Table VI. Average Amounts of DDT Recovered in Soils from Different Crops in 1954

Crop	Years Treated with DDT	Soil Depth, Inches	DDT Recovered P.P.M.	DDT Recovered Lb./acre
Apple				
Under trees	7	6	34.7	62.2
Between trees	7	6	19.7	35.5
Peach				
Under trees	6–7	8	7.9	19.0
Between trees	6–7	8	3.9	9.4
Potatoes				
Annual	8	9	4.5	12.2
2-year rotation	3	9	1.2	3.2
Corn	3–6	9	5.1	13.7

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PESTICIDE TOXICITY

Chronic Toxicity for Rats of Food Treated with Hydrogen Cyanide

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A study of the chronic toxicity to rats of food fumigated with hydrogen cyanide showed that food containing 100 and 300 p.p.m. of hydrogen cyanide produced no signs of cyanide toxicity during a 2-year feeding period. At termination hematological values were within normal limits and neither gross nor microscopic examination of tissues revealed evidence of pathology due to hydrogen cyanide feeding. Definite increases in thiocyanate concentrations were found in the tissues of the experimental animals. The results of this investigation provide data important in the evaluation of the safety and hazards of hydrogen cyanide in view of its varied uses in agriculture and industry.

HYDROGEN CYANIDE has been used for fumigation for almost 60 years, being introduced originally in California for the fumigation of citrus trees infested with scale insects. Coquillet, 1886, is given credit for being the first to suggest its use for destroying insects on plants (*12, 14*).

Since these early investigations the use of hydrogen cyanide as a fumigant has been extended until it now includes the fumigation of dwellings and barracks (*11*) for the destruction of roaches, water bugs, and bedbugs, and the fumigation of warehouses and mills (*7, 8*) against certain insects that destroy food products. The gas has also been employed at ports of entry to combat the introduction of injurious insects from foreign countries (*9, 13*). Some of the more important of these pests are the pink boll worm and the citrus black fly. Fumigation with hydrogen cyanide is also used to prevent the spread of yellow fever (*5*) and bubonic plague epidemics (*6*).

Cyanides are used extensively in electroplating, photography, extraction of precious metals from ores, and case hardening of steel. In these uses there

is the possibility of the liberation of hydrogen cyanide.

Hydrogen cyanide is not absorbed by dry foodstuffs to any great degree, whereas fruits and leafy succulent vegetables may take up large quantities of the gas. Heerdt (*10*) states that foods with a high moisture content take up notable quantities of hydrogen cyanide on fumigation. Bail and Cancik (*3*) say that fluids and moist foods should not be left in rooms which are being fumigated.

Because hydrogen cyanide is extremely poisonous to man, and is extensively used as a fumigant, this investigation was designed to study the chronic toxicity in rats of food fumigated with hydrogen cyanide when fed over practically their entire life span.

