Detection of Cyolane in Alfalfa Pellets and Rumen Content of a Cow

by G. S. TADJER
Leopold Greenberg Institute of Forensic Medicine, The Hebrew University, Jaffa, Abu Kabir, Israel
and
M. N. EGYED
Kimron Veterinary Institute, Bet Dagan, affiliated with the Tel-Aviv University, Israel

Cyolane[+], an organophosphorus insecticide, is restricted in this country to use against Prodenia litura infestations on cotton. It has been established, however, that contrary to instructions this insecticide has been used against the same pest in alfalfa. Cyolane-contaminated alfalfa meal or alfalfa meal pellets in cattle feed have been responsible for poisoning in cattle (Egyed et al. 1971). The clinical symptoms included anorexia, apathy, and watery diarrhea. In various outbreaks the morbidity rate was very high, while the mortality rate was extremely low. When three calves were experimentally fed with the Cyolane-treated alfalfa hay, mild diarrhea lasting 3-4 days and significantly depressed blood cholinesterase activity was observed (Egyed et al. 1972). In this particular case alfalfa had been sprayed at the growing stage and the hay was fed approximately one year after spraying.

Spontaneous intussusception of the small intestine in guinea pigs was observed by feeding alfalfa hay contaminated with aflatoxin and Cyolane (Schoenbaum et al. 1972). To our knowledge there are no other references related to the toxic effects of Cyolane in ruminants. T.L.C., G.L.C. and colorimetric procedures are given for the estimation of Cyolane residues in alfalfa and rumen contents of a fatally poisoned cow which was suspected of having ingested Cyolane-contaminated alfalfa.

Methods and Results

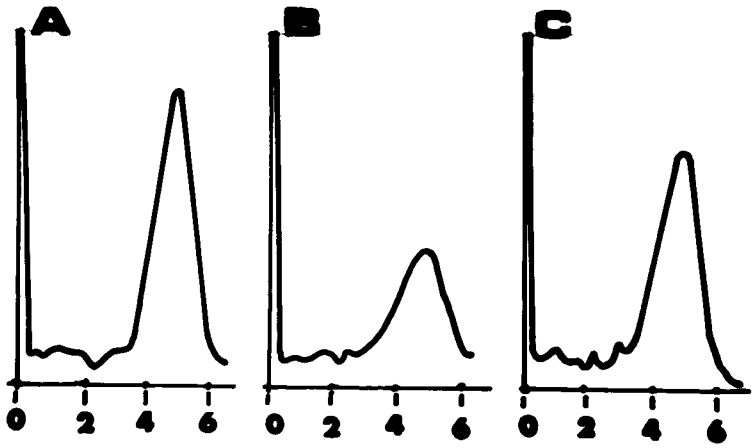
Alfalfa pellets and rumen contents, 100 g. each, were extracted with n-hexane. The extract of each was re-extracted with acetonitrile-hexane (De Faubert Maunder et al. 1964) and samples were passed through an activated alumina column (Getz and Wheeler 1968) topped with a 2-cm high layer of charcoal. The effluent solution was evaporated in a Kuderna-Danish concentrator to 1 ml. The concentrate was used for thin-layer chromatography (T.L.C.) and gas-liquid chromatography (G.L.C.) methods.

[+] 2-(diethoxyphosphinylimino)-1,3-dithiolane. Manufactured by American Cyanamid Company, Wayne, N.J., U.S.A.

Bulletin of Environmental Contamination & Toxicology, Vol. 12, No. 2 © 1974 by Springer-Verlag New York Inc.

1. T.L.C.

The plates were prepared, dried, and activated according to standard methods (Stahl 1965). The T.L.C. absorbent was silica gel GF254 and the mobile phase was acetone-benzene (15+35). A volume of 50 l. of the concentrate was spotted on the plate. The plate was kept in the developing tank until the solvent front advanced 10 cm. The spots were visualized both by ultraviolet light (366 nm) and by spraying the plate with 2% 4-(p-nitrobenzyl) pyridine in acetone (Tadjer and Lustig 1971). After heating the plate for 5 minutes at 105°C, it was resprayed with acetone containing 10% tetraethylene pentamine (Getz and Wheeler 1968). The extracts from the rumen contents and alfalfa pellets, as well as the reference standard+ of Cyolane, were visualized as purple-blue spots with Rf_{100}=48 (Tadjer and Lustig 1971).



