

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231548456>

Persistence and fate of ethylenethiourea in tomato sauce and paste

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 1984

Impact Factor: 2.91 · DOI: 10.1021/jf00125a066

CITATIONS

11

READS

36

2 AUTHORS:



[Ramble O. Ankumah](#)

Tuskegee University

51 PUBLICATIONS 263 CITATIONS

SEE PROFILE



[William Marshall](#)

McGill University

133 PUBLICATIONS 1,707 CITATIONS

SEE PROFILE

Table IV. Comparison of Cellulose Index with Cellulose Values

sample	I, % cellulose	II, cellulose index	I/II ratio	cellulose index \times 0.8
bright lamina	5.9	7.1	0.83	5.7
bright stems	11.7	14.8	0.79	11.8
burley lamina	6.4	8.3	0.77	6.6
burley stems	15.4	19.1	0.81	15.3
\bar{X} : 0.80				

milliliter for the various tobacco samples and demonstrates the stability of the solutions over a period of at least 8 days.

Comparison of Methods. The cellulose index values obtained by the procedure described here were compared with the cellulose values reported by Bokelman and Ryan (1983) for the same four samples. The cellulose value can be closely approximated by taking 80% of the cellulose index. Data are given in Table IV.

CONCLUSIONS

A relatively rapid and simple procedure has been developed for the cellulose index of various cured tobacco materials. The RSD at 2σ is $\pm 5.0\%$ for a ground, uncased bright lamina with a cellulose index of 7.1. The cellulose index values are approximately 25% higher than the cellulose values for samples analyzed by the fractionation method of Bokelman and Ryan (1983).

ACKNOWLEDGMENT

I thank A. B. Smiley and Shirley Langley for the analysis of many samples for total reducing sugars and for conducting the stability study of the hydrolysates.

Registry No. Cellulose, 9004-34-6.

LITERATURE CITED

- American Society for Testing and Materials. Standard Test Methods D1102-56, D1103-60, D1104-56, D1105-56, D1106-56, and D1107-56.
- Bokelman, G. H.; Ryan, W. S., Jr. submitted for publication in *Beitr. Tabakforsch.*, 1983.
- Bokelman, G. H.; Ryan, W. S., Jr.; Oakley, E. T. *J. Agric. Food Chem.* 1983, 31, 897-901.
- Christy, M. G.; Samfield, M. *Tob. Sci.* 1960, 4, 33-37.
- Davis, R. E. *Tob. Sci.* 1976, 20, 139-144.
- Fieser, L. F.; Fieser, M. "Introduction to Organic Chemistry"; D. C. Heath and Co.: Boston, MA, 1957; p 280.
- Hellendoorn, E. W.; Noordhoff, M. G.; Slagman, J. *J. Sci. Food Agric.* 1975, 26, 1461-1468.
- Ryan, W. S., Jr. *Beitr. Tabakforsch.* 1984, 12, 105-111.
- Tso, T. C. "Physiology and Biochemistry of Tobacco Plants"; Dowden, Hutchinson & Ross: Stroudsburg, PA, 1972; p 199.
- Updegraff, D. M. *Anal. Biochem.* 1969, 32, 420-424.
- Van Soest, P. J.; Wine, R. H. *J. Assoc. Off. Anal. Chem.* 1968, 51, 780-785.

Received for review December 8, 1983. Revised manuscript received March 23, 1984. Accepted April 19, 1984.

Persistence and Fate of Ethylenethiourea in Tomato Sauce and Paste

Ramble O. Ankumah and William D. Marshall*

Tomato sauce was fortified (at 5.00, 0.50, or 0.05 ppm) with [4,5- ^{14}C]ethylenethiourea (ETU), and samples were analyzed weekly for 3 months. Recovered products were separated by thin-layer chromatography, located by autoradiography and quantified by liquid scintillation counting. The toxicant was rather stable in this matrix over the course of the investigation. The rate of decomposition depended upon the level of spiking and on batch to batch differences in the matrix. Five decomposition products identified by TLC; they were 2-imidazolyl hydrosulfate, 2-imidazolidinesulfonate, 2-imidazolidone, 2-(methylthio)imidazolidine, and 1-(2-imidazolin-2-yl)-2-imidazolidinethione. As a consequence of the persistence of ETU in this matrix, a quarantine or holding time for canned tomato products prior to distribution or sale would not represent a suitable decontamination technique of this commodity.