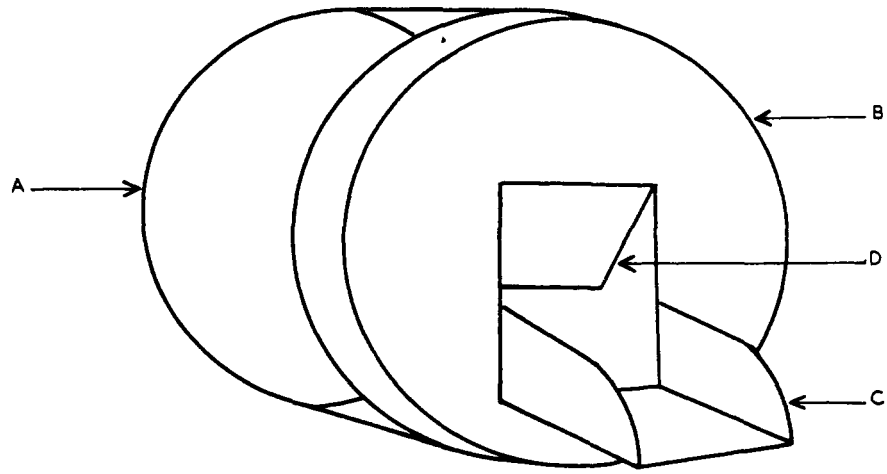


Figure 2. Feeding jar

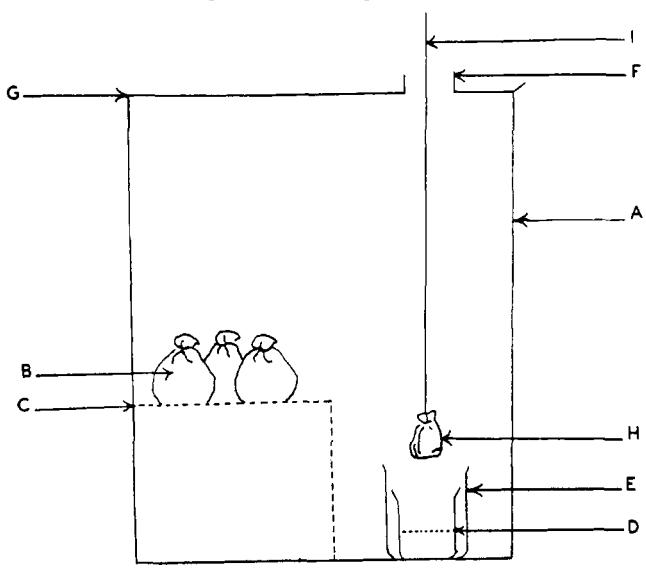
Methods

Weanling male and female albino rats of the Carworth Farms strain were used. The rats were individually housed in wire-mesh cages elevated above the droppings, with water and the appropriate diets available at all times.

The food for the test animals was fumigated as follows: Ten 200-gram portions of the stock rat food (a finely ground, commercially available dog meal) were tied in cheesecloth bags and placed in a Rezyl pail wherein hydrogen cyanide was generated by the action of sulfuric acid on sodium cyanide "aeroids" (supplied by the American Cyanamid Co.).

Figure 1 illustrates the fumigation apparatus. The Rezyl pail, *A*, had a capacity of 20.6 liters. The bags of food, *B*, were placed upon *C*, a wire-cloth platform elevated about 6 inches above the bottom of the pail. A 250 ml. beaker, *D*, containing 16.5 ml. of concentrated sulfuric acid and 22 ml. of water was set inside a 1000-ml. beaker, *E*, and placed in the bottom of the pail so that it would be directly under the Plex spout, *F*, when the lid, *G*, was placed in position.

Figure 1. Fumigation can



Ten grams of the sodium cyanide aeroids were tied in a kraft paper bag, *H*, and suspended by a piece of cord, *I*, about 24 inches long. The suspending cord was passed up through the Plex spout aperture.

While the cord suspending the bag of aeroids was firmly held, lid *G* was placed in position and secured by means of 8 C-clamps (not shown in the diagram). The bag of aeroids was then lowered into the beaker containing the sulfuric acid and the cap of the Plex spout (not shown in the diagram) was quickly screwed on. The entire process of fumigation was carried out in a hood with adequate ventilation. The food was fumigated for 24 hours. At the end of this period, the pail was opened and the fumigated food was removed and immediately placed in a friction-lid can of about 20-liter capacity, where it was allowed to age for 72 hours before use.

A weighed quantity of the food (35 grams) was placed in a 3-liter round-bottomed flask containing 600 ml. of distilled water and 5 grams of tartaric acid. The hydrogen cyanide was distilled with steam into 100 ml. of a 2.0% solution of sodium hydroxide. A Kjeldahl connecting bulb was placed between the distillation flask and the condenser to prevent any spray from entering the condenser. A glass tube attached to the adapter at the end of the condenser reached below the liquid level in the receiver. The distillation was continued at a slow rate until the total volume of the distillate was about 400 ml. The volume of distillate was measured and a 100-ml. aliquot taken for analysis. The sodium cyanide formed in the distillate was titrated with 0.02*N* silver nitrate, using 2 to 3 drops of 20% potassium iodide as an indicator.

