content would, as suggested by Bretzloff (1970), make less calcium available to the cell wall. A high starch content could therefore be expected to result in mealiness. Since total solids and specific gravity are almost uniquely dependent on the starch content, similar positive correlations between these factors and mealiness would follow as a matter of course.

Age and Storage Time. The changes that occur in potato tubers during storage are complex and incompletely understood. But perhaps most significant are the reduction in turgor due to dehydration and loss of starch due to respiration. The first factor would be expected to result in a relaxation of shear stresses in the cell surface and an ability to tolerate thermal expansion without exceeding the elasticity limit. Loss of starch would result in release of calcium, which now becomes available for reinforcement of the cell wall constituents. Both of these factors would therefore be expected to result in loss of mealiness which is, in fact, the common experience.

Starch Retrogradation. Gel formation and retrogradation of the gel has been shown to influence texture in products subject to chilling or extended cooling periods. The extent to which retrogradation takes place is evidently a function of the nature of the starch, particularly the amylose–amylopectin ratio.

Diffusion of amylose has been postulated to strengthen intercellular adhesion, and releasable amylose was observed to be particularly prominent in low-starch tubers (Reeve, 1954). The latter two effects will tend to obscure or alter the overall effect of the cell wall-related factors discussed earlier.

Texture of the cooked potato is obviously influenced by numerous factors, and no attempt has here been made to treat this subject exhaustively. The purpose of this communication is rather to focus on those factors that appear to be of major importance and to try to interpret experimental results in terms of a coherent concept. In that context, the starch gelation pressure should only be thought to exist when there is a net influx of mass to the tissue and not as phenomenon that inevitably takes place when gelation of the starch grains occurs.

LITERATURE CITED

Atwater, W. O., *U.S.D.A. Exp. Sta. Bull.* **21,** 88 (1895).
Barrios, E. P., Newsom, D. W., Miller, J. C., *Amer. Potato J.* **38,** 183 (1961b).
Barrios, E. P., Newsom, D. W., Miller, J. C., *Proc. Amer. Soc. Hort. Sci.* **78,** 413 (1961a).
Barrios, E. P., Newsom, D. W., Miller, J. C., *Amer. Potato J.* **40,** 200 (1963).
Bartolome, L. G., Hoff, J. E., J. AGR. FOOD CHEM. **20,** 266 (1972).
Bettelheim, F. A., Sterling, C., *Food Res.* **20,** 71 (1955).
Bretzloff, C. W., *Amer. Potato J.* **47,** 176 (1970).
Fraser, S., "The Potato," Orange Judd, New York, N. Y., 1910, p 169.
Gilbert, A. W., "The Potato," Macmillan, New York, N. Y., 1920, p 263.
Le Tourneau, D. J., Zaehringer, M. V., *Idaho Agr. Exp. Sta. Res. Bull.* No. 64 (1965).
Linehan, D. J., Hughes, J. C., *J. Sci. Food Agr.* **20,** 113 (1969).
Personius, C. J., Sharp, P. F., *Food Res.* **4,** 229 (1939).
Rathsack, K., "Der Speisewert der Kartoffel," Berlin, 1935.
Reeve, R. M., *Food Res.* **19,** 333 (1954).
Reeve, R. M., *Econ. Bot.* **21,** 294 (1967).
Reeve, R. M., J. AGR. FOOD CHEM. **20,** 1282 (1972).
Schwartz, J. H., Greenspun, R. B., Porter, W. L., *Food Technol.* **14,** 364 (1961).
Talburt, W. T., Smith, O., "Potato Processing," AVI, Westport, Conn., 1959, p 18.
Unrau, A. M., Nylund, R. E., *Amer. Potato J.* **34,** 303 (1957).
Wager, H., *J. Sci. Food Agr.* **14,** 583 (1963).
Whittenberger, R. T., Nutting, G. C., *Food Res.* **15,** 331 (1950).
Whittenberger, R. T., *Amer. Potato J.* **28,** 738 (1951).