Ethylenethiourea (ETU, 2-imidazolidinethione) is a toxicologically significant decomposition product of ethylenebis(dithiocarbamate) (EBDC) fungicides. EBDCs are widely used in fruit and vegetable production to control a variety of fungal pathogens. These fungicides are not systemic; however, surface residues on fresh produce can be converted to ETU during normal industrial processing involving heat treatment (Watts et al., 1974; Newsome and Laver 1973; Philips et al., 1977). The fate of ETU in the sterile environment of a processed food is controversial. It has been reported (Han, 1977) that ETU, during a 4-week storage (at 1.0 or 0.1 ppm), decreased to 1% of the initial amount in pickles, 1-5% in apple sauce, 0.1-0.2% in tomato sauce, and 9-12% in spinach. In contrast, Uno et al. (1978) have reported that ETU in tomato puree was stable for up to 200 days. Efficient decontamination

procedures are available for the removal of EBDC surface residues from tomatoes and green beans (Marshall and Jarvis, 1979; Marshall, 1982) prior to processing. However, if a suitable holding time for a potentially contaminated food could be defined, these preprocessing washes would not be necessary. Most processed fruits and vegetables are canned during a short 2-3-week period of each year and sufficient storage facilities are available. Thus, a suitable holding time would represent an attractive decontamination procedure provided that the degradation products were innocuous.

Abiotic transformations of ETU have been described as being mainly oxidative (Rose et al., 1980). Ethyleneurea (2-imidazolidone, EU) results directly from the oxidation of ETU or from partial oxidation (to sulfinate or sulfonate) followed by hydrolysis (Marshall, 1979). In contrast, ETU itself is quite stable to hydrolysis. The interaction of ETU with substrates that function as sulfur acceptors has been less well studied. Thiourea reacts with a variety of halogenated heterocycles (Boarland and McOmbie, 1951; Polonovski and Schmitt, 1950; Scott and Watt, 1937; Ro-

*Department of Agricultural Chemistry and Physics, Macdonald College, Ste. Anne de Bellevue, Quebec H9C 1C0.

senhauer et al., 1929) in alcohols to form isothiuronium salts. These salts are decomposed *in situ* by the reaction medium (or may be readily hydrolyzed in dilute base) to form thiolated heterocycle and presumably urea. Kirkman and Wolfenden (1978) have used 6-chloropurine ribonucleoside as a substrate for the "chemical mutation" in water of a variety of cysteine derivatives to serines, and S-alkylated derivatives of ETU have been reported to be unstable in the presence of crop extracts. Phenylmercuric acetate complexes strongly with several oxazolidine-2-thiones and has been used as a phase transfer reagent for their recovery at ppb levels from plasma, urine, milk, and fodder (de Brabander and Verbeke, 1982).

In these studies we wished to further clarify the persistence of ETU in tomato sauce and paste and to identify the products resulting from the decomposition of this toxicant. In addition, evidence was sought for interactions of ETU with components of sauce that might accelerate the decomposition process.

MATERIALS AND METHODS

Fortification and Simulated Sterilization. Tomato sauce, purchased locally (100 mL), was fortified at 0.05, at 0.50, or at 5.00 ppm with [4,5-¹⁴C]ETU. After thorough mixing with a magnetic stirrer for 10 min, 2-mL portions were transferred to individual Pyrex (13 × 100 mm) tubes and loosely sealed with screw caps. The resulting 40 samples were placed in an oven at 80 °C for 1 h to mimic industrial processing. Nitrogen was blown over the surface of each sample for 2 min to displace air and the vials were tightly sealed while still hot. The pool of individual samples was maintained in the dark at room temperature. Three samples were removed from the pool at time zero and at 7-day intervals. These samples were analyzed immediately as described below.

Recovery of Labeled Products. Individual samples were rapidly shell frozen and then freeze-dried for 12 h at -40 °C. The residue was triturated in 5 mL of methanol. A 100-μL portion of the methanolic extract was counted directly, and the remainder was reduced in volume under a gentle stream of nitrogen. Equal volumes of the concentrate were applied (as a streak) to precoated 5 × 20 cm silica gel (Whatman LK5F, 0.25 mm or Uniplat GHLF, 0.25 mm, or SIL UV-254, 0.20 mm) and to precoated cellulose (Cell 300 UV-254, 0.1 mm, or Cell 400 UV-254 or Cell 300 DEAE Polygram, Macherey Nagel and co.) thin-layer plates. The plates were eluted with ethyl acetate-ammonia-water (90:6:6 v/v/v (silica gel) or with 1-propanol-water (85:15 v/v) (cellulose). Individual plates were eluted for approximately 15 cm.

