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VIBRIO PARAHAEMOLYTICUS: INFECTION OR TOXICOSIS?[1]

ROBERT M. TWEDT AND DAVID F. BROWN
Division of Microbiology, Bureau of Foods
Food and Drug Administration, Cincinnati, Ohio 45226

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ABSTRACT

The facultative halophile *Vibrio parahaemolyticus*, since its first isolation, has been shown to be toxic for mice and has produced experimental infection when fed to animals and humans. Pathogenicity has been shown to be associated with the presence of a heat-stable hemolysin-the Kanagawa-hemolysin. Previously, investigators reported that most strains of *V. parahaemolyticus*, regardless of virulence, could produce dilatation in the ligated rabbit gut. We have shown however, that ileal loop reactivity is strongly associated with Kanagawa-hemolysin and, thus, with virulence. Reactivity is obtained with live cell suspensions and the reaction time is dose dependent. Other investigators have reported that preparations of heated cells, cell lysates, culture filtrates, and purified Kanagawa-hemolysin will not produce dilatation in rabbit ileum nor edema in the mouse foot pad. In our studies, cell-free culture filtrates were without effect in the ligated ileal loop, suggesting absence of enterotoxin. Cell wall and heated cell preparations yielded varying ileal reactions. Present data are insufficient to describe the mechanism of virulence of *V. parahaemolyticus*.

Although many characteristics of *Vibrio parahaemolyticus* have been revealed during two decades of investigation, the question "Infection or Toxicosis?" remains unanswered. I would like to review briefly the progress made by other investigators as well as ourselves toward discovering the answer.

In their pioneer study of the Shirasu food-poisoning epidemic in Japan in 1950, Fujino et al. *(8)* described the causative organism as highly toxic. When viable cells were administered to mice, either orally or by injection, death resulted swiftly from septicemia. Subsequently, other investigators in Japan and elsewhere have reported that the lethal dose for mice falls in the range of 10^5 to 10^8 viable cells *(3, 16, 23)*.

Experimental infections which closely duplicate the natural course of illness have been produced (Table 1) when broth cultures were fed to experimental subjects. Typical gastroenteritis symptoms have resulted, including diarrhea, vomiting, fever, and isolation of the infecting organism in the stools. Takikawa *(21)* reported immediate temperature rise and increase in white blood cell count of peripheral blood when cats were fed either live or heated cells. This suggests involvement of endotoxin. Takikawa also produced illness in human volunteers fed live whole cells. Aiso and Matsuno *(2)* obtained symptoms of gastroenteritis when culture filtrates were fed to both monkeys and cats, which suggests the presence of an enterotoxin. Aiso and Fujiwara *(1)* failed to produce gastroenteritis in dogs fed patient isolates, however.

The illness which resulted from an accidental ingestion of 3×10^5 viable cells by a laboratory worker was documented by Sakazaki et al. *(19)*. Typical symptoms occurred after a 6-hr incubation period. The organism, recovered from the diarrheal stools, was an 02:K3 Kanagawa-positive. Sakazaki pointed out that 15 Kanagawa-negative strains, isolated primarily from fish, failed to produce illness even when more than 10^9 cells were fed to human volunteers.

The association of Kanagawa-type hemolysis with pathogenicity of strains of *V. parahaemolyticus* was first observed by Kato et al. *(11)*. Using the special blood agar formulation of Wagatsuma *(25)*, which contains a monosaccharide and 7% NaCl, Sakazaki et al. *(19)* demonstrated that 96.5% of 2,720 cultures isolated from diarrheal stools were hemolytic, whereas only 1% of 650 sea-fish isolates were hemolytic. The relationship between source of isolate and hemolytic activity, the Kanagawa phenomenon, was observed by Miyamoto et al. *(15)* and others.

In 1970, we *(24)* compared the hemolytic activities (Table 2) of 91 strains of *V. parahaemolyticus* isolated from human diarrheal stools, sea fish, and sea water; 21 isolated from wound infections; 14 from non-pathogenic, marine vibrios; and 21 from moribund blue crabs *(14)*. We found that the percentage of Kanagawa-positive organisms not associated with human gastroenteritis was significantly higher than the 1% reported by Sakazaki. Alternatively, Kanagawa-negative strains were recently isolated from patients and food from epidemics of gastroenteritis. Teramoto et al. *(22)* in 1971 reported isolating 10 serotypes from three outbreaks involving 59 cases of illness. All were Kanagawa-negative.

The virulence of *V. parahaemolyticus*, when inoculated into the ligated rabbit ileal loop (Table 3), utilizing the method of De and Chatterje *(5)*, was demonstrated by Sakazaki et al. *(18)*. Broth cultures of almost all *V. parahaemolyticus*, regardless of Kanagawa type, produced a severe reaction. In addition, some strains of *V. alginolyticus* could produce similar

[1]Presented at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania, April 23-28, 1972.

TABLE 1. VIRULENCE OF *Vibrio parahaemolyticus* FED TO EXPERIMENTAL ANIMALS

Strains tested[a]	Origin of strains	Experimental animal	Material fed[b]	Amount[a]	Presence of gastro-enteritis symptoms	Recovery from stools	Remarks[c]	Investigator	Year
NS	Patients	Cat	WC	NS	−		Rise in temperature and WBC	Takikawa (Ref. *21*)	1958
		Cat	WC heated	NS	−				
		Dog	WC	NS	+				
		Human	WC	0.001-1 ml	+	+			
NS	Patients	Dog	WC	2-3 mg	+	+		Aiso and Matsuno	1961
		Cat	WC	2-3 mg	+	+			
		Cat	CF	NS	+				
		Monkey	CF	NS	+				
7	Patients (5) and fish (2)	Dog	WC	5×10^{10}	−		Diarrhea with a non-pathogen	Aiso and Fujiwara (Ref. *1*)	1963
4	Patients (3) and fish (1)	Monkey	WC	$2\text{-}5 \times 10^{10}$	+	+	Only 1 patient isolate produced illness		
		Human	WC	1×10^{9}	+				
1	Patient *(1)*	Human	WC	3×10^{5}	+	+	Kanagawa +	Sakazaki et al. (Ref. *19*)	1968
	Fish and patient *(11)*	Human	WC	$0.2\text{-}6.6 \times 10^{10}$	−		Kanagawa −		

[a]NS – not stated.
[b]WC – whole cells.
CF – culture filtrate.
[c]WBC – white blood cell count.

TABLE 2. HEMOLYSIS OF SPECIAL HUMAN BLOOD AGAR PLATES BY VIBRIOS[a]

Vibrio group	Blood group	Media[b]	Number of strains tested	Strains grown		Hemolysis		
				Numbers	% of strains tested	Number hemolytic	% of strains tested	% of strains grown
I	0 Rh+	W	91	69	76	54	59	78
	0 Rh+	K		86	95	66	73	77
	0 Rh−	W		62	68	40	44	65
II	0 Rh+	W	19	18	95	14	74	78
	0 Rh+	K		19	100	17	90	90
	0 Rh−	W		17	90	14	74	82
III	0 Rh+	W	14	12	86	1	7	8
	0 Rh+	K		7	50	1	7	14
	0 Rh−	W		12	86	1	7	8
IV	0 Rh+	W	20	17	85	11	55	65
	0 Rh+	K		17	85	17	90	100
	0 Rh−	W		15	75	11	60	74

[a]Taken from Ref. 24.
[b]W – Wagatsuma agar; K – Kato medium.

dilatation. From his data, Sakazaki suggested that the ileal loop test had limited value for pathogenicity testing of *V. parahaemolyticus* cultures *(17)*.

Zen Yoji *(28, 29)* described the *in vivo* reactions which resulted from inoculating strains of *V. parahaemolyticus*, *V. alginolyticus*, and related marine vibrios into ligated rabbit ileal loops. He also stated that almost all of the *V. parahaemolyticus* cultures, regardless of Kanagawa type, showed remarkable dilatation, while all other vibrios showed little response. Zen Yoji et al. *(31)* obtained similar results using the mouse foot edema test.

We utilized the modification of the De-Test described by Ghoda et al. *(9)* to study the reactions in the rabbit ileal loop of two groups of *V. parahaemolyticus* strains. These were designated virulent or nonvirulent on the basis of their Kanagawa reaction and their source of isolation. After a 48-hr starvation period and 24 hr without water, laparotomy was performed following barbiturate anaesthesia. Commencing at least 30 cm above the ileocecal junction, six to eight 12-cm loops were ligated. All cell and fraction preparations were inoculated in 1-ml volume, and the injection site was tied off. Animals were sacrificed after incubation periods from 6-30 hr. The ileal fluid accumulation for each loop was scored ac-

cording to Drucker et al. *(6)*.

The ratios of loop fluid volume to dry weight of tissue were compared. To be scored as positive, strains must have yielded a dilatation at least 3-fold greater than the suspensate or bacterial control in at least two out of three tests with separate animals.

We compared the ileal loop reactions (Table 4) of 19 Kanagawa positive strains of *V. parahaemolyticus* isolated from patients' diarrheal stools with those of 10 Kanagawa negative strains isolated from sea fish. *In vivo* reactions differed significantly between the two groups when 10^8 washed cells were inoculated in 1 ml of buffered physiological saline and were incubated within loops for 18-24 hr. Most Kanagawa-positive strains produced a severe dilatation in ligated loops, whereas most Kanagawa-negative strains yielded none. The correlation observed between *in vitro* and *in vivo* tests for virulence was positive in 25 of 29 strains tested, or 86%.

When Sasaki et al. *(20)* inoculated massive numbers of *V. parahaemolyticus* K-33 into ligated rabbit ileal loops, no variation in bacterial population size per milliliter could be observed at 3-hr intervals up to 24 hr. Of course, since there was great dilatation as early as 3 hr, the total number of cells would have increased.

To determine the effect of inoculum size and incubation time upon ileal loop reactivity (Table 5), we inoculated 10^5 to 10^8 cells of *V. parahaemolyticus* strains per loop and examined for loop reaction and population size at 6-hr intervals for 30 hr.

When massive inocula of 10^7 or 18^8 cells of either hemolytic strain were injected, a drop of approximately two logs in bacterial cell population occurred at 6 hr with no dilatation. Recovery from this initial cell loss was observed as early as 12 hr. The greatest number of cells was recovered at 18 hr, and the cell population in these heavily inoculated loops then decreased steadily at 24 and 30 hr. Dilatation was seen after the 12-hr observation, concurrent with bacterial cell multiplication.