Johan E. Hoff

Department of Horticulture
Purdue University
Lafayette, Indiana 47907

Received for review June 28, 1972. Accepted August 8, 1972. Purdue University Agricultural Experiment Station Journal Article No. 4798.

Effect of DDT on Rumen Fermentation

The effect of DDT, DDD, other metabolites and analogs of DDT, chlordane, dieldrin, aldrin, and DDVP on *in vitro* fermentation and methane production by rumen microbes was studied. DDT inhibited methane production only at very high levels (240 ppm) in contrast to chloral hydrate, which is a potent methane inhibitor at 12 ppm. DDT and other pesticides studied had no effect on fermentation gases or volatile fatty acid metabolites at 120 ppm.

Trei *et al.* (1971a,b, 1972) have shown that when sheep and cattle are given certain compounds in the diet which inhibit methane production in the rumen, an improvement in growth rate and efficiency of utilization of feedstuffs results. The performance response is at least partially due to inhibition of rumen methane production. A recent report (McBride, 1970; McBride and Wolfe, 1971b) that DDT is a potent inhibitor of methanogenesis by certain methanogenic bacteria prompts us to report our observations of the effect of DDT, some of its metabolites and analogs, and five other polychlorinated insecticides on fermentation and methane production by rumen microbes.

In vitro fermentations were carried out as described in a previous report (Trei *et al.*, 1971b). Strained rumen fluid (75 ml) obtained from a fistulated steer was incubated with a grain substrate (2.4 g) and the chemical additives for 3 hr. The resulting gaseous and liquid metabolites are shown in Table I.

When compared with other polyhalogenated compounds which inhibit methane production at concentrations of 1–10 ppm [*e.g.*, chloroform (Trei and Olson, 1969), bromochloromethane (Trei *et al.*, 1970), and some haloacetic acids and derivatives (Trei *et al.*, 1971b)] both pure and technical grade DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] have little antimethanogenic activity in rumen fluid. When added to rumen fluid at 240 ppm, DDT inhibited methane production only to the extent of 20% (experiment 4). At 120 ppm DDT had no effect on the fermentation as measured by pro-

Table I. Effect of DDT and Related Compounds on *in vitro* Rumen Fermentation Parameters

Compound[a]	Concn,[b] ppm	Total[c] gas, cm³	Volume % gas			Total VFA, μm/ml[d]	Molar % VFA			
			CO_2	CH_4	H_2		C_2	C_3	C_4	C_5
Experiment 1										
Control zero time		0				113	71.4	17.6	9.5	
Control 3 hr		83	72	28	0	159	67.1	16.5	13.3	1.8
DDT (pure)	60	77	72	28	0	168	68.0	16.7	12.5	1.8
DDT (pure)	12	76	70	30	0	169	67.2	16.6	13.0	1.9
DDT (tech)	60	80	71	29	0	158	67.5	16.8	12.8	1.8
DDD	60	82	71	29	0	159	67.7	16.5	12.9	1.8
Chlordane	120	75	72	28	0	163	68.2	16.3	12.6	1.8
Dieldrin	120	82	72	28	0	154	67.4	16.5	13.0	1.9
Aldrin	120	83	72	28	0	160	67.4	16.6	13.0	1.9
DDVP	60	70	72	27	1	153	66.8	16.5	13.4	1.9
p-CTBA	12	75	73	16[f]	11[f]					
Experiment 2										
Control zero time		0				143	65.1	19.9	13.3	
Control 3 hr		69	64	36	0	188	60.9	19.3	17.1	1.5
DDT (pure)	120	68	63	37	0	189	61.0	19.5	16.9	1.5
DDT (tech)	120	68	62	38	0					
Chloral hydrate	12	87	64	4[f]	32[f]	187	55.0[f]	21.0[f]	19.0[f]	1.6
Experiment 3										
Control zero time		0				120	63.9	18.7	15.5	
Control 3 hr		58	66	34	0	163	58.7	18.3	18.8	2.4
DDT (tech)	120	56	66	34	0	172	58.8	19.0	18.6	2.3
DDT (tech in EtOH)	120	56	70	30	0	170	57.0	18.3	20.0	2.3
DDE	120	59	65	35	0	156	58.5	19.0	20.0	
BCT	120	59	65	35	0	176	58.9	18.2	18.8	2.6
DCA	120	59	68	32	0	172	59.1	18.2	18.8	2.4
DDM	120	58	66	34	0	170	59.0	18.3	18.3	2.5
o-CTBA	24	65	75	15[f]	10[f]	164	55.5[f]	19.2	22.4[f]	3.0
Experiment 4										
Control zero time										
Control 3 hr		100	72	28	0	142	60.4	18.2	17.0	2.4
DDT (tech in EtOH)	240	93	72	5[f]	23[f]	130	56.7[f]	19.8[f]	19.2[f]	2.2
DDT (tech)	240	89	76	22[e]	2[e]	133	59.0	18.9	16.8	2.8
Chloral hydrate	12	105	72	4[f]	24[f]	135	56.5[f]	19.8[f]	19.0[f]	2.6