Autoradiographic Detection and Quantitation. Eluted plates were covered with X-ray film (Kodak XAR-2) in light-tight cassettes for 7 or 14 days. On the basis of the pattern of the developed film, radioactive areas on the chromatographic plates were located. The adsorbent from these radioactive areas was removed, suspended in cocktail, and quantified by scintillation counting. The methanol-insoluble residue from the freeze-dried sample was solubilized by digestion (1 h at 90 °C) with tetramethylammonium hydroxide (0.5 mL). Hydrogen peroxide (0.1 mL of 35% solution) was added at 0.5-h intervals. The resulting clear hydrolysate was diluted with scintillation cocktail and counted directly. All counts were corrected for background and quenching.

Association Experiments. Size exclusion studies were performed by placing test mixtures directly on the head of a Sephadex G-25 column (10 × 30 cm) and eluting (gravity flow) with acetate buffer (pH 4.2). Ten-drop fractions were collected in individual scintillation vials,

diluted with scintillation cocktail, and assayed for activity. The column was calibrated with dextrin blue (void volume) and with cobaltous chloride (totally retained fraction). Standard ETU was observed to elute in the totally retained fraction.

Partitioning experiments using ethyl acetate or dichloroacetic acid in acetonitrile (Singh et al., 1979) were performed using 1 mL of sauce fortified with 0.50 ppm of [4,5-¹⁴C]ETU and 3.0 mL of solvent. The mixtures were vortexed for 0.5 min and then centrifuged for 5 min to hasten phase separation. The organic supernatant was transferred to scintillation vials, reduced in volume under nitrogen, and counted after dilution with scintillation cocktail. Distilled, deionized water that had been similarly spiked with labeled ETU served as a basis for comparison.

Labeled Reagents. Specifically labeled [4,5-¹⁴C]ETU, 8.35 Ci/mol, was demonstrated to be 98.5 ± 2.5% pure by thin-layer chromatography and reisolation of the product. No labeled impurities could be detected by autoradiography in either system. EU (4,5-¹⁴C) was prepared by reacting a 20-μL aliquot of [4,5-¹⁴C]ETU in 1% sodium bicarbonate with excess 35% hydrogen peroxide. The reaction mixture was maintained in the dark at room temperature and then concentrated on a rotary evaporator. The radiochemical yield of the crude product by reisolation from silica gel TLC plate was 92 ± 3%.

RESULTS AND DISCUSSION

Persistence Trials. Plots of the persistence of ETU (at 0.05, 0.50, or 5.00 ppm) in tomato sauce as a function of time (week 0 to week 12) are presented in Figure 1. In these graphs the total activity in the methanolic extract, the ETU, and other products are expressed as a percentage of the activity initially added to each sample. Each entry in these graphs represents the analysis of a separate sample (three replicate samples in the 0.50- and 5.00-ppm trials and duplicates in the 0.05-ppm trial were analyzed). "Other products" represent the sum of origin material, suborigin material from the initial site of application on the preadsorbent layer, and products that were less mobile than ETU. These results were based on activity recovered from silica gel plates by using chromatographic conditions in which ETU was separated from all the known transformation products.

Recovery of activity from the sauce was reproducible and ranged from 91 to 76% over the 12-week course of these trials. Linear regression analyses for both the percent extractable activity and the percent ETU recovered at all spiking levels were performed; the relevant statistical parameters are recorded in Table I. Although the goodness of fit of the linear regression model (R^2) was only moderate, the coefficient of variation was acceptably low. These data indicate that at the 5.00-ppm level neither the total activity extracted nor the percent ETU recovered varied systematically with time. In contrast, at the two lower levels of spiking there was a modest but steady decline with time of both the ETU and the total activity recovered. Within the constraints of the regression model, no significant rate differences between the 0.50- and 0.05-ppm levels could be detected for either analyte. A possible explanation for the differences between the higher and the two lower spiking levels would involve the participation of a limited number of active sites within the sauce. At the highest level these sites were overloaded.