When smaller inocula from 10^5 to 10^6 cells were injected, the bacterial cells decreased to less than recoverable numbers from 6 through 18 hr. No loop dilatation occurred at these times. Recovery by multiplication was nearly complete at 24 hr, and loop dilatation had begun. Reactions were severe by 30 hr.

Yahagi *(26)* reported that *V. parahaemolyticus* K-33 penetrated epithelial cells and lamina propria of the ileum when ligated rabbit small intestine was inoculated. Perhaps this accounts for the decrease in numbers which we have reported with large inocula after 18 hr. Yahagi's finding *(26)* suggests that *V. parahaemolyticus* exhibits a *Shigella*-like virulence pattern, i.e., an infection characterized by epithelial cell penetration, disturbance of bowel function, and consequent fluid loss. Such a mechanism contrasts with the cholera-like virulence pattern of enterotoxin elaboration.

Some investigators have examined cells, cell fractions, and culture filtrates in an attempt to identify a *V. parahaemolyticus* toxin. Much of this work has stemmed from the association of the hemolysin responsible for the Kanagawa reaction with pathogenicity. Japanese workers have hypothesized that the hemolysin was responsible for the pathologic syndrome or was intimately associated with an enterotoxin.

In general, four types of hemolysin have been described by Japanese workers *(17)*. Two of these are contained in the bacterial cell. The third hemolytic fraction has been found in broth culture supernate and has been demonstrated to be heat labile *(27)*. The fourth hemolytic fraction can be recovered from culture supernate of Kanagawa-positive cells but never from Kanagawa-negative cells.

This fourth heat-stable Kanagawa hemolysin was studied by Kato et al. *(12, 13)*. They purified both hemolytic and toxic activity by ammonium sulfate

TABLE 3. TOXICITY FOR MICE AND THE LIGATED RABBIT GUT[a]

V. parahaemolyticus			*V. alginolyticus*			*V. anguillarum*		
Culture no.	Mice[b]	Ligated rabbit gut	Culture no.	Mice[b]	Ligated rabbit gut	Culture no.	Mice[b]	Ligated rabbit gut
1	5/5	+	1	5/5	+	1	0/5	−
2	5/5	+	2	5/5	+	2	0/5	−
3	4/5	+	3	5/5	+	3	0/5	−
4	5/5	+	4	5/5	±	4	0/5	−
5	5/5	+	5	3/5	+	5	0/5	−
6	4/5	±	6	5/5	±	6	0/5	−
7	5/5	+	7	5/5	+	7	0/5	−
8	5/5	+	8	5/5	+	8	0/5	−
9	5/5	+	9	3/5	+	9	0/5	−
10	5/5	+	10	4/5	±	10	0/5	−

[a]Taken from Ref. *18*.
[b]Ratios are number dead over number injected with 0.5 ml broth.

TABLE 4. CORRELATION OF *in vitro* AND *in vivo* VIRULENCE TESTS FOR *V. parahaemolyticus*[a]

Strain designation	Serotype K-antigen[b]	Kanagawa reaction	Ileal loop reaction	Virulence *in vivo*	Correlation
M229	15	+	1/4	−	−
M235	15	+	3/5	+	+
T3600-1	2	+	2/3	+	+
3142-60	NT	+	0/1	−	−
Ansai	11	+	5/8	+	+
Hirakata	NT	+	2/3	+	+
Nakatsukawa	3	+	2/4	+	+
Oda	11	+	3/4	+	+
Toyonaka	NT	+	2/4	+	+
Yanagisawa	9	+	4/6	+	+
4750	3	+	3/4	+	+
9121	3	+	2/3	+	+
9187	13	+	3/3	+	+
9312	7	+	3/4	+	+
9337	38	+	5/7	+	+
9345	45	+	4/4	+	+
9379	7	+	2/2	+	+
9382	11	+	4/7	+	+
9384	15	+	3/4	+	+
EB101	NT	−	0/4	−	+
S-42-87	3	−	0/6	−	+
6301-67	NT	−	1/3	−	+
8967	NT	−	0/3	−	+
9020	NT	−	0/3	−	+
9064	NT	−	4/6	+	−
9166	12	−	3/4	+	−
9365	46	−	0/2	−	+
9369	28	−	0/2	−	+
9381	NT	−	0/2	−	+

[a]—Percent correlation $= \frac{25}{29} = 86\%$.
[b]—NT = Not typed.

precipitation followed by zone chromatography. Kato et al., as reported by Sakazaki *(17)*, demonstrated an increase in titer in patients' sera of antibodies to the purified thermostable hemolysin. The titer increased 50% after 1 week and 70% after 2 weeks, and antibody could not be detected in normal sera. These workers thus confirmed the association between the Kanagawa hemolytic activity and enteropathogenicity.

In the same study, Kato et al. discovered that a variant strain which lacked K-antigen could no longer produce enteritis when inoculated intraduodenally into dogs, although the organism was still strongly hemolytic. This suggests that K-antigen, hemolysin, and toxin are closely associated but separate entities. Nevertheless, no relationship has ever been reported between serotype and virulence. This may mean only that a smooth encapsulated cell causes disease regardless of serotype.

Zen Yoji *(29)* tested toxigenicity of the hemolytic fraction, which he and his co-workers purified by a technique similar to Kato's method *(30)*. He found that inoculation of lyophilized hemolysin failed to show the dilation of ligated rabbit ileal loops which live cells had shown. He suggested that the toxin responsible for fluid accumulation is different from the thermostable Kanagawa hemolysin and "is present in the culture fluid or in the bacterial cell."

In later experiments, Zen Yoji et al. *(31)* injected heated *V. parahaemolyticus* cells, cell lysates, or culture filtrates into the mouse foot pad. None of these yielded a positive reaction, with the exception that a 10-fold concentrated culture filtrate yielded an atypical edema. These results further substantiated Zen Yoji's earlier suggestion that the toxic factor is different from the Kanagawa toxin.

Recently, Bhattacharya et al. *(4)* demonstrated an apparent vascular permeability factor but not an enterotoxic factor in the culture supernate of *V. parahaemolyticus*. This contrasts with *V. cholerae*, in which the two factors are thought to be simply two manifestations of the same enterotoxin *(7)*.

We inoculated cell-free culture filtrates, heated cells, and cellwall preparations into the ligated rabbit ileal loop (Table 6) in an effort to discover a cell-free or cell-bound toxin. Supernates of centrifuged 24-hr broth cultures were filtered under positive

pressure through 0.45 mμ membrane filters. Suspensions of 10^8 whole live cells in buffered physiological saline were heated to 60 C for 10 min. Concentrated cell suspensions were shaken with glass beads; the resulting broken cells were washed by dialysis and centrifugation and the cell walls suspended in buffered physiological saline *(10)*. We were unable to recover live cells from any of these preparations.

The reactions observed when these preparations were inoculated contrasted sharply with reactions seen with suspensions of live whole cells. The cell-free culture filtrate is without effect in the ileal loop, suggesting the absence of an enterotoxin, at least in sufficient concentration. In addition, only certain heated cell or cell-wall preparations are effective, suggesting that endotoxin is present, if at all, in minimal concentration.

We are continuing our study of cell fractions and cell-free preparations. However, we are using concentrated preparations, under the assumption that our negative data may result from minimal concentrations of a toxin; if, on the other hand, toxin production requires a host factor, we are studying the ileal loop reactivity to positive ileal loop lumen fluid. Cell-free filtrates of positive loops taken at 12, 18, and 24 hr are concentrated and reinjected into the ligated rabbit loop. We are also investigating the ability of homologous and heterologous antibody, either active or passive, to protect the rabbit from the effects of ileal loop inoculation with live cells.

TABLE 5. RELATIONSHIP OF ILEAL LOOP REACTIVITY TO INOCULUM SIZE AND INCUBATION TIME

Strain designation	Bacterial population and ileal loop reactivity											
	0 hr	6 hr		12 hr		18 hr		24 hr		30 hr		
	Org/ml	Org/ml	Reactivity[a]	Org/ml	Reactivity	Org/ml	Reactivity	Org/ml	Reactivity	Org/ml	Reactivity
Yanagisawa	3.6×10^5	100	−	<100	−	<100	−	1.4×10^7	+	1×10^6	+
Yanagisawa	8.8×10^5	<100	−	<100	−	<100	−	4×10^7	+	7.8×10^6	+
Yanagisawa	5.8×10^7	5.4×10^5	LA	5.4×10^7 (Est.)	+	4×10^8	+	8.4×10^8	+	8.9×10^7	+
Yanagisawa	6.3×10^8	5.3×10^6	LA	6.3×10^7 (Est.)	+	1.5×10^9	+	7.8×10^8	+	5.0×10^7	+
9379	3.7×10^5	6×10^4	−	<100	−	2×10^3	−	4.5×10^4	±	1.7×10^5	+
9379	4.8×10^5	2×10^3	−	<10	−	<100	−	6.2×10^4	±	LA	+
9379	5.8×10^8	1.3×10^6	LA	5.3×10^7	+	5.7×10^8	+	1.4×10^8	+	9×10^7	+
9379	7.2×10^8	6.5×10^6 (Est.)	LA	6.2×10^7	+	1.5×10^9	+	7×10^8	+	1.2×10^8	+

[a]LA = Laboratory accident.

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TABLE 6. ILEAL LOOP REACTIVITY WITH VARIOUS PREPARATIONS INJECTED

Strain designation	Whole cells			Culture filtrates		Heated cells		Cell walls	
	Times tested	Times positive	Reactivity	Times tested	Times positive	Times tested	Times positive	Times tested	Times positive
M235	5	3	+	2	0	1	0	1	0
Ansai	6	4	+	2	0	1	0	1	0
Hirakata	3	2	+	1	0	1	1	1	0
Toyonaka	4	2	+	1	0	1	1	1	0
4750	4	3	+	2	0	1	1	1	1
9121	3	2	+	1	0	1	1	1	1
9187	3	3	+	1	0	1	1	1	1
9312	4	3	+	1	0	1	0	1	0
9337	7	5	+	1	0	1	0	2	0
9379	2	2	+	1	0	1	0	1	1
9382	7	4	+	1	1	1	0	1	0
9384	4	3	+	2	0	1	0	1	0
M229	4	1	−	2	0	1	0	1	0
6301-67	3	1	−	3	1	1	0	1	0

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VIBRIO PARAHAEMOLYTICUS: A PROBLEM IN MARICULTURE?[1]

C. VANDERZANT

Department of Animal Science

AND

R. NICKELSON

Texas Agricultural Extension Service
Texas A&M University, College Station, Texas 77843

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ABSTRACT

Vibrio species are associated with numerous outbreaks of diseases in marine fishes. In such instances, *Vibrio anguillarum, Vibrio alginolyticus,* or *Vibrio parahaemolyticus* have been isolated from diseased species. In pond-cultivated brown shrimp *(Penaeus aztecus)* coryneform bacteria and *Vibrio* predominated. Although mortalities among brown shrimp caused by *V. parahaemolyticus* have been observed in laboratory aquaria, there is at present no firm indication that this organism is a major problem in pond-cultivated shrimp in Texas.