[a] DDT (tech) was obtained from Eastman Organic Chemicals. DDT (pure) was homogeneous by gas chromatography. Aldrin, dieldrin, and DDVP were obtained from Shell Chemical Company. Chlordane was obtained from S. B. Penick and Company. *o*-CTBA and *p*-CTBA were obtained from Pennsalt Manufacturing Company. DCA, BCT, and TBA were obtained from Aldrich Chemical Company. DDM was obtained from Maybridge Chemical Company. DDD was obtained from Rohm and Haas Company. [b] Chemical concentration expressed on a volume basis. [c] Total cubic centimeters of gas produced during an incubation time of 3 hr. [d] *In vitro* volatile fatty acid production is difference between concentration of VFA of incubated sample less concentration of strained rumen fluid inoculum (control zero time) × volume (75 ml). [e] Significantly different from control ($p < 0.05$). [f] Significantly different from control ($p < 0.01$).

duction or composition of fermentation gases or volatile fatty acid (VFA) metabolites (experiments 2 and 3). These concentrations (120 and 240 ppm) exceed the solubility of DDT in water (45 ± 1 ppm at 37.5°; Babers, 1955). DDT was considerably more active when added at a level of 240 ppm in ethanol solution (2 ml in 75 ml of rumen fluid), which probably significantly increased the solubility of DDT. Even at the highest concentration studied (240 ppm), the overall level of fermentation activity did not appear to be greatly inhibited as measured by total gas and VFA production.

Technical grade DDT is generally contaminated with various impurities including *o,p* and *o,o* isomers of DDT, DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], plus *o*- and *p*-chloro-α-trichloromethylbenzyl alcohol (CTBA) (Haller *et al.*, 1945). Both *o*- and *p*-CTBA have significant methane-inhibiting activity at low concentrations, but are less active than the known methane inhibitor chloral hydrate (Van Nevel *et al.*, 1969). As is usually the case when methane is inhibited, molecular hydrogen is observed in the fermentation gases and there is a significant shift in VFA composition from acetate toward propionate and butyrate. This effect has been discussed (Czerkawski, 1969; Rufener and Wolin, 1968; Trei *et al.*, 1970).

