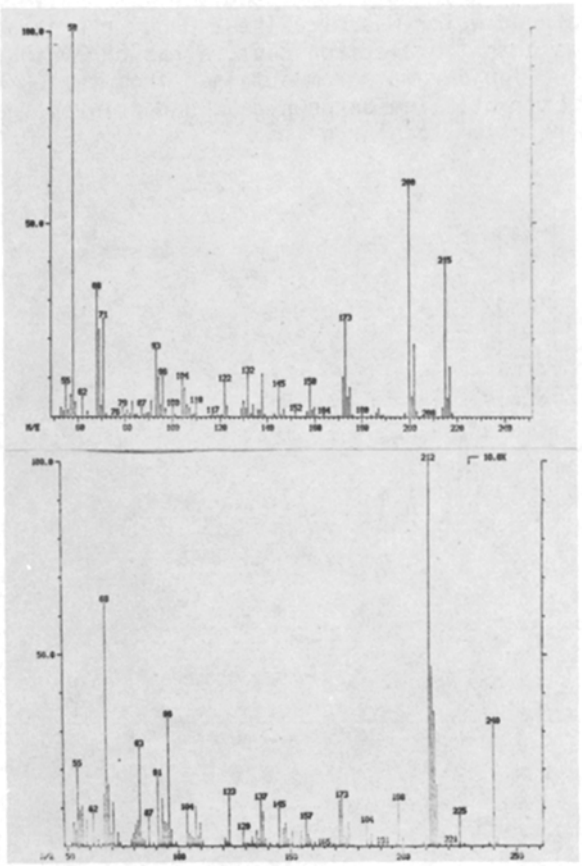

Figure 2. Top, mass spectrum of atrazine; bottom, mass spectrum of cyanazine.

The mass spectrum of the unknown peak with a RRT=0.58 is shown in Figure 3. This spectrum also indicates an apparent molecular ion, M^+, at m/e 215 with major fragments at m/e 200, 173, 93, 68, and 58. This mass spectrum was searched against the 24,409 compound NBS library. The library search report is shown in Table 1. The five library compounds whose spectral characteristics most closely matched those of the unknown spectrum are listed in the report. The library index number, molecular formula, molecular weight and the base peak ion are also tabulated for each possible match. The PURITY, FIT, and RFIT values are indicative of the similarity between the unknown spectrum and the library spectrum. All three numbers are in the range of 0 to 1000 based on perfect spectral match equaling 1000. For the PURITY calculations all masses and intensities of the unknown spectrum are taken into consideration, whereas for the FIT calculation only the masses in the unknown spectrum which

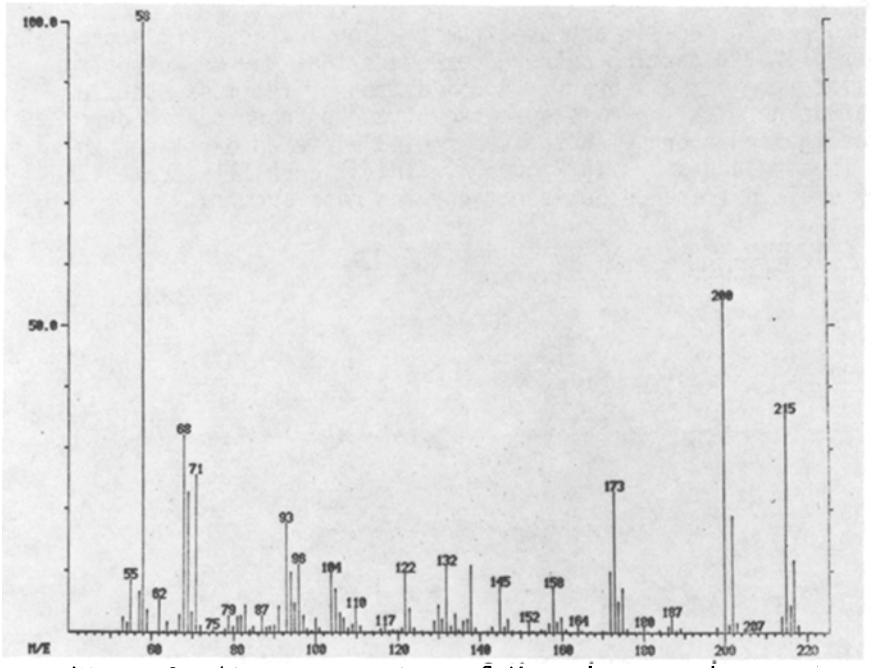

Figure 3. The mass spectrum of the unknown peak.

Table 1. Library search report for the mass spectrum of the unknown peak with a RRT=0.58.