In recent years numerous isolations of *Vibrio parahaemolyticus* or organisms resembling or related to *V. parahaemolyticus* from marine environments and marine species have been reported in the United States and Canada *(4, 5, 6, 9, 11, 12, 13, 15, 16, 20, 23, 25, 27, 28).* Some of the materials from which isolates were obtained are listed in Table 1. A more detailed discussion on the distribution of *V. parahaemolyticus* is presented in another paper (this symposium) by J. Liston and J. A. Baross. It has been suggested that certain strains identified as *V. parahaemolyticus* on the basis of morphological, biochemical, and serological reactions may be strains of *Vibrio anguillarum* or members of as yet unnamed groups as determined by DNA homology experiments *(3).*

VIBRIOS AS FISH PATHOGENS

Numerous outbreaks of diseases in marine fishes associated with *Vibrio* species have been described in the literature. Marine vibrios were first reported to be pathogenic to fish in 1909 when Bergman *(7)* described an outbreak of disease in eels from the Baltic Sea. It involved hemorrhagic lesions in the musculature of the fish. *Vibrio anguillarum* was reported the etiological agent of this disease. In the Pacific Northwest, Rucker et al. *(18)* reported diseases caused by marine vibrios among Pacific salmon, rainbow steelhead trout, and herring. Extensive hemorrhages in the musculature and internal organs were noted. Pacha and Kiehn *(17)* studied the cultural characteristics and serological relationship of pathogenic marine vibrios isolated from fish in the Pacific Northwest. They showed that these Pacific Northwest strains of *Vibrio* were closely related to *V. anguillarum.* Tubiash *(21)* reported on the activity of *Vibrio alginolyticus* and *V. anguillarum* against soft-shell clams. These were species that caused bacillary necrosis in larval and juvenile bivalve mollusks. Heaviest losses occurred from heart and excurrent siphon injections. Adult soft-shell clams, eastern oysters, hard clams, and blue mussels were refractory to 24 hr of exposure in large concentrations of these vibrios.

For detailed information on vibrio diseases in marine fishes including the species involved, geographical range of the disease, characteristics and pathology of the disease, the reader is referred to a recent review by Anderson and Conroy *(2),* and *Principal Diseases of Marine Fish and Shellfish* by Sindermann *(19).* Recently Evelyn *(8)* reported vibriosis by *V. anguillarum* in four species of Pacific salmon that were held in sea water facilities. Haastein and Holt *(10)* described vibriosis in coalfish, cod, dab, flounder, plaice, and salmon from Norwegian waters. The causative agent was *V. anguillarum.* In both reports, hemorrhagic lesions of the skin and musculature were observed. Death in rainbow trout occurred when *V. anguillarum* was given *per os* at a water temperature of 15 C. When the bacteria were added to the water, fish succumbed to infection after 10 to 27 days at a water temperature of 10 C.

The Japanese have made great advances in mariculture. They recognize diseases caused by *Vibrio* species as a major problem in fish-farming operations. Akazawa *(1)* and Kusuda *(14)* reported an ulcer-type disease caused by *Vibrio* species. The organisms existed year around in Wakasa Bay. It was found in cultivated fish and also on wrasses, mackerel, dolphins, and yellowtail. The bacteria attacked wounded or weakened fish easily. *Vibrio parahaemolyticus,*

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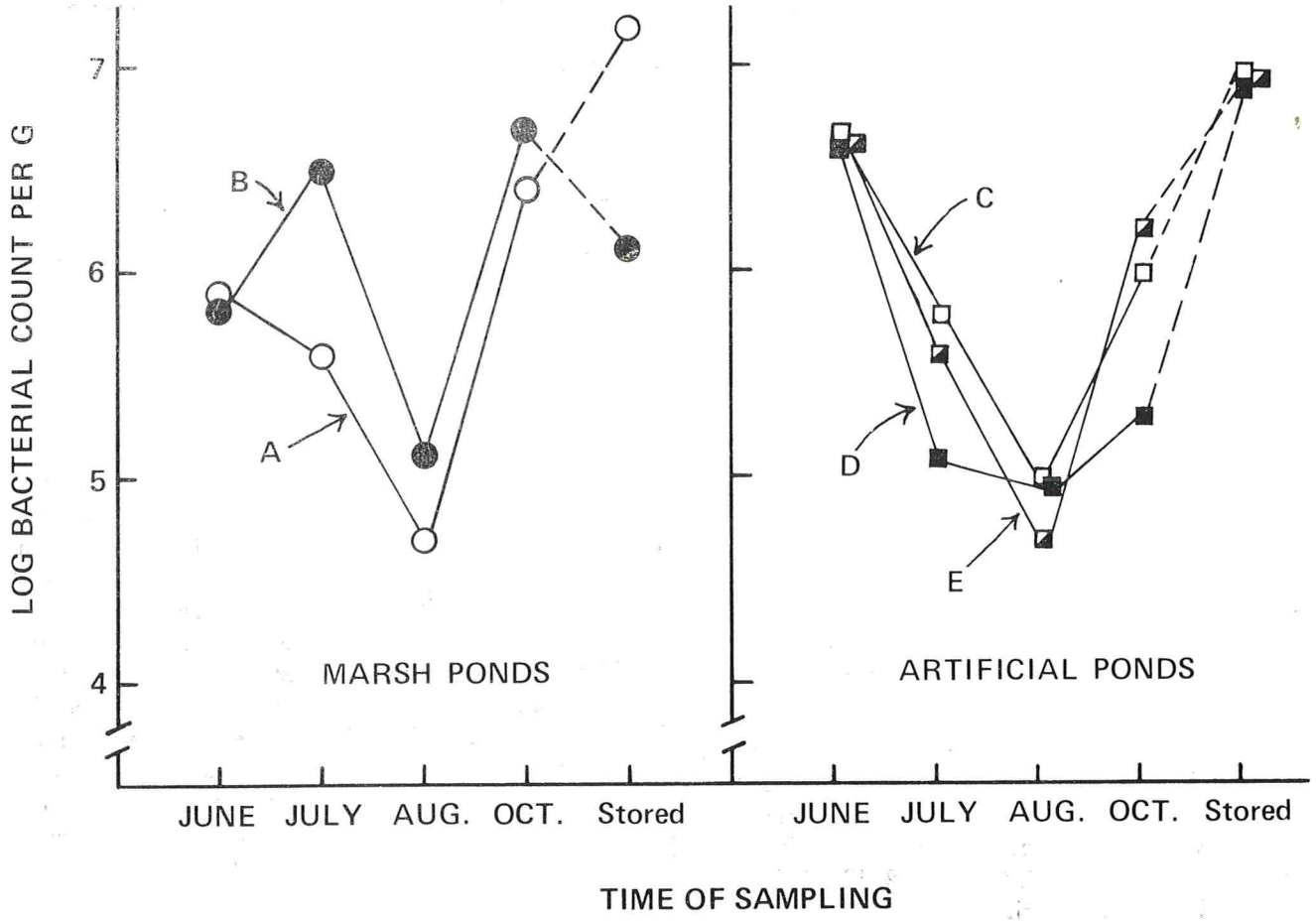

Figure 1. Agar plate counts of fresh pond shrimp and pond shrimp harvested in October and stored at 3 to 5 C for 14 days. [Data from Vanderzant et al. *(26)*.]

V. alginolyticus, and *V. anguillarum* were isolated. There is some question as to which species was responsible for the outbreaks.

Information linking *V. parahaemolyticus* to specific disease problems among marine species is sparse. In most instances there is no clear relationship between this isolate and disease problems. [For an interesting discussion on this subject see "Infection of goldfish with *Vibrio anguillarum*," Umbreit and Ordal *(22)*.] Krantz et al. *(13)* isolated *V. parahaemolyticus* from lethargic and moribund crabs. Examination of the weak animals revealed large numbers of bacteria in their hemolymph. Broken claws and appendages consistently contained necrotic liquified tissue and large numbers of bacteria. Lipovsky and Chew *(16)* reported isolation of *V. parahaemolyticus* from Pacific oysters among which mortalities occurred in the laboratory. There is, however, some question about the specific species involved.

Vibrios and Mariculture

Our interest in *V. parahaemolyticus* is focussed primarily on the distribution of this organism in marine species of the Gulf of Mexico and in shrimp from pond cultivation. In addition information is sought about *(a)* the possible effect of this organism on marine species particularly shrimp, and *(b)* the public health significance of this organism on shrimp harvested from ponds.

Nearly all shrimp harvested commercially in the United States are caught in nets by trawlers on nearshore fishing grounds. Although pond cultivation of shrimp in the United States is almost entirely limited to experimental trials, research on shrimp mariculture has become increasingly important in recent years. One of the objectives of the Mariculture program sponsored by the Sea Grant Program at Texas A&M University is to develop an economically feasible shrimp culture industry. Since microbial activity is one of the main sources of quality deterioration of shrimp, information is needed about the microbial flora of fresh and refrigerated pond-reared shrimp. In these studies natural or artificial ponds along the Texas Gulf Coast filled with brackish water are stocked with either postlarval or juvenile shrimp.