The metabolism of DDT by rumen microbes (Fries *et al.*, 1969; Kutches and Church, 1971; Miskus *et al.*, 1965; Sink *et al.*, 1972), and the effect of DDT upon rumen dry matter disappearance, VFA production, and protozoal numbers has been studied (Kutches *et al.*, 1970). At low concentrations (250 ppm) DDT has a negligible effect upon rumen function *in vitro* (Kutches *et al.*, 1970). Sink *et al.* (1972) showed that DDT is slowly metabolized primarily to DDD and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene]. The rate of metabolism appears to be greater on high grain rations than on roughage rations. We did not determine the extent of DDT metabolized in our 3-hr incubation but literature suggests that metabolism should have been less that 10%. Neither DDD nor DDE inhibited methanogenesis when added at 120 ppm. Another DDT metabolite bis(*p*-chlorophenyl)acetic acid (DCA) was also inactive at 120 ppm, as were analogs 2,2-bis(*p*-chlorophenyl)-1,1,1-trifluoroethane (BCT) and dichlorodiphenylmethane (DDM).

Four other well-known polychlorinated insecticides—chlordane, aldrin, dieldrin, and DDVP (2,2-dichlorovinyl dimethyl phosphate)—also had no effect upon the fermentation when added at 120 ppm.

McBride and Wolfe (1971b) reported that DDT is an inhibitor of methane production by cell-free extracts of *Methanobacterium* strain M.o.H. and whole cells of *Methanobacterium ruminantium* strain P.S. Both of these organisms were originally isolated from sludge and are not believed to be present in the normal rumen (Bryant *et al.*, 1971). The predominant rumen methanogen *Methanobacterium ruminantium*

strain M1 is taxonomically related, but has additional requirements for growth (Bryant *et al.*, 1971). These include an exogenous source of Cofactor M, a recently identified cofactor required for methane formation (McBride and Wolfe, 1971a). This surprising difference in the susceptibility of rumen microbes and pure cultures of nonrumen methanogens to inhibition by DDT may be due to differences in cell membrane permeability, preferential uptake by nonmethanogenic rumen microbes, or nonspecific absorption by other components of crude rumen fluid. Cellular uptake of DDT in rumen fluid is reported to be rapid and greater than 95% (Kutches and Church, 1971).

Some polychlorinated compounds are potent inhibitors of rumen methanogenesis and alter the composition of rumen fermentation metabolites. However, a single exposure to relatively high concentrations of DDT and several other polychlorinated insecticides does not appear to have a major direct effect upon *in vitro* rumen fermentations. The effect of long-term exposure *in vivo* is unknown.

LITERATURE CITED

Babers, F. H., *J. Amer. Chem. Soc.* **77,** 4666 (1955).
Bryant, M. P., Tzeng, S. F., Robinson, I. M., Joyner, A. E., Jr., *Advan. Chem. Ser.* **105,** 23 (1971).
Czerkawski, J. W., *World Rev. Nutr. Diet.* **11,** 240 (1969).
Fries, G. R., Marrow, G. S., Gordon, C. H., J. AGR. FOOD CHEM. **17,** 860 (1969).
Haller, H. L., Bartlett, P. D., Drake, N. L., Newman, M. S., Cristol, S. J., Eaker, C. M., Hayes, R. A., Kilmer, G. W., Magerlein, B., Mueller, G. P., Schneider, A., Wheatley, W., *J. Amer. Chem. Soc.* **67,** 1591 (1945).
Kutches, A. J., Church, D. C., Duryee, F. L., J. AGR. FOOD CHEM. **18,** 430 (1970).
Kutches, A. J., Church, D. C., *J. Dairy Sci.* **54,** 540 (1971).
McBride, B. C., Ph.D. Thesis, Department of Microbiology, University of Illinois, 1970.
McBride, B. C., Wolfe, R. S., *Biochemistry* **10,** 2317 (1971a).
McBride, B. C., Wolfe, R. S., *Nature (London)* **234,** 551 (1971b).
Miskus, R. P., Blair, D. P., Casida, J. E., J. AGR. FOOD CHEM. **13,** 481 (1965).
Rufener, W. H., Jr., Wolin, M. J., *Appl. Microbiol.* **16,** 1955 (1968).
Sink, J. D., Varela-Alverez, H., Hess, C., J. AGR. FOOD CHEM. **20,** 7 (1972).
Trei, J. E., Olson, W. A., *J. Anim. Sci.* **29,** 173 (1969).
Trei, J. E., Parish, R. C., Scott, G. C., *J. Anim. Sci.* **33,** 1171 (1971a).
Trei, J. E., Parish, R. C., Singh, Y. K., Scott, G. C., *J. Dairy Sci.* **54,** 536 (1971b).
Trei, J. E., Scott, G. C., Parish, R. C., *J. Anim. Sci.* **34,** 510 (1972).
Trei, J. E., Singh, Y. K., Scott, G. C., *J. Anim. Sci.* **31,** 256 (1970).
Van Nevel, C. J., Hendrix, H. K., DeMeyer, D. I., Martin, J., *Appl. Microbiol.* **117,** 695 (1969).