It was further observed that the sum of the total extractable activity and methanol-insoluble activity (residual activity) was equal to the activity originally added to each sample. This was true for all three spiking levels and indicates that no activity was being lost during the

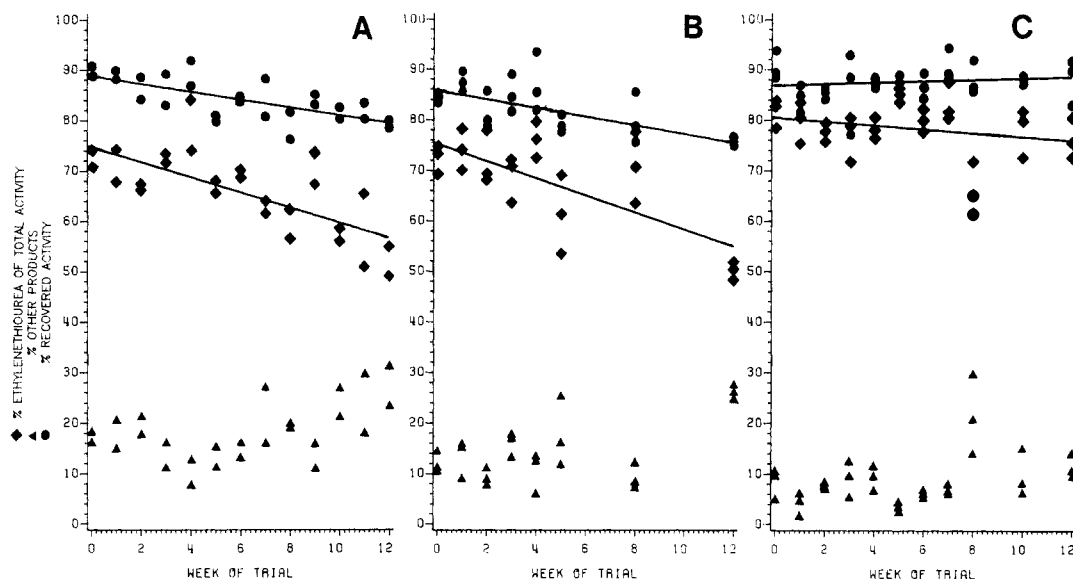


Figure 1. Variation with time of ETU recovered (♦), of total activity recovered (●), and of other products recovered (▲) from tomato sauce fortified with 0.05 (A), 0.50 (B), or 5.00 (C) ppm of [4,5-¹⁴C]ETU.

Table I. Regression Analyses for the Recovery of Analytes from Tomato Sauce as a Function of Time

analyte	spiking level, ppm	slope	linear regression analysis			
			y intercept	R ² ^a	CV, % ^b	confidence interval ^c for slope
% ETU recovered	5.00	-0.38	80.6	0.06	6.91	-1.02 < -0.38 < 0.25
	0.50	-1.69	75.5	0.48	9.95	-2.48 < -1.69 < -1.15
	0.05	-1.52	75.1	0.49	0.09	-2.16 < -1.52 < -0.88
% activity extracted	5.00	0.15	86.8	0.03	3.93	-0.19 < 0.15 < 0.49
	0.50	-0.82	86.0	0.39	4.73	-1.26 < -0.82 < 0.37
	0.05	-0.78	88.8	0.49	1.11	-1.11 < -0.78 < -0.45

^a Measure of goodness of fit. ^b Coefficient of variation. ^c 95% confidence interval.

Table II. Range of Percent Recovery of Total Activity and of ETU from Tomato Sauce, Paste, and Buffers

expt	batch no.	fortification level, ppm	matrix	duration, weeks	replicates	range of act. recovered ^a		decrease in ETU, ^a %	
						B ^b	T ^c	B ^b	T ^c
I	1	5.00	sauce	12	3	91 ± 3	89 ± 5	82 ± 3	76 ± 4 (6)
II	1	0.50	sauce	12	3	85 ± 3	76 ± 2	72 ± 3	50 ± 2 (31)
III	1	0.05	sauce	12	2	90 ± 2	79 ± 3	73 ± 2	52 ± 4 (29)
IV		0.50	phosphate	8	2	100	100	87 ± 3	90 ± 1 (0)
V		0.50	acetate	8	2	100	100	84 ± 0	74 ± 1 (12)
VI	2	0.50	sauce (O ₂)	8	2	91 ± 3	78 ± 4	75 ± 2	46 ± 2 (39)
VII	2	0.50	sauce (N ₂)	8	2	90 ± 2	82 ± 5	73 ± 4	42 ± 2 (42)
VIII	2	0.50	sauce	8	2	88 ± 1	78 ± 2	73 ± 0	45 ± 1 (38)
IX	3	0.50	sauce	5	3	88 ± 3	78 ± 4	80 ± 2	19 ± 4 (76)
X	4	0.50	paste	3	3	88 ± 5	84 ± 3	72 ± 3	69 ± 5 (4)
XI	5	0.50	paste	3	3	93 ± 2	85 ± 4	75 ± 2	67 ± 4 (11)
XII	6	0.50	paste	3	3	98 ± 3	83 ± 5	69 ± 4	67 ± 4 (3)