Bacterial counts of fresh pond shrimp sampled

from June to October ranged from 5×10^1 to 5.5×10^6 per g *(26)*. Counts usually decreased sharply in July, reached their lowest level in August, and increased in October (Fig. 1). Coryneform bacteria and, to a lesser extent, *Vibrio* species were the predominant isolates from fresh pond shrimp (Table 2). On refrigerated storage, coryneform bacteria predominated. A comparison of the microbial flora of shrimp at the beginning and end of the experimental period (June versus October) showed a decrease in coryneform bacteria and an increase in *Vibrio* and *Flavobacterium* species. Changes in numbers and types of microorganisms probably are related to some extent to changes in characteristics of the water such as temperature, salinity, oxygen level, phytoplankton activity, and pH. In August when the counts were low, the salinity and temperature of the water were high. Although bacterial counts of pond shrimp were comparable to those of many commercial boat samples at time of landing, the striking difference in microbial flora between Gulf and pond-reared samples was the lack of *Pseudomonas* in the latter. In Gulf shrimp *(24)* coryneform bacteria and species of *Pseudomonas, Moraxella,* and *Micrococcus* predominated (Table 3).

In the course of our shrimp mariculture operations high mortalities have occurred at the hatchery operation level and in the ponds. A protozoan (*Epistylus* sp.) has been identified as an organism causing shrimp mortalities in ponds. However, at the same time, *V. parahaemolyticus* was isolated from pond shrimp and pond water. Our first study was prompted by high mortalities among brown shrimp *(Penaeus aztecus)* which were kept for nutritional experiments in laboratory aquaria *(27)*. The predominant isolate on the plates with seawater was a gram-negative pleomorphic rod with a single polar flagellum with biochemical reactions characteristic of *V. parahaemolyticus*. The DNA base composition was 45.4 mole percent guanine plus cytosine. It reacted with O III - K30 antiserum. The isolate was pathogenic for mice when administered intraperitoneally. An 18-24 hr brain heart infusion broth culture of the isolate was added to aquaria with brown shrimp to yield a concentration of 10^4 and 10^5 cells per milliliter of water. Within 0.5 to 3 hr, the shrimp in the inoculated tanks reacted differently than the control shrimp. Death followed usually within 3 hr. *Vibrio parahaemolyticus* was recovered in almost pure culture from the interior of the cephalothorax region. When similar experiments were conducted with a coryneform bacterium isolated from shrimp, no mortalities occurred.

In subsequent experiments adult and postlarval shrimp were subjected to *V. parahaemolyticus*. In the tests with adults, the shrimp were placed in gallon jars, one per jar, in aerated Instant Ocean (Aquarium Systems Inc., Wickliffe, Ohio). In the experiments with postlarvae, 25 were placed in each jar. The temperature was kept at 28 C in a temperature controlled bath. Results of these preliminary experiments are presented in Tables 4 and 5. Culture SAK8 produced significant mortalities in adult shrimp when a broth culture was added to obtain a cell concentration of 3×10^6 per ml aquarium water. Cultures 5A and 17802 produced no marked mortalities at a cell concentration of 10^4/ml. When cultures were added to jars each with 25 postlarval shrimp (Table 5), significant mortalities were observed with broth cultures of 5A, 0, and 9. Except for culture 9, addition of cells only did not cause mortalities.

Although the results indicate that *V. parahaemolyticus* can cause death of postlarval and adult brown shrimp under certain conditions, there is at present no proof that this is a major problem in pond cultivation. In addition, experimental conditions in the present laboratory trials are less complex than those encountered in ponds. The conditions in the shrimp ponds, particularly from June to October, appear ideal for a build up of halophilic *Vibrio*. Cultivation of marine species under crowded conditions in a confined body of brackish water with temperatures as high as 25 to 30 C can be expected to create disease

TABLE 1. ISOLATION OF *V. parahaemolyticus* OR ORGANISMS RESEMBLING *V. parahaemolyticus* FROM VARIOUS MARINE ENVIRONMENTS

Materials	Investigators
Sediment (Gulf and South Atlantic Coast)	B. Q. Ward, 1968.
Seawater, sediment, shellfish	J. Baross, and J. Liston, 1968; 1970.
Blue crabs	G. E. Krantz, R. R. Colwell, and E. Lovelace, 1969.
Oysters, crabs	W. L. Landry, B. Wentz, and M. Fishbein, 1970.
Water, oysters	V. P. Lipovsky, and K. K. Chew, 1970.
Shrimp	C. Vanderzant, R. Nickelson, and J. C. Parker, 1970.
Processed blue crab meat	M. Fishbein, I. J. Mehlman, and J. Pitcher, 1970.
Crabs, oysters; frozen shrimp, oysters and crabs	H. C. Johnson, J. A. Baross, and J. Liston, 1971.
Water, oysters	C. H. Bartley, and L. W. Slanetz, 1971.
Clams, mussels, oysters, periwinkles, snails; frozen clams, mussels and periwinkles	W. K. Thomson, and D. A. Trenholm, 1971.
Water (Chesapeake Bay) plankton	T. Kaneto, and R. R. Colwell, 1971.
Crabs	U. S. Dept. of HEW. Morbidity and mortality, 1971.
Clams, oysters, crabs	C. Vanderzant, and R. Nickelson, 1972.

TABLE 2. DISTRIBUTION OF MICROBIAL FLORA OF POND-REARED SHRIMP

Pond	Sample	Percentage distribution									
		Coryneform	*Vibrio*	*Flavobacterium*	*Aeromonas*	*Achromobacter*	*Moraxella*	*Alcaligenes*	*Bacillus*	*Micrococcus*	Unidentified
A	June	90	2.5	7.5							
	July	72.5	10	12.5	2.5		2.5				
	August	55	22.5	10	7.5			2.5	2.5		
	October	55	20	10			15				
	Stored	91.3								8.7	
B	June	77.5	2.5	7.5			5		5		2.5
	July	85.7	7.1		3.6				3.6		
	August	67.5	20	2.5	5		5				
	October	30	20	45					5		
	Stored	54				15.4				23	7.6
C	June	82.5	7.5	2.5	5		2.5				
	July	80.2	6.6	6.6			6.6				
	August	45	30	2.5	7.5		12.5		2.5		
	October	45	15	5	10		20			5	
	Stored	100									
D	June	82.5	7.5	2.5	5		2.5				
	July	72.5	17.5	5	2.5		2.5				
	August	57.5	20	5	5		2.5		10		
	October	50	20						30		
	Stored	100									
E	June	82.5	7.5	2.5	5		2.5				
	July	87.5	2.5	7.5			2.5				
	August	60	5	2.5			2.5		30		
	October	50.4	8.4	16.8			16.8		8.4		
	Stored	94.5								5.5	

Data from Vanderzant, C., R. Nickelson, and P. W. Judkins *(26)*.

TABLE 3. DISTRIBUTION OF MICROORGANISMS FROM FRESH SHRIMP SAMPLES ISOLATED FROM MEDIA WITH REGULAR SEAWATER

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
Achromobacter	0	0	0	0	0	0	2.5	0	0	0	0	0
Alcaligenes	5.7	0	2.5	0	0	0	0	0	0	0	0	0
Bacillus	2.9	0	0	0	0	0	0	0	0	0	0	0
Coryneform	22.9	37.5	65.0	45.0	75.7	35.0	25.0	35.0	15.0	42.5	37.5	47.5
Flavobacterium	11.4	5.0	0	2.5	2.7	7.5	7.5	7.5	2.5	45.0	7.5	2.5
Lactobacillus	0	0	0	0	2.7	2.5	7.5	2.5	0	0	0	0
Microbacterium	0	0	0	0	0	0	2.5	0	0	0	0	0
Micrococcus	14.3	10.0	17.5	10.0	18.9	5.0	10.0	2.5	0	2.5	7.5	7.5
Moraxella	8.6	25.0	7.5	12.5	0	12.5	30.0	5.0	2.5	10.0	22.5	32.5
Pseudomonas	31.4	17.5	0	27.5	0	30.0	15.0	47.5	65.0	0	25.0	2.5
Staphylococcus	2.9	5.0	7.5	2.5	0	0	0	0	0	0	0	7.5
Vibrio	0	0	0	0	0	7.5	0	0	15.0	0	0	0

Data from C. Vanderzant, E. Mroz, and R. Nickelson *(24)*.

problems. In our present pond experiments emphasis is placed on a microbiological-histopathological examination of kills encountered among juveniles and adult shrimp.

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TABLE 4. EFFECT OF *V. parahaemolyticus* ON ADULT BROWN SHRIMP

Culture[a]	Inoculum/ml	Mortality
SAK 8	3×10^6 (broth and cells)	3/3
5A	10^4 (cells)	0/3
ATCC 17802	(cells)	0/3
	(broth and cells)	1/3
Control		0/3

[a]For sources of cultures see ref. 25.

TABLE 5. EFFECT OF *V. parahaemolyticus* ON POSTLARVAL BROWN SHRIMP

Culture	Inoculum/ml	Mortality after 24 hr
ATCC 17802	3×10^6 (cells)	1/25
	1×10^4 (broth and cells)	1/25
5A	1×10^7 (cells)	0/25
	3×10^6 (broth and cells)	25/25
0	1×10^7 (cells)	0/25
	1×10^7 (broth and cells)	25/25
9 (recent oyster isolate)	2×10^6 (cells)	16/25
	2×10^7 (broth and cells)	18/25
Control		0/25

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INHIBITORY SUBSTANCES IN HUMAN MILK[1]

MANFRED KROGER AND GEORGE H. WATROUS, JR.
Division of Food Science and Industry, Pennsylvania State University
University Park, Pennsylvania 16802

(Received for publication November 27, 1972)

ABSTRACT

An extremely high incidence of antibiotic or antibiotic-like inhibitory substances in 158 human milk samples is reported. Employing the disc assay method as recommended for cows' milk, 97% of the samples were found to be positive for inhibitory substances, and 46% positive for penicillin. The method may not be suitable for human milk because of naturally occurring (interfering) inhibitory substances. The possibility of the presence of dietary antibiotics should not be ruled out, however, this subject was not pursued further.

Increasing attention is being devoted to residual drugs in our food supply. Widespread and indiscriminate use of pharmaceuticals is said to have led to problems which parallel those brought about by so-called environmental contaminants. Use of drugs in animal agriculture is meant to reduce mortality and morbidity from infections, increase growth rates, improve feed conversion, relieve stress or tranquilize, and improve productivity in general. The subject of drugs in animal feeds has been summarized by the National Academy of Sciences *(15)*.