This method differs only in minor details from the A.O.A.C. method (*2*).

Special feeding jars were designed to provide a minimum of air circulation and to prevent, as much as possible, the loss of hydrogen cyanide vapors. Details of the jar are shown in Figure 2. The feeding jars, *A*, were made from 8-ounce ointment jars with metal screw-top lids. A rectangular opening, 30 by 40 mm., was cut in the lid, *B*. A trough, *C*, was soldered to the bottom of this opening, extending outward. A flap, *D*, protruding inward, was attached to the upper edge of the opening. The feeding jar was firmly held in place in the cage by means of coiled springs.

Preliminary Feeding Experiments

In a preliminary feeding experiment using three adult male albino rats, fumigated food containing 934 p.p.m. of hydrogen cyanide resulted in food refusal the first 2 days, after which time the level of hydrogen cyanide was found to have dropped to 286 p.p.m. During the next 2 days the hydrogen cyanide level dropped to 106 p.p.m. and the food consumption of the rats increased.

In view of the above results, another preliminary experiment was set up wherein the fumigated, aged food was diluted with the stock food to attain hydrogen cyanide concentrations of about 100 and 300 p.p.m. This was fed to rats and the residues were analyzed 24 and 48 hours later. The initial average hydrogen cyanide concentrations in the diluted food were 105 and 311 p.p.m. When analyzed after 24 and 48 hours, the lower level had dropped from 105 to 84 p.p.m. in 24 hours and to 38 p.p.m. in 48 hours. The higher level had dropped from 311 to 266 p.p.m. in 24 hours, and to 85 p.p.m. in 48 hours. The results of these studies (Table I) showed that fresh food would have to be prepared and fed on

Table I. Determination of Hydrogen Cyanide in Fumigated Food

HCN Content of Fumigated Food, P.P.M. After Dilution		HCN Content of Food Residues after Feeding, P.P.M. 24 Hours		48 Hours	
		Desired Levels			
After 3 days' aging	100	300	100	300	100	300
1050	97	309	80	260	41	94
1140	112	306	86	268	38	88
996	107	316	79	260	34	72
1060	102	314	90	275	37	86
Average	105	311	84	266	38	85
Drop, %			20	14.5	63.8	72.6

alternate days in order to keep the hydrogen cyanide concentrations at approximately the correct levels. During this preliminary study no food refusal or toxic signs were noted.

Two-Year Feeding Experiment

A control and two experimental groups of 10 males and 10 females were initiated on the 2-year feeding study. One experimental group was placed on a 100 p.p.m. feeding level, and the other received a diet containing 300 p.p.m. of hydrogen cyanide.

The food of the test rats was prepared fresh every 2 days for the 2-year period and analyzed for its initial hydrogen cyanide content. The initial hydrogen cyanide concentrations found in the diets for the first seven feeding periods are presented in Table II. These are representative of the values found during the 2-year study. The averages of the first seven feedings were found to be 106 and 301 p.p.m. for the 100 and 300 p.p.m. levels, respectively. At intervals of 3 weeks during the 2-year feeding period the residue foods of each level, after 2 days' feeding, were pooled and analyzed for their hydrogen cyanide content. An average of all residue foods analyzed over the 2-year period reveals that the 300 p.p.m. residues dropped to 80.1 p.p.m. and the 100 p.p.m. to 51.9 p.p.m., after 2 days of feeding.

A comparison of the residues analyzed in the summer months (June, July, August, and September) with those analyzed in the winter months (November, December, January, and February) over the 2-year period did not reveal a significant difference. An average of the summer months' residues after 2 days' feeding showed the 300 p.p.m. level to have dropped to 73 p.p.m. and the 100 p.p.m. level to 55 p.p.m. The winter months' residues dropped to 82 p.p.m. at the 300 p.p.m. level and to 46 p.p.m. at the 100 p.p.m. level. In order to be certain that the rats were not refusing the fumigated food, additional food checks were conducted to determine the uniformity of the intake on the first day as compared to that on the second day of each feeding period (Table III). This study showed that not only was the feeding uniform over a 20-day period, but the test rats actually consumed slightly more food on the first day of each feeding period.