^a The uncertainty represents 1 SD for triplicates and 1/2 the range for duplicates. ^b B = beginning of trial. ^c T = termination of trial.

freeze-drying or during the subsequent sample workup. Typical autoradiograms of ETU spiking standard chromatographed on silica gel (LK5B plate) and of the 0.50-ppm plate trial after 11 weeks (plate 5) chromatographed on cellulose (Cell 300) are reproduced in Figure 2.

A further series of nine persistence trials was conducted using identical procedures and methodologies; these results are included in Table II. In these trials tomato paste and aqueous buffers (0.1 M phosphate or acetate, pH 4.18), as well as tomato sauce, were used as substrates; however, the fortification level was maintained constant at 0.50 ppm. Ranges of recovery of both total activity and of ETU (for the beginning and termination of each trial) as well as the percent reduction of recovered ETU are included in Table II. Considering trials II, III, VI, VII, VIII, and IX in which tomato sauce served as the matrix, the range of initial

recoveries of both total activity (91 ± 3 to 85 ± 3%) and of ETU (80 ± 2 to 72 ± 3%) between different trials was minimal. Upon termination of these trials the recovery of total activity was also reproducible (82 ± 5 to 76 ± 2%); however, the percent recovery of ETU varied considerably (52 ± 4 to 19 ± 4%). Thus, the decrease in ETU ranged from 29% after 12 weeks to 76% after 5 weeks. Because replicate samples within the same trial gave reproducible results and because different trials (same batch of sauce) performed at the same time (VI vs. VII vs. VIII) or at different times (trial II vs. III) gave reproducible results, it is considered that differences in recoveries were due to differences in the separate batches of sauce.

Within the same batch of sauce, oxygenation of the substrate appeared to have little effect. In trial VI the spiked sauce was aerated for 10 min, spiked, and placed

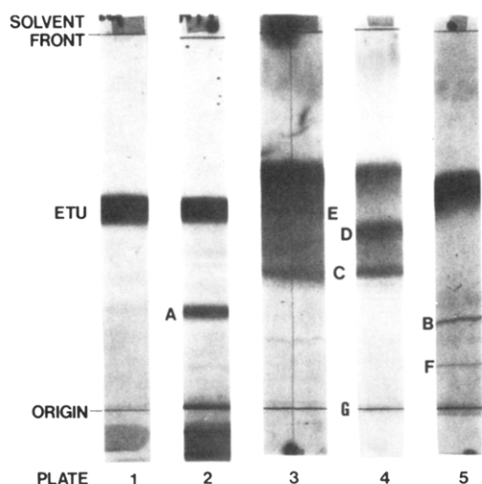


Figure 2. Representative autoradiograms of silica gel (1, 2) and cellulose (3, 4, 5) TLC plates showing the distribution of degradation products A to F. Plate 1 is ETU spiking standard, 2 and 3 are activity from acetate buffer at week 4, 4 is activity from phosphate buffer at week 8, and 5 is activity from the 0.50-ppm stability trial in tomato sauce.

PROCEDURE	SUBSTRATE	TREATMENT	
A	Sauce	1 \diamond 2 \diamond 3 \diamond 4 \diamond	T
B	Sauce	2 \diamond 3 \diamond 4 \diamond 1 \diamond	L
C	Sauce	1 \diamond 3 \diamond 4 \diamond	C
D	Water	1 \diamond 2 \diamond 3 \diamond 4 \diamond	

Figure 3. Variation of procedures for the recovery of activity from tomato sauce spiked with [4,5- 14 C]ETU.

in individual containers. The samples were heat inactivated, and air was blown over the surface of each sample prior to sealing. In trial VII the sauce and individual samples were treated in an identical fashion with nitrogen. Trial VIII served as a control in which no special precautions were taken to include or exclude molecular oxygen. These trials were run concurrently, and no differences in initial or final recoveries of analytes were apparent.