Antibiotics are part of this picture. They may be naturally occurring in a food, may have developed secondarily in it, or may have been added *(14)*. Antibiotics have been used widely in dairy cattle management since the late 1940s. With lactating cows they are administered mainly to treat mastitis. Some of the dose subsequently appears in the milk, no matter what the route of administration. Such milk may not be sold. The presence of antibiotics in market milk was a serious dairy industry problem two decades ago and it took a number of years to bring it under control. These aspects were reviewed by Cuthbert *(6)* and general data on the subject are compiled in a FAO/WHO publication *(7)*.

Presence of antibiotics in human milk has been reported by Höppner et al. *(11)* who examined 27 randomly selected samples of human milk and found 13 to contain inhibitory substances, antibiotics, chemotherapeutic or other bacteriostatic substances, while the milk samples from 14 mothers recently treated with antibiotics showed 12 positive ones in the standard test for antibiotics.

It is believed by some that the normal human diet is now responsible for most of these antibiotic residues in human milk and the population in general. It remains to be determined, however, where these antibiotics originate and whether the positive tests reported are unequivocally due to specific antibiotics and not due to other bacteriostatic agents.

Some data and observations have been reported on the excretion of antibiotic substances in human milk after administration *(9, 10, 17)* and the subject has often been included in reviews dealing with the transfer of drugs into human milk *(2, 4, 12, 16)*.

This paper is a report on a relatively large number of human milk samples with positive evidence for the presence of inhibitory substances.

SUBJECTS AND METHODS

All milk samples were donated by members of the Greater Philadelphia Childbirth Education Association and collected, stored, and transported as previously described *(13)*. Samples were delivered frozen in a variety of containers. These were checked later for possible contributions of inhibitory substances to the samples.

The standard filter paper disc method to detect inhibitory substances (and identity test for penicillin) in milk was employed *(3)*. A spore suspension of *Bacillus subtilis* (culture ATCC 6633) was used.

A preliminary examination was conducted in October 1970 on 37 human milk samples. When 21 of these proved to be positive and only 16 samples yielded negative results, this matter was pursued further. During December 1971 and May 1972 another 158 samples were examined.

These samples were tested in the raw state (not heat-treated) for inhibitory substances and also specifically for the presence of penicillin with the use of penicillinase-impregnated discs. If both these tests yield positive results with a milk sample, a zone of inhibition surrounding the discs indicates that an inhibitor other than penicillin may be present. If the first general test is positive, and the penicillinase-treated disc shows no zone of inhibition, then penicillin is assumed to be present.

Further, to eliminate occurrence of so-called false-positives, all samples were reexamined after heating to 85 C for 5 min. False-positive tests may result from natural inhibitory substances in raw milk. Pasteurization is known to reduce but not completely eliminate such substances. In routine laboratory work usually only positive samples are heat-treated and reexamined.

Employing these methods it is possible to report positive samples as containing: *(a)* inhibitory substances in general; *(b)* penicillin; *(c)* penicillin plus other inhibitory substances; and *(d)* inhibitory substances other than penicillin.

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TABLE 1. INCIDENCE OF INHIBITORY SUBSTANCES IN HUMAN MILK (158 SAMPLES) AS DETERMINED BY THE APHA DISC ASSAY METHOD

	No. of samples	Percent
Before heating:		
Positive for inhibitory substances	153	96.8
Negative for inhibitory substances	5	3.2
Positive for penicillin	73	46.2
Positive for penicillin plus one or more other inhibitory substances	50	31.7
Positive for inhibitory substances other than penicillin	80	50.6
After heating:		
Positive for inhibitory substances	141	89.2
Negative for inhibitory substances	17	10.8
Positive for penicillin	99	62.6
Positive for penicillin plus one or more other inhibitory substances	57	36.0
Positive for inhibitory substances other than penicillin	42	26.6

RESULTS

Of the first set of 37 samples examined in 1970, 21 were positive, while 16 showed no evidence of inhibitory effects.

The 99 and 59 samples studied more closely in 1971 and 1972, respectively, led to observations as disturbing as those found in the preliminary check and as those by Höppner and associates *(11)*.

Table 1 is a condensation of our findings. The extremely high incidence of positive results requires a great deal of explanation and gives rise to considerable speculation until further research is carried out on this subject. One unexpected phenomenon was the increase from 73 samples positive for penicillin before heating to 99 after heating. These 26 samples were negative for penicillin before heating, but they were positive for inhibitory substances. Heating apparently initiated a reaction or created reaction products that yielded what must be considered "false-positives," although the objective of heating samples is to eliminate so-called false-positives.

The different kinds of containers in which the samples were kept until analysis, as well as their lids and liners underwent a screening for presence of inhibitory substances that could have diffused from container material to milk. All these were found to be negative for inhibitory substances when raw cows' milk was stored in them for several days and then assayed.

DISCUSSION

Cow milk samples have been routinely examined for the presence of antibiotics in our laboratory during the last two decades. The incidence of positive samples has lately been about 0.1%; nationally it is about 0.3%.

Fresh cows' milk is known to contain several substances capable of bacterial inhibition *(5)*. These are believed to be "natural biological bacteriostats." Lactenin, lysozyme (also in saliva, tears, and egg white) and other, still unidentified, "substances" have such inhibitory properties and have been demonstrated in milk *(5, 8)*.

Bacterial growth in milk may lead to production of certain antibiotic or antibiotic-like substances. Nisin is an example and has been thoroughly studied *(14)*.

Antibiotic production has been associated with the lactic streptococci, lactobacilli, propionibacteria, acetobacters, and some other microorganisms responsible for food fermentation (e.g., yogurt).

Occurrence of other natural inhibitory substances, as in vegetables, fruits, cereals, spices, and honey, has been well documented *(14)*.

At this time no definite statement can be made about the origin of the inhibitory substances detected by us in human milk. Several possibilities exist.

(a) Human milk, and possibly also the milk of other mammals, may occasionally, or quite frequently, contain one or several natural antibiotic-like substances.

(b) Human milk samples, usually not obtained under aseptic conditions, can be expected to be grossly contaminated with microorganisms. Höppner and associates *(11)* counted between 150 and 1,000,000 bacteria/ml in 13 of the human milk samples they examined for inhibitory substances. The possibility of bacteria contributing to the inhibitory effect in human milk is possible but remote. No such effect has been reported for cows' milk delivered to dairy plants. One could also argue that cosmetics, detergents, cleansers, etc., present on skin or breast pump, are responsible for positive results.

(c) The mothers could have received doses of antibiotics before providing their milk samples. It is highly unlikely that so many mothers were under treatment at the same time. It is equally unlikely for any nursing mother to allow herself to be treated with antibiotics. As an active member of a Childbirth Education Association each mother can be expected to know about the transfer of drugs to her milk, including antibiotics, and the possible ill effects they may have on her baby.

(d) Certain foods could be indicted for transferring antibiotics and antibiotic-like substances to the mothers and their milk. We do not intend to lay the blame on any food here, since evidence for it is lacking. Further studies are required to investigate this point.

Pending further research on this matter the possi-

bility of dietary antibiotic residues entering human milk can not be ruled out. More information on the nature of the inhibitory substances must also be generated. Until that time, the appearance of such substances in human milk must be viewed with caution.

Finally, our data may suggest that the disc assay method as used by the dairy industry for cows' milk yields very questionable results when applied to human milk. Since penicillinase is specific for penicillin, some factor in human milk seems to produce results similar to penicillin. To compound the matter, heating human milk seems to enhance the activity of this unknown inhibitor.

Of some interest was the fact that zones of inhibition surrounding the milk-impregnated discs were relatively constant in size.

It is strongly suggested here to study further the quality of human milk and also to establish guidelines and quality control measures for human milk used by so-called milk banks of hospitals. The U.S.A. may be lagging behind Europe where the Committee of Ministers of the Council of Europe at the 186th - 189th meetings (Strasbourg, January - May, 1970) adopted Resolution (70) 9 dealing with human milk: "Governments are invited in this resolution to ensure that better arrangements are made for the distribution of mother's milk, and that such milk is available for babies needing it urgently, from one member country to another. Member states are invited to ask for no more than the refund of the cost of collecting, processing, and transporting the milk. They are also asked to establish common rules for the labeling, packing, and dispatch of mother's milk, to make it exempt from import duties, and to provide for its speedy delivery to the consignees by the most direct route" *(1)*.

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AEROBIC MICROBIAL FLORA OF SMOKED SALMON[1]

J. S. Lee and D. K. Pfeifer
Department of Food Science and Technology
Oregon State University, Corvallis, Oregon 97331

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Abstract

Aerobic microbial flora, moisture, and NaCl contents of smoked salmon samples, obtained from retail outlets along the coast of the Pacific Northwest, were examined. The microbial loads ranged from 1.3×10^2 to 2.2×10^6. The moisture levels were from 48 to 64%, and the water phase salts from 3.2 to 8.2. Regularly recoverable microorganisms in most samples were gram-positive cocci. They were either mostly staphylococci micrococci, depending on the sample. *Bacillus*, *Pseudomonas*, and yeasts were predominant in some samples.

The gram-positive cocci were able to grow in 10 to 25% NaCl, but the majority of them did not multiply at 4 C and were readily inactivated by mild heat (D_{52C} of 1.5 to 47.9 min).

The essential features of the newly adapted Good Manufacturing Practice (GMP, 6) for smoked fish are: hot smoking at 82 C (180 F), for a minimum of 30 min, if the water phase salt (WPS) of the finished product is 3.5 or above; or 66 C (150 F) treatment for 30 min, if WPS is at least 5.0. Storage temperature of 3.3 C (38 F) or lower is also specified for the smoked fish.

While this GMP is intended to remove the hazard of *Clostridium botulinum* type E from smoked fish, the accompanying improvement in sanitary quality of smoked fish would be beneficial.

Little information on the microbial flora, other than *C. botulinum* type E, in smoked fish is available. The purpose of this investigation was to isolate and identify the microbial flora of smoked salmon from various processors before the GMP became effective. The gram-positive cocci isolated were further characterized to determine their potential health hazard and their abilities to survive or multiply during the hot smoking process.

Materials and Methods

Smoked salmon

Six smoked salmon samples were purchased from various roadside retail counters, which represented all known processors of the survey area, along the southern Washington and northern Oregon coasts. An additional sample (Sample G) was an experimental product not being offered for sale. All samples were well iced and held for no longer than 48 hr before examination.