RETENTION TIME

Figure 1

Gas Chromatograms of (A) Cyolane standard; (B) extraction rumen contents; (C) alfalfa pellets.

2. G.L.C.

A volume of 5 l. of the extract from the alfalfa pellets and rumen contents was injected. The instrument was equipped with an electron-capture detector (E.C.D.), the glass column (6 ft. x 1/8 in.) with built-in glass injector port was packed with 10% QF 1 on Chromosorb W (80-100 mesh). The operating temperatures for injector, column, and E.C.D. were 240°, 210°,

+ Approx. 95% purity. Kindly supplied by the Ministry of Health, Jerusalem.

and 180°C, respectively. Carrier gas was nitrogen. The retention times for the reference standard (Fig. 1A), for the extract of rumen contents (Fig. 1B), and for the alfalfa pellets (Fig. 1C) were between 5 and 5.5 minutes giving a definite peak.

3. Colorimetric method

A quantitative colorimetric procedure was originally devised to determine residues of dithiolane insecticides in cottonseed and on cotton foliage (Blinn and Boyd 1964). The chromogenic reaction is based on the acid hydrolysis of Cyolane to a product which is converted to thiocyanate by treatment with alkali. The thiocyanate is then converted to cyanogen bromide and reacted with benzidine in pyridine solution to form an intense red solution (max 530nm).

Alfalfa pellets and rumen contents of one cow contained 1.26 and 0.3 p.p.m. Cyolane, respectively. The same methods were used on alfalfa pellets and rumen contents of cows with no known history of Cyolane contamination, and in these samples no Cyolane was detected taking into account background values.

Discussion and Comments

Many organophosphorus insecticides are rapidly metabolized and eliminated from the animal body. Cyolane residues, however, may remain in alfalfa for a long time in sufficient quantities capable of causing toxic symptoms in cattle (Egyed et al. 1972). Cyolane has a relatively high toxicity to laboratory animals and marked cholinesterase inhibiting ability not only in laboratory animals (Anon 1966) and dogs (Noel et al. 1966) but also in cattle (Egyed et al. 1972). Cholinesterase activity returned to normal levels some three months after feeding of Cyolane contaminated alfalfa hay had been discontinued. It could well be that the apparent high toxicity is not directly related to the small amounts of Cyolane which were found on analysis but could be due to a relatively high amount of metabolite with potent ability to depress the cholinesterase activity. It cannot be excluded that drying alfalfa under dry climate prevents rapid degradation of the metabolite.

Our T.L.C. and G.L.C. methods were possibly unsuitable for the detection of this metabolite if it is a relatively polar substance. This point, however, should be further investigated.

References

ANON. Technical Information. Cyanamid International. Wayne, N.J., U.S.A. (1966).

BLINN, R.C., and J.E. BOYD: JAOAC 47, 1106 (1964).

DE FAUBERT MAUNDER, M.J., H. EGAN, E.W. GODLY, J. ROBURN, and J. THOMPSON: Analyst 89, 174 (1964).

EGYED, M.N., J. BARANCHUK, M. SCHOENBAUM: Refuah vet. 29, 158 (1972).

EGYED, M.N., G.S. TADJER, J. BARANCHUK: Refuah vet. 28, 106 (1971).

GETZ, M.E., and E.G. WHEELER: JAOAC 51, 1101 (1968).

NOEL, P.R.B., M. CHESTERMAN, D. JACOBSON, A.E. STREET: CG 47031 toxicity studies in dogs. Final report. Huntingson Research Centre, Huntingdon, England (1966).

SCHOENBAUM, M., U. KLOPFER, M.N. EGYED: Lab. anim. 6, 327 (1972).

STAHL, E. (Ed.): Thin Layer Chromatography. Springer Verlag, Berlin, Heidelberg, New York, 1965.

TADJER, G.S., A. LUSTIG: J. Chromat. 56, Table 380 D., 48 (1971).