These observations are in sharp contrast to a series of extraction experiments in which a gentle stream of air instead of nitrogen was used to reduce the volume of the final methanolic extract. Tomato sauce was treated following procedure A, B, or C of Figure 3, whereas distilled water served as the substrate for the companion control experiment (procedure D). Following these procedures 96.5, 99.3, and 97.8% (for A, B, and C respectively) of the total activity in the methanolic extract was found within 2 cm of the origin. In contrast, only 11.7% of the activity from procedure D was observed within the origin region. Moreover, 83.5% of the total activity (procedure D) was observed to cochromatograph with authentic ETU. These observations suggest that there is an extractable prooxidant in the sauce that is responsible for the decreased stability of ETU. This extractable prooxidant from the sauce appears to be unaffected by heat treatment (procedure A vs. C).

Transformation of ETU to EU by reaction with sulfur acceptors was achieved in two model systems. ETU reacted rapidly in ethanol at room temperature with 6-chloropurine (but not 5-bromouracil) to form an addition product. The precipitated product was readily hydrolyzed by 0.1 M NaOH, resulting in thiolated purine and EU as evidenced by cochromatography with standards. ETU was also rapidly complexed by phenylmercuric acetate in alkaline media. The complex was organosoluble, reversible (pH adjustment), and susceptible to hydrolysis or air ox-

Table III. Partition Coefficients of ETU Extracts from Tomato Sauce and Water

extraction no.	ethyl acetate ^d		DCAA-CH ₂ Cl ₂ ^{a,d}	
	tomato sauce	water	CH ₃ CN-tomato sauce ^b (1:2)	CH ₃ CN-water ^c (1:10)
1	0.23	0.29	0.18	0.25
2	0.24	0.31	0.17	0.25
3	0.25	0.29	0.18	0.24
4	0.24	0.29	0.16	0.22
5	0.22	0.29	0.18	0.25
6	0.24	0.29	0.17	0.23
7	0.24	0.32	0.19	0.24

^aDCAA-CH₂Cl₂ = dichloroacetic acid (0.01%) in methylene chloride. ^bCH₃CN-tomato sauce = acetonitrile-tomato sauce (1:2). ^cCH₃CN-water : acetonitrile-water (1:10). ^dExtraction solvent.

idation, resulting in phenylmercuric thiol and EU. Complexes of ETU with zinc and cobalt have been described (Eaton and Zaw, 1971). Although these conversions required the participation of thiolate anion, it is postulated that similar interactions occur with the extractable prooxidant in the sauce despite the rather acidic conditions of our trials.

Aqueous buffers (trials IV and V, Table II) were not good model substrates for the decomposition of ETU in sauce or paste. ETU was appreciably more stable in both phosphate and acetate buffers than in tomato products, suggesting that pH alone is not a major factor in decomposition. In short-term trials three separate brands of tomato (trials X, XI, and XII) were studied concurrently. No appreciable differences in the rates of decomposition of ETU were observed. A series of companion trials was conducted in which the same spiked substrates were maintained at 60 °C for 3 weeks. Although ETU decomposition was somewhat more rapid at this temperature, the samples themselves were considerably darkened, indicating extensive degradation of the matrix. Accelerated decomposition studies were therefore abandoned.

Association and Partitioning Experiments. To determine whether an association or complexation of ETU with components of the sauce could be detected, the following experiments were performed. In size exclusion experiments sauce fortified with 0.5 ppm of ETU, or the supernatant from fortified sauce was placed directly on the head of a Sephadex G-25 column (exclusion limit 2500). The presence of sauce or supernatant had no effect on the chromatography of ETU. Association would have been evidenced by activity in the totally excluded fraction or increased activity between the totally excluded and totally retained fractions. It was further demonstrated that the eluted activity within the totally retained fraction (greater than 97% of the activity initially spiked into each sample) corresponded to ETU as evidenced by cochromatography with authentic standard on silica gel TLC. Thus, no on-column decomposition of ETU occurred and recoveries were virtually quantitative.