Roger C. Parish*
John E. Trei

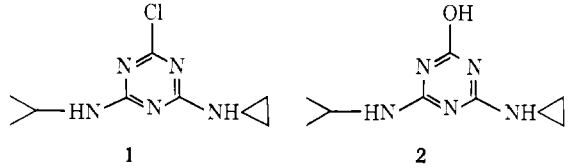
Smith Kline and French
1600 Paoli Pike
Applebrook Animal Health Research Center
West Chester, Pennsylvania 19830

Received for review May 9, 1972. Accepted July 12, 1972.

Analytical Method for Determination of Residue Levels of Hydroxycyprazine (2-Hydroxy-4-cyclopropylamino-6-isopropylamino-*s*-triazine) in Corn

An analytical procedure for determination of residue levels of hydroxycyprazine (2-hydroxy-4-cyclopropylamino-6-isopropylamino-*s*-triazine) in corn silage and grain has been developed. The procedure is based on extraction and separation of hydroxycyprazine, followed by its conversion to cyprazine (a chlorotriazine), which subsequently is determined gas chromatographically.

Chloro-*s*-triazines are widely used as herbicides, primarily on corn, *Zea mays*. Cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine, **1**) is a recently introduced member of this class of compounds. The metabolism of cyprazine in corn plants was investigated using ^{14}C ring-labeled cyprazine applied both foliarly and to the soil. Cyprazine, upon foliar application to corn plants in the 3–6 leaf stage (the recommended time of treatment), is converted rapidly and nearly quantitatively to a polar metabolite(s) (Riden and Asbell, 1969), later found to be analogous to the glutathione conjugate reported for atrazine (Lamoureux *et al.*, 1970). Time studies showed that this conjugate hydrolyzes sequentially through the γ-glutamylcysteine conjugate to hydroxycyprazine (2-hydroxy-4-cyclopropylamino-6-isopropylamino-*s*-triazine, **2**) and then to dealkylated products (Riden *et al.*, 1970). These metabolic products, containing the *s*-triazine ring, do not translocate from the treated leaves and are lost from the corn plant when these leaves naturally senesce and are shed. A field study on the uptake of soil-applied ^{14}C ring-labeled cyprazine into young corn plants showed the possibility of **2** being present in the leaves. The difficulties inherent in low level radioassay techniques prevented determination of an exact level of **2**. However, this study indicated that no more than 0.05 ppm could be present in the corn leaves with none found in the stems or ears. It was, therefore, necessary to develop an analytical procedure to determine the levels of **2**, if any, which might appear in the grain and silage of corn treated with **1**.



1 **2**

Identification techniques for hydroxy-*s*-triazines have been reported, including various types of chromatography (Fishbein, 1970; Flint and Aue, 1970; Harris, 1967; Montgomery and Freed, 1964), and mass spectrometry (Montgomery *et al.*, 1969). None of these methods were found to be suitable to residue analysis, due either to lack of sensitivity or interference by naturally-occurring materials.

Hydroxycyprazine exists as a tautomeric mixture involving ring-protonated structures similar to those reported for other hydroxy-*s*-triazines (Chen, 1967). Its chemistry, therefore,