Table II. Hydrogen Cyanide Content of Fumigated Food

Feeding Period	HCN Content of Fumigated Food, P.P.M. After 3 days' aging	After Dilution Feeding Levels 100	300
1	904	113	286
2	812	104	312
3	1064	89	285
4	536	107	303
5	1158	103	306
6	1258	110	312
7	1327	116	309
	Average	106	301

Results

The growth curves, at quarterly intervals, of the male and female groups are shown in Figures 3 and 4. The growth curves are almost identical for the three groups of males (Figure 3) throughout the 104-week feeding period. The females, however (Figure 4), showed considerable variation. The controls of this group exhibited an abnormal rise after 91 weeks of feeding, accompanied by 2 deaths. The 100 p.p.m. females reached an abnormal peak at 78 weeks; this peak was due to tumor development in 1 rat which died after 78 weeks, with a terminal weight of 759 grams. The dashed line is the curve for the entire 100 p.p.m. group; the dotted line from the 52nd week to the 91st week represents the group exclusive of this tumored rat. The sudden decline after 1.5 years of feeding appeared to be due to rapid loss of weight, particularly by two rats, but there were also a general senility and weight loss in this group. The 300 p.p.m. level appeared to be of normal nature, reaching a peak at the end of 78 weeks of feeding and declining slowly as the rats approached the 104th week.

A comparison of survival data of the control animals with the experimental ones indicates a random distribution of mortality. During the first 75 weeks of feeding the total mortality of each group of 20 rats was as follows: three in the control group, two at the 100 p.p.m. level, and two at the 300 p.p.m. level. In the ensuing 29 weeks of feeding, deaths became more frequent and a final analysis of survival data shows that seven control animals had died as compared with eight in the 100 p.p.m. group and five in the 300 p.p.m. group. The higher incidence in the latter months of the study is probably due to the increasing age of the rats.

Table IV presents additional survival data and a final compilation of the rat food consumption. "Theoretical rat days" indicates the number of days involved if all the rats survive the entire feeding period. "Actual rat days" indi-

Table III. Average Grams Daily Food Consumption, Based on 10 Consecutive 2-Day Feeding Periods

	Control Group				100 P.P.M. Group				300 P.P.M. Group			
	Male		Female		Male		Female		Male		Female	
Rat No.	1st[a]	2nd[a]	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	19	18	14	13	20	15	15	10	19	12	14	15
2	19	15	14	12	18	18	13	12	18	17	15	14
3	16	14	16	12	22	20	13	11	16	15	18	14
4	13	10	12	11	17	13	12	14	17	15	19	18
5	17	17	15	12	19	18	15	12	17	14	14	12
6	18	16	13	12	19	16	13	11	15	18	17	16
7	22	16	14	12	20	15	14	11	16	16	21	17
8	18	18	14	13	18	18	12	11	17	18	11	11
9	17	16	20	16	20	21	11	12	18	16	16	14
10	17	15	13	10	Died		16	15	19	11	21	22
Av. gram/rat/day	17	15	14	12	19	17	13	12	17	15	16	15

[a] Day of feeding.

Table IV. Data on Food Consumption and Survival

HCN Level, P.P.M.	Sex	No. of Rats		Av. Body Weight, G.		Rat Days			Food Consumption, G.	
		Start	Finish	Start	Finish	Theor.	Actual	% survival	Total	Av./rat/day
Control	M	10	7	58	482	7340	6420	87	118,249	18.42
	F	10	6	54	361	7340	6893	94	104,996	15.23
100	M	10	5	57	446	7340	6206	85	120,739	19.46
	F	10	7	55	248	7340	6995	95	102,754	14.69
300	M	10	9	57	436	7340	6915	94	127,953	18.50
	F	10	6	55	303	7340	6764	92	116,585	17.24

cates the number of days that the rats actually lived. "Per cent of survival" is obtained from these two values. The food consumption data indicate that the intake of the experimental rats was comparable to that of the control rats. Hematological values determined on representative rats initially and at termination of the study appear to be within normal limits.