In partitioning experiments tomato sauce or distilled water fortified with 0.5 ppm of [14 C]ETU was extracted 7 successive times with ethyl acetate or with dichloroacetic acid-methylene chloride. Partition coefficients calculated from the activity in each extract of sauce are compared in Table III with partition coefficients from identical extractions using water as a substrate. The absence of any appreciable change in the partition coefficients with successive extractions indicates a homogeneous environment within the sauce. If association between the toxicant and the sauce did occur, it must have been readily reversible

Table IV. Thin-Layer Chromatographic Behavior of ETU and Transformation Products on Silica Gel or on Cellulose Thin-Layer Plates

standards	solvent system A (EtOAc-NH ₃ -H ₂ O, 15:1:1)			solvent system B (1-propanol- H ₂ O, 95:15)		
	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a
ethylenethiourea (ETU)	0.55	0.42	0.32	0.64	0.77	0.65
2-imidazolidone (EU)	0.28	0.16	0.15	0.58	0.72	0.61
2-imidazoline	0.00 (p) ^b	0.00 (p)	0.00	0.23	0.32	0.27
Jaffe's base	0.00	0.00	0.00	0.37	0.55	0.38
N-acetyl-ETU	0.67	0.55				0.83
ETU-sulfonate (ETU-O ₃)	0.00	0.00	0.05	0.44	0.43	0.40
2-thiohydantoin	0.70			0.61		
2-imidazolone	0.33			0.61		
oxalic acid	0.00	0.00	0.00			0.17
hydantoin		0.37			0.57	0.28
glycine		0.00 (p)			0.09	0.07
S-methyl-ETU	0.00	0.00	0.05	0.49	0.63	0.43
biimidazoline			0.00		0.00	0.10
ethylenediamine (EDA)			0.00		0.10	0.07

^a Plate 1 = silica LK5F (Whatman); plate 2 = silica GHLF (Uniplate, Analtech); plate 3 = SILUV₂₅₄ (E. M. Merck); plate 4 = Cell 300 UV₂₅₄; plate 5 = Cell 300 DEAE; plate 6 = Cell 400 UV₂₅₄ (Polygram, Machery Nagel). ^b P = preabsorbent area.

(at least in the range 0.50–0.075 ppm that was examined in these experiments). This study was replicated 3 times with identical results. The observations from the association and partitioning experiments suggest that ETU in tomato sauce does not bind strongly to constituents of the sauce. By inference, this contaminant, if present, will be bioavailable to organisms ingesting the sauce.

Degradation Product Identification. One of the objectives of this work was to characterize and identify the products of ETU degradation in tomato preparations. At least seven degradation products were detected in the stability trials of Table II. Five of these products were tentatively identified on the basis of cochromatography with authentic standards and known oxidative degradation patterns of ETU (Marshall, 1979; Rhodes, 1977). The chromatographic behavior of standards is summarized in Table IV. Typical autoradiograms indicating the spatial distribution of these degradation products on TLC plates are reproduced in Figure 2. Plates 2 and 3 of this figure represent the distribution of activity (on silica gel and cellulose TLC plates, respectively) recovered after 4 weeks in acetate buffer. Plate 4 is an autoradiogram obtained at week 8 of the phosphate buffer experiment, and plate 5 was obtained from the 0.50-ppm trial in tomato sauce at week 11.

Unknown A was observed in all trials (6–9% of the extracted activity) and cochromatographed with EU (2-imidazolidone). Unknown B was also observed in all trials and cochromatographed with authentic 2-imidazolinyl hydrosulfate (0.5–4% of the extracted activity). Unknown C was more prevalent in the buffers than in tomato preparations and was present in quantities representing 0–5% of the extracted activity. Unknown C cochromatographed with Jaffe's base, 1-(2-imidazolin-2-yl)-2-imidazolidinethione. Unknown D was also observed in both buffers and sauce (0–3%) and cochromatographed with 2-imidazolidinesulfonate. Unknown E was only observed in acetate buffer (4–6%) and was observed to cochromatograph with authentic S-methyl-ETU [2-(methylthio)imidazolidine]. Unknown F was transitory and was only observed in tomato products (4–6%). It was also

observed in EU preparations from ETU reactions with H₂O₂. It disappeared rapidly with the formation of more polar products. Its chromatographic behavior was similar to that of 2-imidazolidinesulfonate (ETU-O₂). However, this was not confirmed. Product G was the most polar of the products and was observed only in the buffers (0–3.5%). It cochromatographed with several potential products (glycine, biimidazoline, oxalic acid, and ethylenediamine) in the solvent systems of this study. The degradation products of ETU characterized in these trials can be described as oxidative. They have been observed previously in degradation studies (Marshall, 1979) and in metabolism studies in plants (Vonk, 1970; Rhodes, 1977). EU has also been characterized in stability trials of ETU in tomato products (Han, 1977).