Microbiological examinations

All smoked salmon pieces were finely sliced aseptically under a flow of sterile air (Bioquest Sterility Test Cabinet). Slices from different pieces were pooled and 50 g of this mixture were blended in an Osterizer with 50 ml of Butterfield's phosphate buffer. Appropriate dilutions were spread plated on agar containing Bacto tryptone (0.5%), Bacto-peptone (0.5%), Bacto-yeast extract (0.25%), glucose (0.1%), and NaCl (0.5%). The plates were incubated at 25 C for 72 hr Bacto tryptone and counted *(7)*.

A dilution which yielded isolated colonies was chosen and all colonies from the plates were transferred on a master plate with sterile toothpicks. Except for two samples with low counts, the number of colonies for each sample identified was over 100.

Microbial isolates were then identified by the modified replicaplating procedure *(5, 9)*. The gram-positive cocci were further identified into 6 groups of *Staphylococcus* and 8 *Micrococcus* groups according to the scheme proposed by Baird-Parker *(3)*. The following tests were also conducted: coagulase test by the tube method using Bacto-DNAse agar, and hemolysis on basal agar plus citrated human red blood cells.

The maximum NaCl levels tolerated by selected *Staphylococcus* and *Micrococcus* species were determined by the growth of stationary cultures in tryptone-peptone-yeast extract-glucose broth plus various concentrations of NaCl. Tubes were incubated at 25 C for 4 weeks and growth verified by the microscopic examination. Growth at 4 C was determined in the above medium plus 4% NaCl and incubating at 4 C for 3 weeks.

Thermal inactivation characteristics at 52 C were determined by withdrawing and plating, periodically, samples from a flask containing a known number of cells in above media held at a constant temperature of 52 ± 1 C *(10)*.

Moisture content

The total moisture of the samples was determined by the AOAC procedure *(1)*. The smoked salmon was dried to constant weight in a 102 C vacuum oven at ≥8.5 inches of Hg.

NaCl

The sodium chloride content of smoked fish was measured by the AOAC procedure *(2)*. Chlorine in the sample was precipitated with $AgNO_3$ and titrated with NH_4SCN solution. The water phase salt (WPS) was calculated according to the formula: NaCl (%)/H_2O (%) + NaCl (%) × 100 *(6)*.

Results and Discussion

Microbial load, moisture and NaCl content

Table 1 shows the microbial counts, and moisture and NaCl levels of samples examined. Despite the small number of samples, the data clearly point out the extreme variability among smoked salmon. The

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TABLE 1. MICROBIAL LOAD, MOISTURE, NaCl, AND WATER PHASE SALT (WPS) OF SMOKED SALMON

Sample	Microbial load (count/g)	Moisture (%)	NaCl (%)	WPS[a]
A	2.2×10^6	48	4.3	8.2
B	1.9×10^3	61	3.7	5.7
C	6.5×10^4	56	2.0	3.4
D	1.8×10^2	64	2.1	3.2
E	4.1×10^4	53	4.2	7.3
F	2.7×10^4	61	2.7	4.2
G	1.3×10^2	60	2.2	3.5
Range	1.3×10^2-2.2×10^6	48-64	2.0-4.3	3.2-8.2

[a]Water phase salt (% brine) = $\frac{\text{NaCl (\%)}}{\text{H}_2\text{O (\%)} + \text{NaCl (\%)}} \times 100$

TABLE 2. AEROBIC MICROBIAL FLORA OF SMOKED SALMON

Microorganisms	Percent in samples						
	A	B	C	D	E	F	G
Gram-positive cocci	1	94	95	13	36	38	100
Bacillus	98	4	0	25	0	19	0
Pseudomonas	1	1	0	50	5	37	0
Yeasts	0	0	3	0	59	0	0
Unidentified	0	1	2	12	0	6	0
No isolates examined	166	143	150	16	237	147	12

aerobic microbial count ranged from 1.3×10^2 to 2.2×10^6/g. The count also appeared to be inversely related to the moisture level. It is worth noting, however, that 5 of 7 samples met the minimum WPS level of 3.5% specified by GMP and 3 of 7 samples could have met the milder heating alternative (150 F vs. 180 F) that requires 5.0% WPS *(6)*. Samples with higher WPS levels did not necessarily contain fewer aerobic microorganisms. For some unknown reason, the reverse appeared to be true.

Microbial flora

The identities of microorganisms isolated from the smoked salmon are presented in Table 2. The most frequently isolated microorganisms, except for Sample A, were gram-positive cocci. *Bacillus* species, *Pseudomonas* species, and yeasts were found in different proportions in different samples.

The uneven distribution of *Pseudomonas* species and yeasts would indicate post-processing contamination and the presence of *Bacillus* species could indicate underprocessing. All spores of *Bacillus* species isolated readily survived heating at 65 C (149 F) for 30 min but not 80 C (176 F). Conspicuously absent from the smoked salmon were *Acinetobacter-Moraxella* species, that are common in seafoods.

Gram-positive cocci

The gram-positive cocci were further characterized because of their suspected human origin. Table 3 lists the percent distribution of *Staphylococcus* and *Micrococcus* species isolated from each smoked salmon. *Staphylococcus* subgroup I, or *Staphylococcus aureus*, were found in 3 samples and all isolates were positive for coagulase, DNAse, and were β-hemolytic on human blood agar. The high percentage of *S. aureus* recorded for Sample G is somewhat misleading as the microbial count of this sample was extremely low (130/g). The *Staphylococcus* subgroups II through VI or *Staphylococcus epidermidis* species were found in various proportions in 3 samples. They could also have originated from human skin.

The origin of the *Micrococcus* species in smoked salmon is difficult to speculate. Baird-Parker *(4)* studied gram-positive cocci isolated from human skin, pig skin, bacon, and household dusts and reported that most of *Micrococcus* isolates were from the bacon. *Micrococcus* spp. may be uniquely adapted to the smoked foods.

Another possible source of gram-positive cocci in smoked salmon is the brine. Table 4 shows some of the physiological characteristics of gram-positive cocci isolated in relation to their potential for growth and survival in brine. Many gram-positive cocci grew readily in media containing nearly 20% NaCl and all isolates tested grew at or above 10% NaCl. The level of NaCl tolerated was equivalent to the salt content of the 42 to 95° salometer brine, in contrast to the 40 to 80° salometer brine generally used

TABLE 3. IDENTITIES[a] OF GRAM-POSITIVE COCCI ISOLATED FROM SMOKED SALMON

Sample	Percent distribution[b]															
	Staphylococcus							*Micrococcus*								
	I	II	III	IV	V	VI	Unident.	1	2	3	4	5	6	7	8	Unident.
B	1	0	4	14	0	34	5	4	0	3	0	0	22	11	0	4
C	0	0	0	0	0	0	0	0	0	0	0	7	71	1	0	21
D	0	0	0	0	0	0	0	25	0	75	0	0	0	0	0	0
E	0	0	0	0	0	0	0	11	0	17	0	0	0	0	0	70
F	3	0	3	31	3	15	31	0	0	0	0	0	0	3	0	10
G	58	0	0	8	33	0	0	0	0	0	0	0	0	0	0	0

[a]Based on the identification scheme of Baird-Parker *(3)*.
[b]Sample A contained only unidentifiable *Micrococcus* spp.

TABLE 4. SELECTED CHARACTERISTICS OF GRAM-POSITIVE COCCI ISOLATED FROM SMOKED SALMON

Strain		Identity	Max. NaCl (%) tolerated	D_{52} (C) in min	4 C growth
A-4	*Staphylococcus*	I	19	1.5	−
A2-10		II	20	3.6	−
B-99		III	10	−	−
D-32		III	11	−	−
B1-1		IV	16	16.7	−
A-3		V	20	6.9	−
A2-58		V	19	−	−
B1-3		VI	16	−	−
D-38		VI	21	13.8	−
I-26	*Micrococcus*	1	22	27.6	−
C2-43		1	11	−	−
A2-92		2	12	−	−
A2-158		2	20	26.1	−
C2-73		3	21	47.9	−
C2-82		3	22	−	−
B2-43		5	25	11.7	+
B2-49		5	24	−	+
I-7		6	21	−	−
BI-32		3	24	6.3	+

in the industry. They could not, however, have grown excessively in the brine if it had been at 3.33 C as the GMP specified. Only 3 *Micrococcus* spp. out of 19 gram-positive cocci tested grew at 4 C after 3 weeks of incubation (Table 4). All gram-positive coccus isolates examined were also very sensitive to heat (Table 4).

Micrococcus spp. were slightly more heat resistant than *Staphylococcus*. The average exposure time required to inactivate 90% of *Micrococcus* spp. at 52±1 C was 24 min, while the average D_{52} for *Staphylococcus* spp. was 8.5 min.

The most heat resistant gram-positive coccus (*Micrococcus* C2-73) registered the D_{52} value of 47.9 (Table 4). At the lowest heat level of 66 C (150 F) permitted by the GMP, even the inactivation of *Micrococcus* C2-73 proceeded so rapidly that the accurate inactivation data could not be obtained.

Owing to the high NaCl tolerance, the gram-positive cocci could accumulate or grow in brine, especially if brine was used repeatedly at temperature above 3.3 C (38 F). Their sensitivity to heat, however, is such that they are not likely to survive either the 66 or 82 C smoking process specified by the GMP. Other aerobic microorganisms isolated from the smoked salmon also appear to have resulted from contamination after smoking. Their patterns of distribution, however, were too irregular to be used as a general indicator of cross-contamination.

The most consistently recoverable group was the gram-positive cocci. Their physiological characteristics are such that these bacteria may be suited as indication of post-processing contamination. In addition, their presence in substantial numbers may also indicate the extent of temperature abuse of the product.

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MICROBIAL COUNTS OF INDIVIDUAL PRODUCER AND COMMINGLED GRADE A RAW MILK[1]

H. E. Randolph, B. K. Chakraborty[2], Otto Hampton[3], and D. L. Bogart[4]
Department of Animal Science
Texas A&M University, College Station 77843

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Abstract

Microbial populations of Grade A raw milk samples from 105 individual producers and 74 bulk tank trucks (commingled) were enumerated by Standard Plate Count (SPC), psychrotrophic count (PBC), coliform count (CC), laboratory pasteurized count (LPC), thermophilic count (TBC), yeast and mold count (Y&M), and special penicillin (PEN) and crystal violet tetrazolium (CVT) agar count procedures. In addition, microbial populations were determined by the SPC, PBC, PEN, and CVT procedures after preliminary incubation (PI) of samples. Initial mean counts obtained on individual producer samples were generally lower than those for commingled samples. However, producer samples had higher mean counts after PI. Growth ratios were lower for commingled than for individual producer samples indicating slower growth during PI. Results obtained by the PBC, PEN, and CVT procedures were similar when viewed as correlation coefficients, distribution of samples according to microbial counts, mean counts, and growth ratios during PI. Before PI, the correlation between these three tests was poor and lacked statistical significance when the PBC was $<50{,}000$/ml. After PI, the tests were highly correlated ($P<0.01$) and the r values ranged from 0.8 to 0.9 for samples with PBC levels above 10^6/ml.