During the 2 years of feeding no gross signs of cyanide toxicity were observed. In the latter stages of the study a number of the rats were unthrifty in appearance, with alopecia, emaciation, tumors, and sores on their bodies. In most of the animals that died autolysis prevented an accurate evaluation of the autopsy findings. Under the conditions of this investigation it is recognized that concentrations of hydrogen cyanide in the diet must be expressed in ranges rather than as definite values; however, within these limits there appears to be no recognizable toxicity either in the survival longevity data or in the growth rates during 104 weeks of feeding.

At termination of this study the surviving rats were sacrificed, blood was collected for cyanide and thiocyanate studies, and autopsies were performed. The liver, kidneys, spleen, brain, heart, adrenals, and testes or ovaries of each animal were weighed, and the ratios of organ weight to body weight appeared to be within normal limits.

Autopsies revealed the same general abnormalities and signs of senility in the control and experimental rats. Gross findings which were frequently encountered were: pale, granular, and thickened livers, congestion of the medulla of the kidney, abnormally small spleens, enlarged adrenals, atrophied, encysted, and inflamed genital organs, and enlarged, hemorrhagic pituitaries. Many nodes and tumors were found throughout the viscera. Infection of the ears was also in evidence.

The following tissues were taken from each of a representative number of rats and studied histologically: heart, lung, liver, spleen, stomach, small and large intestines, kidney, adrenal, thyroid, testes or uterus and ovary, and the cerebrum and cerebellum of the brain. The microscopic examination of these tissues revealed no evidence of pathology due to hydrogen cyanide feeding. All findings were compatible with those usually seen in aging animals, and the same general changes were found in both the control and experimental animals.

Cyanide and Thiocyanate Determinations

The objective of this portion of the study was to determine the presence of cyanide and any increase of thiocyanate in various tissues of male and female rats after 104 weeks of chronic feeding of fumigated food containing 100 and 300 p.p.m. of hydrogen cyanide.

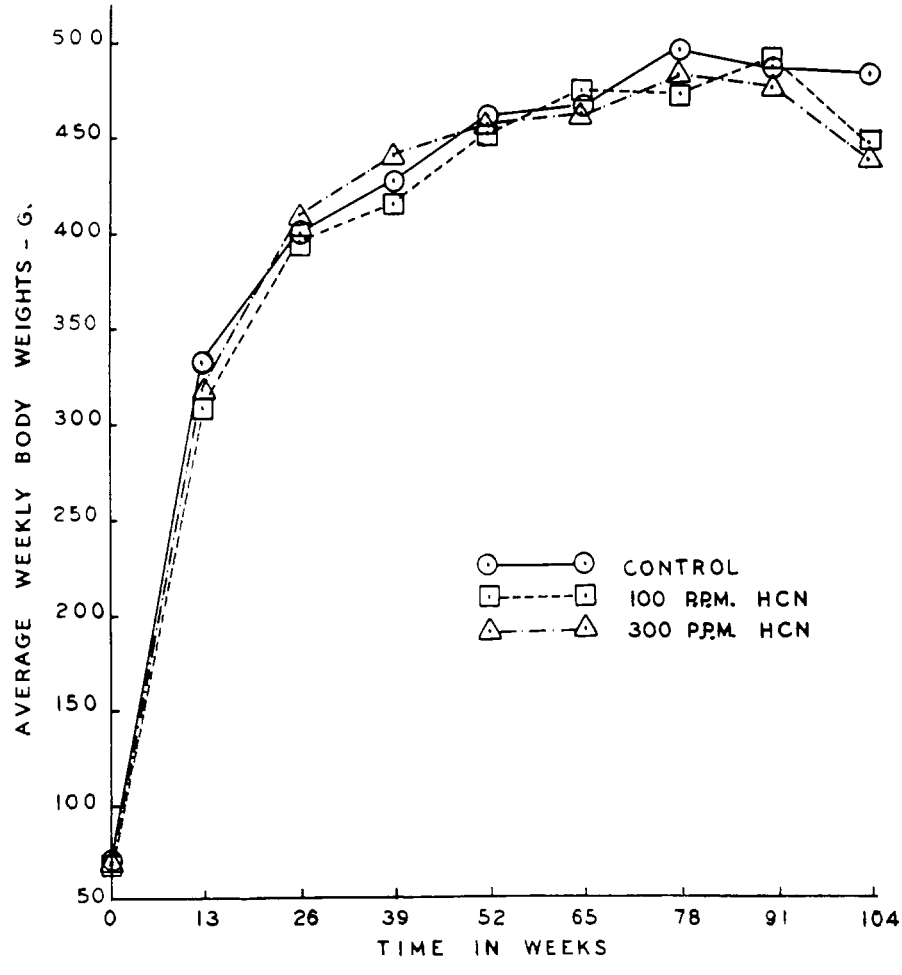
All animals were sacrificed by decapitation and blood was collected in small beakers containing heparin. The plasma and red blood cells were separated by centrifugal action. The liver and kidneys were also removed and homogenized with an equal amount of water. Suitable aliquots were taken for analysis. The method of Aldridge (*1*) as modified by Bruce, Howard, and Hanzal (*4*) was used for the estimation of cyanide and thiocyanate in the tissues.