Library Search — Data: MS47 # 93 — Base M/E: 58
02/22/79 15:11:00 + 3:06 — Cali: 11079 # 8 — Ric: 176127.
Sample: McCreedy Water 79-6
Enhanced (S 15B 2N OT)

25409 Spectra in LibraryNB Searched For Maximum Purity
297 Matched at Least 3 of the 16 Largest Peaks in the Unknown

Rank	Name
1	5729 1,3,5-triazine-2,4-diamine,6-chloro-n-ethyl-n'-(1-methylethyl)-
2	1521 1,3,5-triazine-2,4-diamine,6-chloro-n,n'-diethyl-
3	4813 hydrouracil,5,5-dibromo-6-hydroxy-
4	21547 1H-indole-3-ethanamine,1-(acetyloxy)-5-methoxy-n-n-dimethyl-
5	3070 benzene,1-bromo-4-ethoxy-

Rank	Formula	M.Wt	B.Pk	PURITY	FIT	RFIT
1	C8.H14.N5.CL	215	58	808	986	808
2	C7.H12.N5.CL	201	44	349	619	368
3	C4.H4.O3.N2.BR2	286	46	289	595	404
4	C15.H20.O3.N2	276	58	288	662	345
5	C8.H9.O.BR	200	200	284	724	319

also occur in the library spectrum are considered. RFIT complements FIT by detecting unknown compounds that may be components of mixtures in the library. A comparison of the mass spectrum of atrazine with the mass spectrum of the unknown peak under investigation along with the subtracted difference between these two spectra are shown in Figure 4. This figure illustrates the very small difference between these two mass spectra.

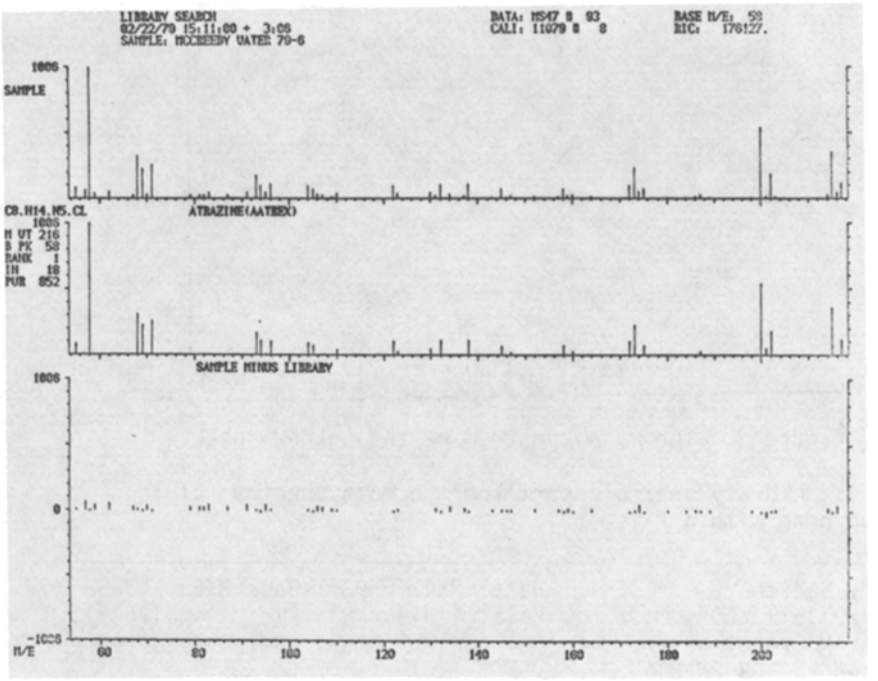

Figure 4. A comparison of the mass spectrum of atrazine with the mass spectrum of the unknown peak along with the subtracted difference between these two spectra.

KLAASSEN and KADOUM (1979) examined farm ponds during and after application of atrazine over a period of two years. Residues were found in all physical and biological components of the system immediately after application and for continuing periods. The possibility of atrazine entering the aquatic ecosystem appears to be very high.

CORKE and PALMATEER (1973) in a personal communication to SIRRONS et al. (1973) proposed an alternate method of forming atrazine in soils by means of microbiological activities. Chemical modification would follow the sequence of degradation of the nitrile to an amide and then to the acid with subsequent decarboxylation. Thus atrazine would derive from cyanazine.

Considering the persistence and stability of atrazine, the surrounding farmland could also have been treated with atrazine

in previous years and still have been in suspension in the farm pond.

Based on the relative retention times and the mass spectral evidence presented, atrazine was positively identified and confirmed in a farm pond when the surrounding farmland was treated with cyanazine. The origin of the atrazine is open for speculation.

ACKNOWLEDGEMENT

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