Milk may be subjected to a variety of tests to evaluate its microbiological quality, compliance with regulatory classifications, and conditions of sanitation during production *(1, 3, 4, 6, 7, 8)*. The Standard Plate Count (SPC) is used to determine compliance with health authority grade classifications *(18)*. Other investigators *(4, 6, 8, 10, 11)* have shown that the SPC is of limited value in assessing the microbiological quality of milk or the conditions of farm sanitation. However, Johns and Berzins *(11)* found that a comparison of microbiological counts before and after incubation at 12.8 C for 18 hr reflects conditions of sanitation on the farm.

Modern production and handling procedures have caused changes in the microbiological populations of raw milk *(9, 11)*. Improved cooling and sanitation procedures have made it possible to produce raw milk with lower bacterial counts, but have also increased the relative importance of psychrotrophic bacteria *(8, 10)*. Johns *(10)* indicated that for an accurate assessment of the microbiological quality of raw milk, test results should indicate the level of psychrotrophic organisms. Hartley et al. *(8)* found a significant relationship between the psychrotrophic count of raw milk and farm sanitation conditions. However, practical utilization of psychrotrophic counts is limited by the time required to obtain results *(10)*.

Modified procedures to detect gram-negative bacteria in pasteurized dairy products have been reported *(12, 15)*. Olson *(15)* employed crystal violet and Lightbody *(12)* utilized penicillin in the agar as selective inhibitors of gram-positive bacteria. In both instances, 2, 3, 5 triphenyl tetrazolium chloride was incorporated into media to make colonies of gram-negative organisms more distinct. Their results were encouraging even though these tests since have been shown to be somewhat unreliable *(13, 16)*. Usefulness of these modified tests in estimating psychrotrophic bacteria in raw milk has not been investigated. This paper presents data on the microbiological analysis of 105 producer and 74 bulk tank truck samples of Grade A Raw Milk and the correlation of counts obtained by various procedures.

Materials and Methods

Collection of samples

Grade A raw milk samples were collected through the Associated Milk Producers, Inc. (AMPI) in Houston, San Antonio, Austin, Waco, and Bryan, Texas. Bulk tank samples from individual producers were obtained during June and July of 1970 by AMPI representatives and stored in commercially sterile plastic sample bags (Whirl-Pak). The commingled samples were collected with a stainless steel dipper chemically sanitized with a 25 ppm iodophor solution *(1)*, between November 1969 and April 1970 from bulk tank trucks at AMPI receiving stations or plants. These samples were stored in previously autoclaved polypropylene screw-top bottles.

Treatment of samples

Samples were held in ice water until all tests were completed. Tests were done before and after preliminary incuba-

[1]Technical paper 10052 of the Texas Agricultural Experiment Station, College Station 77843.
[2]Present address: Bio-Engineering, Texas A&M University, College Station 77843.
[3]Present address: Pet Incorporated, Johnson City, Tennessee 37601.
[4]Present address: U.S. Army Vet. Det. (MEDDAC) NCID, Patton USAR Center, Bell, California 90201.

TABLE 1. MICROBIOLOGICAL COUNTS OBTAINED ON GRADE A RAW MILK SAMPLES[a]

Microbiological test[b]	Producer samples		Commingled samples	
	Range	Mean	Range	Mean
	(count/ml)			
SPC	800-630,000	70,000	2,200-940,000	100,000
PBC	5-430,000	25,000	20-760,000	34,000
PEN	5-330,000	27,000	10-580,000	32,000
CVT	10-460,000	17,000	30-610,000	18,000
CC	5- 49,000	1,200	5- 10,000	830
LPC	5- 54,000	1,200	60- 29,000	2,600
TBC	-	-	5- 700	41
Y&M	-	-	<1- 900	110

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.
[b]SPC-Standard Plate Count; PBC-Psychrotrophic Bacteria Count; PEN-Penicillin Count; CVT-Crystal Violet Count; CC-Coliform Count; LPC-Laboratory Pasteurized Count; TBC-Thermophilic Bacteria Count; Y&M-Yeast and Mold Count.

tion (PI) as described by Johns and Berzin *(11)*. Samples were transferred to sterile screw-top test tubes for PI. After PI, samples were cooled immediately and stored in ice water. All laboratory tests on commingled and individual producer samples were initiated within 24 and 48 hr, respectively, after sampling.

Microbiological analysis

The Standard Agar Plate Method *(1)* was used for the microbiological analysis of samples. Standard plating media and incubation temperatures were used for Standard Plate (SPC), psychrotrophic (PBC), laboratory pasteurized (LPC), thermophilic (TBC), coliform (CC), and yeast and mold count (Y&M) procedures *(1)*.

Special plating media, crystal violet tetrazolium agar (CVT) described by Olson *(15)* and penicillin (10 IU/ml) tetrazolium agar (PEN) described by Lightbody *(12)* were used to obtain additional counts. Conditions of plate incubation used with these media were modified from 32 C for 48 hr *(12, 15)* to 21 C for 72 hr.

Analysis of data

Data were subjected to computer analysis to obtain the distribution of samples according to microbial counts, mean counts, and linear correlation coefficients between counts obtained by various tests.

RESULTS AND DISCUSSION

Microbial counts of raw milk samples

The ranges and mean microbiological counts obtained on 105 individual producer and 74 commingled milk samples are in Table 1. Observed ranges for the various microbiological counts were quite broad for both producer and commingled samples. In general, counts for producer samples were lower than for commingled samples. The higher SPC's noted for commingled samples are consistent with previous reports *(2, 3)*. Since commingled samples did not represent individual producer samples used in this investigation, it is not possible to make definite conclusions regarding the reason for higher counts in commingled samples. However, it is possible that higher counts were related to sanitary conditions in handling, poor refrigeration, breaking of clumps during pumping, or contamination from the truck tank.

Counts obtained by PBC, PEN, and CVT procedures were quite similar from the standpoint of range and mean values for both producer and commingled samples. The mean PBC's for producer and commingled samples were 25,000 and 34,000/ml compared to mean PEN counts of 27,000 and 32,000/ml. Comparative mean counts obtained by the CVT procedure were 17,000 and 18,000/ml. The similarity of counts obtained by the three procedures was not as apparent when data for individual samples, especially those with low counts, were considered.

The PEN and CVT procedures were originally proposed to enumerate gram-negative spoilage organisms in pasteurized dairy products *(12, 15)*. In these tests, crystal violet and penicillin are used as selective inhibitors of gram-positive microorganisms. The major advantage of these procedures is that counts are available in 48-72 hr. However, the effectiveness of these procedures for estimating psychrotrophic contamination has been questioned *(13, 16)*. Sing et al. *(16)* suggested that growth of some bacteria that normally grow at 7.3 C is suppressed in CVT agar. *Micrococcus* organisms have been reported to grow readily in agar containing penicillin *(13)*. Freeman et al. *(5)* reported that crystal violet does not inhibit all gram-positive microorganisms. Therefore, it is quite likely that variations in counts obtained by the three procedures were caused by differences in the microflora of individual samples and by limitations of the selective inhibitors. Such variations would seriously limit the reliability of these procedures for estimating psychrotrophic bacteria in raw milk.

The TBC's of commingled samples ranged from 5 to 700/ml with a mean count of 41/ml. Yeast and mold counts ranged from <1 to 900/ml with the mean count being 110/ml. Occurrence of thermophilic bacteria and yeasts and molds at these relatively low levels appears to be of little practical significance.

Distribution of samples by microbial counts

The distribution of samples on the basis of counts obtained by SPC, PBC, PEN, and CVT procedures is in Table 2. In general, distribution patterns of both producer and commingled samples according to SPC's were asymmetric. Approximately 55% of the producer samples had SPC's <20,000/ml, whereas only about 15% of the commingled samples had counts below this level. The majority (64%) of the commingled samples had SPC's between 21,000 and 100,000/ml. Approximately 71, 81, and 90 of the producer samples had SPC's <50,000, 100,000, and 200,000/ml, respectively. About 78% of the commingled samples had SPC's <100,000/ml and 89% had counts <200,000/ml. The distribution pattern of producer samples according to these levels is quite similar to the calculated distribution pattern of the samples tested by Ohri and Slatter *(14)* while the distribution of commingled samples appeared to be similar to the estimated distribution pattern of the samples tested by Brazis and Black *(2)*.

The PBC, PEN, and CVT counts for producer samples were lower than those of commingled samples. Approximately 79% of the producer samples had PBC's <5,000/ml compared to 68 and 72% of the samples with PEN and CVT counts <5,000/ml. Of the commingled samples, 49, 57, and 72% had PBC, PEN, and CVT counts <5,000/ml. Similar variations in the distribution patterns were apparent for the count levels of 5,000 to 20,000/ml. The The distribution of samples with counts >20,000/ml was similar for the three tests indicating that the PEN and CVT procedures are more reliable on high count milk.

Effect of PI on microbial counts

The effect of PI on counts obtained by the SPC, PBC, PEN, and CVT procedures is in Table 3. Considerable increases in counts and variations in the count ranges were apparent for all four tests. Greater count ranges were observed for commingled than for producer samples. However, mean counts for the four tests were lower for commingled than for producer samples. Calculated growth ratios for the four tests indicate that growth was more rapid in producer than in commingled samples. The PBC, PEN, and CVT growth ratios were higher than the SPC growth ratios for both producer and commingled samples. Growth ratios for PBC, PEN, and CVT were similar.