Table V summarizes the cyanide and thiocyanate contents found in the plasma, red blood cells, liver, and kidneys of male and female rats after 104 weeks of hydrogen cyanide feeding. The plasma and red blood cell cyanide and thiocyanate values are expressed as micrograms per 100 ml. and liver and kidney values as micrograms per 100 grams of tissue.

Twelve rats of the control group were sacrificed. Cyanide was absent in all tissues. The average control thiocyanate values found were as follows: plasma, 361 γ per 100 ml.; red blood cells, 73 γ per 100 ml.; liver, 566 γ per 100 grams; and kidney, 577 γ per 100 grams.

Twelve rats were sacrificed in the 100 p.p.m. group. No free cyanide was found in the plasma, liver, or kidneys of these animals. In most instances cyanide was found in the red blood cells with an average of 5.40 γ per 100 ml. of tissue. An average of the plasma thiocyanate values shows 936 γ per 100 ml. of tissue; and for the liver and kidney, averages of 719 and 1023 γ per 100 grams of tissue, respectively, were obtained.

Figure 3. Growth curves for male albino rats



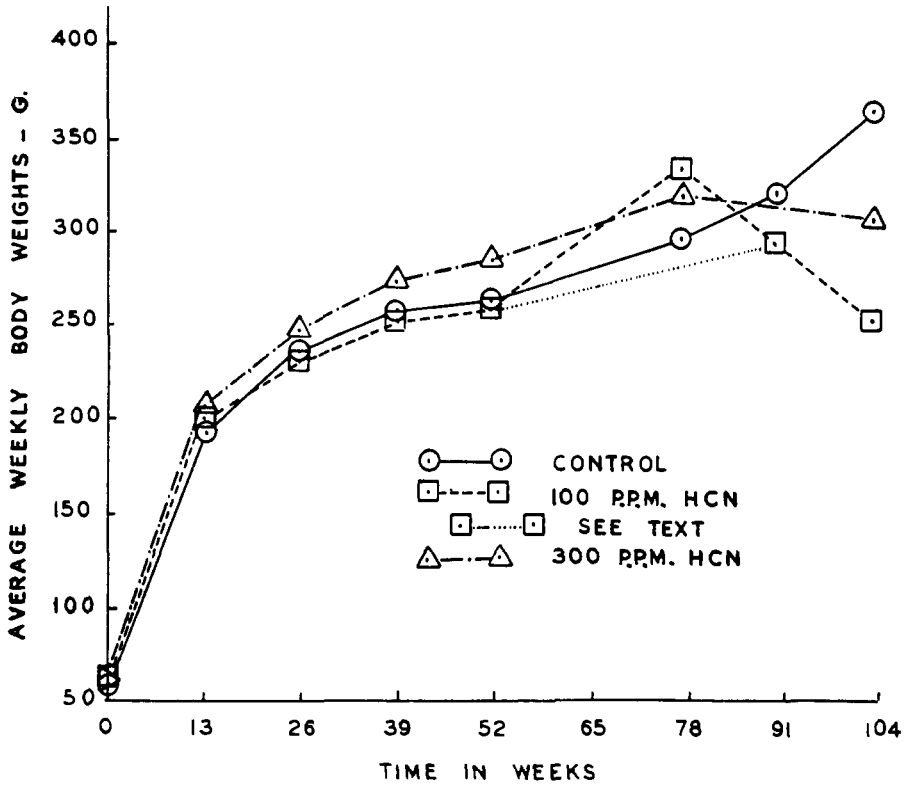


Figure 4. Growth curves for female albino rats

Fifteen rats were sacrificed at the 300 p.p.m. level. No cyanide was found in the plasma or kidneys. It was found in the liver of one rat and in the red blood cells of less than 50% of the animals, showing an average of 1.97 γ per 100 grams of tissue. The plasma and red blood cell thiocyanate values for the 300 p.p.m. level averaged 1123 and 246 γ per 100 ml. of tissue, respectively. The liver and kidney thiocyanate contents revealed averages of 665 and 1188 γ per 100 grams of tissue.

After this study of blood and tissue was initiated, it was found that Aldridge (*1*) had published some data on recovery of thiocyanate in blood. This investigator recovered 93% of the thiocyanate added to plasma, but in acid deproteinization of whole blood found that 80% of the thiocyanate was absorbed by the coagulum. The thiocyanate values for the red blood cells, liver, and kidney in this study are therefore presented as relative values rather than as a true index of thiocyanate content.