In summary, the stability of ETU was observed to vary from batch to batch of test substrate. It was stable at 5.00 ppm but was slowly degraded at 0.50 or 0.05 ppm in sauce. Efforts to remove dissolved oxygen or intentional oxygenation of the sauce had no apparent effect on stability; however, an extractable and heat-insensitive prooxidant from the sauce greatly accelerated the decomposition of ETU in extracts. Transformation products were the result of oxidation of the thiocarbonyl group. It is concluded that the stability of ETU in tomato products (sauce and paste) precludes the use of a holding or storage time as an effective decontamination technique for this toxicant.

Registry No. ETU, 96-45-7; 2-imidazolinyl hydrosulfate, 91158-63-3; 2-imidazolidinesulfonate, 91158-64-4; 2-imidazolidone, 120-93-4; 2-(methylthio)imidazolidine, 45439-05-2; 1-(2-imidazolin-2-yl)-2-imidazolidinethione, 484-92-4.

LITERATURE CITED

- Boarland, M. P. V.; McOmbie, J. F. W. *J. Chem. Soc.* **1951**, 1218.
 de Brabander, H. F.; Verbeke, R. *J. Chromatogr.* **1982**, 252, 239.
 Eaton, D. R.; Zaw, K. *Can. J. Chem.* **1971**, 49, 3315.
 Han, J. C.-Y. "Stability of ¹⁴C maneb residues in canned vegetables", as reported in response of E. I. duPont de Nemours and Co. to RPAR, 1977, Vol. II, Appendix 4.
 Kirkman, S.; Wolfenden, R. *J. Am. Chem. Soc.* **1978**, 100, 5943.
 Marshall, W. D. *J. Agric. Food Chem.* **1979**, 27, 295.
 Marshall, W. D. *J. Agric. Food Chem.* **1982**, 30, 649.
 Marshall, W. D.; Jarvis, W. R. *J. Agric. Food Chem.* **1979**, 27, 766.
 Newsome, W. H.; Laver, G. W. *Bull. Environ. Contam. Toxicol.* **1973**, 10, 151.
 Philips, W. F.; Grady, M. D.; Greudenthal, R. "Effects of Food Processing on Ethylenebis(dithioncarbamate) Fungicides and Ethylenethiourea"; National Technical Information Service, U.S. Department of Commerce: Springfield VA, 1977; PB-268653.
 Polonovski, M.; Schmitt, Hh. *Bull. Soc. Chim. Fr.* **1950**, 17, 616.
 Rhodes, R. C. *J. Agric. Food Chem.* **1977**, 25, 528.
 Rose, D.; Pearson, C. M.; Zuker, M.; Roberts, J. R. "Ethylenethiourea: Criteria for the Assessment of its effects on man"; National Research Council Canada: Ottawa, Ontario, Canada, 1980; Publ. 18469.
 Rosenhauer, R.; Hoffmann, H.; Heuser, W. *Ber. Dtsch. Chem. Ges. B* **1929**, 62, 2730.
 Scott, W.; Watt, G. W. *J. Org. Chem.* **1937**, 2, 143.
 Singh, J.; Cochrane, W. P.; Scott, J. *Bull. Environ. Contam. Toxicol.* **1979**, 23, 470.
 Uno, M.; Okada, T.; Onji, Y.; Matubara, S.; Veda, E. *J. Food Hyg. Soc. Jpn.* **1978**, 19, 397.
 Vonk, J. W. *Ann. Appl. Biol.* **1970**, 65, 489.
 Watts, R. R.; Storrer, R. W.; Onley, J. H. *Bull. Environ. Contam. Toxicol.* **1974**, 12, 244.

Received for review October 26, 1983. Revised manuscript received February 7, 1984. Accepted May 31, 1984. This paper is based on work performed by R.O.A. in partial fulfillment of M.Sc. requirements. This work received financial support in the form of an operating grant (W.D.M.) from NSERC and a UNESCO fellowship (R.O.A.).