This study has shown that diets fumigated with and containing concentrations of 100 and 300 p.p.m. of hydrogen cyanide are nontoxic to male and female albino rats over a 2-year period. From the increased values of thiocyanate found in the tissues of the test animals, it is evident that the cyanide is readily detoxified to thiocyanate.

Summary and Conclusions

In a study conducted for a 2-year period, food containing 100 and 300 p.p.m. of hydrogen cyanide produced no noticeable signs of cyanide toxicity.

Determinations of hydrogen cyanide, made at intervals during the study, assured that the levels offered were uniform and consistent, and the loss of hydrogen cyanide during each 2-day feeding period did not vary with climatic changes.

Hematological values, determined initially and at termination of the study, were within normal limits.

Neither gross nor microscopic examination of the tissues of surviving rats revealed evidence of pathology due to hydrogen cyanide feeding.

No free cyanide was found in the plasma, liver, or kidneys of the rats sacrificed in the 100 p.p.m. group. In most instances cyanide was found in the red blood cells. These four tissues showed a definite rise in thiocyanate content over those of the controls.

Cyanide was not found in the plasma or kidneys of rats sacrificed at the 300 p.p.m. level. It was found in the liver of one rat and in the red blood cells of less than 50% of the rats in this group. Definite rises in thiocyanate were found in all four tissues.

This study has shown that a diet containing 100 or 300 p.p.m. of hydrogen cyanide as a result of fumigation is nontoxic to male and female albino rats over a 2-year period.

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Table V. Average Micrograms of Cyanide and Thiocyanate Found in Tissues of Rats at Termination

Level, P.P.M.	No. of rats	Sex	Plasma		RBC[a]		Liver		Kidneys	
			CN	SCN	CN	SCN	CN	SCN	CN	SCN
Control	6	M	0	415	0	90	0	436	0	421
	6	F	0	307	0	57	0	696	0	733
Group av. (M&F)			0	361	0	73	0	566	0	577
100	5	M	0	982	6.4	168	0	645	0	978
	7	F	0	904	4.6	133	0	772	0	1056
Group av. (M&F)			0	936	5.4	148	0	719	0	1023
300	9	M	0	947	0.72	201	1.6	550	0	1049
	6	F	0	1389	3.9	313	0	837	0	1399
Group av. (M&F)			0	1123	1.97	246	0.97	665	0	1188

[a] Red blood cells.