The large increases in SPC's and PBC's of both producer and commingled samples are consistent with the known influence of PI on proliferation of psychrotrophic microorganisms *(11)*. Comparable PBC, PEN, and CVT growth ratios suggest that these procedures might be suitable to estimate psychrotrophic organisms in milk after PI. For this

TABLE 2. DISTRIBUTION OF GRADE A RAW MILK SAMPLES ON THE BASIS OF MICROBIOLOGICAL COUNTS[a]

Microbiological count range	Microbiological test[b]							
	Producer samples				Commingled samples			
	SPC	PBC	PEN	CVT	SPC	PBC	PEN	CVT
(count/ml)	(% of samples)							
≤ 250	0.0	39.2	24.8	22.9	0.0	4.0	8.1	10.8
260- 1,000	1.0	23.9	16.2	21.9	0.0	8.1	12.2	23.0
1,000- 5,000	17.2	16.2	26.7	27.7	1.3	36.5	36.5	37.8
5,100- 9,900	18.0	4.7	6.7	7.6	2.7	18.9	14.8	9.5
10,000- 20,000	19.1	2.8	7.6	7.6	10.8	13.7	6.8	5.4
21,000- 50,000	16.2	4.7	10.5	7.6	35.2	9.5	9.5	8.1
51,000-100,000	9.5	3.8	1.8	1.9	28.4	2.7	2.7	1.3
110,000-200,000	9.5	0.9	2.8	2.8	10.8	1.3	5.4	2.7
210,000-500,000	7.6	3.8	2.8	0.0	9.4	4.0	2.7	0.0
≥500,000	1.9	0.0	0.0	0.0	1.4	1.4	1.4	1.4

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.
[b]SPC-Standard Plate Count; PBC-Psychrotrophic Bacteria Count; PEN-Penicillin Count; CVT-Crystal Violet Count.

TABLE 3. EFFECT OF PRELIMINARY INCUBATION ON MICROBIOLOGICAL COUNTS OF GRADE A RAW MILK SAMPLES[a]

Microbiological test[b]	Producer samples			Commingled samples		
	Count after preliminary incubation		Growth[c] ratio	Count after preliminary incubation		Growth[c] ratio
	Range	Mean		Range	Mean	
	(Count/ml)			(Count/ml)		
SPC	1,500- 55,000,000	5,000,000	71	3,000-27,000,000	2,900,000	29
PBC	<50->10,000,000	4,000,000	160	900-32,000,000	3,200,000	94
PEN	<50->10,000,000	4,200,000	156	500-37,000,000	2,400,000	75
CVT	<50->10,000,000	3,000,000	177	1,000-36,000,000	1,800,000	100

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.
[b]SPC-Standard Plate Count; PBC-Psychrotrophic Bacteria Count; PEN-Penicillin Count; CVT-Crystal Violet Count.
[c]Growth ratio = $\frac{\text{Mean count after preliminary incubation}}{\text{Mean count before preliminary incubation}}$

TABLE 4. PSYCHROTROPHIC GROWTH RATIO IN GRADE A RAW MILK SAMPLES GROUPED BY INITIAL PSYCHROTROPHIC COUNTS[a]

Initial psychrotrophic count range	Producer samples				Commingled samples			
	Per cent of samples	Mean psychrotrophic count		Growth ratio[b]	Per cent of samples	Mean psychrotrophic count		Growth ratio[b]
		Initial	After preliminary incubation			Initial	After preliminary incubation	
(Count/ml)		(Count/ml)				(Count/ml)		
≤ 100	24.7	43	21,000	489	2.7	50	1,500	30
110- 1,000	37.2	470	120,000	255	9.5	530	99,000	187
1,100-10,000	20.0	3,600	1,700,000	472	55.3	4,300	800,000	186
11,000-50,000	7.6	23,000	6,700,000	291	23.0	25,000	4,100,000	164
>50,000	10.5	210,000	28,700,000	133	9.5	270,000	19,000,000	70

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.

[b]Growth ratio = $\frac{\text{Mean Psychrotrophic Bacteria Count after Preliminary Incubation}}{\text{Mean Initial Psychrophilic Bacteria Count}}$

TABLE 5. CORRELATION COEFFICIENTS BETWEEN COUNTS OBTAINED BY VARIOUS MICROBIOLOGICAL TESTS[a]

Microbiological test[b]	Correlation coefficient (r)										
	SPC	SPC-PI	PBC	PBC-PI	PEN	PEN-PI	CVT	CVT-PI	CC	LPC	TBC
Producer samples											
LPC	−0.02	−0.06	−0.05	−0.05	−0.05	−0.05	−0.05	−0.05	−0.03		
CC	0.36**	0.41**	0.60**	0.32**	0.49**	0.53**	0.58**	0.26**			
CVT-PI	0.69**	0.67**	0.76**	0.96**	0.75**	0.91**	0.83**				
CVT	0.62**	0.65**	0.81**	0.83**	0.82**	0.85**					
PEN-PI	0.69**	0.77**	0.88**	0.94**	0.85**						
PEN	0.72**	0.78**	0.86**	0.84**							
PBC-PI	0.68**	0.77**	0.83**								
PBC	0.69**	0.80**									
SPC-PI	0.64**										
Commingled samples											
Y&M	0.01	−0.01	−0.01	−0.01	−0.04	−0.01	−0.04	−0.04	0.12	0.16	−0.07
TBC	0.08	0.29*	0.07	0.29*	0.08	0.32**	0.15	0.31**	0.56**	−0.03	
LPC	0.20	0.21	0.27*	0.27*	0.11	0.11	0.10	0.23*	0.07		
CC	0.23*	0.45**	0.20	0.46**	0.18	0.47**	0.18	0.45**			
CVT-PI	0.36**	0.89**	0.58	0.84*	0.47**	0.94**	0.39**				
CVT	0.57**	0.47**	0.90**	0.43**	0.85**	0.36**					
PEN-PI	0.43**	0.90**	0.55**	0.91**	0.42**						
PEN	0.53**	0.56**	0.86**	0.51**							
PBC-PI	0.56**	0.90**	0.67**								
PBC	0.57**	0.63**									
SPC-PI	0.52**										

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.

[b]SPC-Standard Plate Count; PBC-Psychrotrophic Bacteria Count; PEN-Penicillin Count; CVT-Crystal Violet Count; CC-Coliform Count; LPC-Laboratory Pasteurized Count; TBC-Thermophilic Bacteria Count; Y&M-Yeast and Mold Count; PI-indicates test conducted after preliminary incubation of the samples.

*Significant ($P<0.05$).

**Significant($P<0.01$).

reason, producer and commingled samples were grouped according to initial PBC's and growth ratios were calculated for each group. Data in Table 4 indicate a general decrease in growth ratios as the initial PBC level increased. This trend was more apparent and consistent for producer than for commingled samples. Furthermore, commingled samples had consistently lower growth ratios at all initial PBC levels than did producer samples. Since commingled samples were not representative of producer samples, it is not possible to assess the significance of commingling to lower growth during PI. Observed differences in counts probably resulted from variations in the microflora of individual producer and commingled samples.

Correlation between microbiological counts

Counts obtained by the various microbiological procedures on producer and commingled samples before and after PI were subjected to statistical analysis to determine the correlation between tests. Linear correlation coefficients are in Table 5. In general, correlation coefficients between the various tests were higher for producer than for commingled samples. For producer samples, the SPC was highly correlated ($P<0.01$) with all other counts except the LPC. The LPC was not related to any other test results obtained on producer samples. With commingled samples, the SPC was correlated with

all counts except LPC, TBC, and Y&M. The low, but statistically significant, correlations observed between CC's, TBC's, and LPC's and some of the other counts on commingled samples is possibly related to conditions of sanitation in handling that could be a common source of the various types of microorganisms. Correlation coefficients between initial SPC's, PBC's and CC's are in agreement with those reported by previous investigators *(8, 17, 19)*.

Correlation coefficients between initial SPC and the initial PBC, PEN, and CVT results ranged from 0.53 (SPC vs. PEN for commingled samples) to 0.72 (SPC vs. PEN for producer samples). Higher correlation coefficients (0.81 to 0.90) were obtained between the PBC, PEN, and CVT tests.

Counts obtained on samples after PI are largely representative of the selected microflora multiplying during incubation. Therefore, as would be expected, highly significant correlations ($P<0.01$) were obtained between SPC, PBC, PEN, and CVT counts on samples after PI. A close comparison of correlation coefficients between these tests indicates that correlations between tests were improved by PI, and that this improvement was more apparent in commingled than in producer samples.

Since the PBC, PEN, and CVT tests showed consistently higher correlation coefficients, the relationship between these tests was examined further by grouping the combined data for producer and commingled samples according to initial PBC's. The correlation coefficients for samples at various count levels both before and after PI are in Table 6. For all of the samples combined, PEN and CVT counts were highly related ($P<0.01$) to PBC's with correlation coefficients ranging from 0.86 to 0.95. However, at low initial PBC levels (<50,000/ml) PEN and CVT counts were not significantly correlated to PBC's. After PI, counts obtained by the different tests were highly related ($P<0.01$) for all count levels. However, correlation coefficients were higher for samples having PBC's after PI of $>10^6$/ml.

The apparent similarity between results obtained by PBC, PEN, and CVT procedures as evidenced by sample distribution patterns, mean counts, growth ratios, and highly significant correlation coefficients is of special interest. Previous studies *(13, 16)* have indicated that the reliability of the PEN and CVT procedures for selective enumeration of gram-negative microorganisms is limited. Therefore, relationships observed between counts obtained by the three procedures might be considered contradictory to previous results. The improved correlation between test results is probably related to use of the lower plate incubation temperature (21 C), as opposed to the higher temperatures (30-32 C) used by previous investigators *(12, 13, 16)* and to high initial PBC's of some samples. Also, lack of significant correlation between the three tests in samples with PBC's <50,000/ml must be emphasized. These results

TABLE 6. EFFECT OF PRELIMINARY INCUBATION ON THE RELATIONSHIP BETWEEN PSYCHROTROPHIC, PENICILLIN, AND CRYSTAL VIOLET COUNTS[a]

Psychrotrophic bacteria counts		Per cent of samples	Correlation between tests[b]	
Range	Mean		PBC vs. PEN	PBC vs. CVT
		Before preliminary incubation		
(Count/ml)			(r value)	
≤ 100	44	15.1	−0.12	−0.15
110- 1,000	470	26.1	0.07	0.15
1,100-10,000	4,100	34.7	0.15	0.10
11,000-50,000	25,000	14.0	−0.10	−0.04
>50,000	240,000	10.1	0.82**	0.85**
Producer samples	25,000	58.6	0.86**	0.81**
Commingled samples	34,000	41.4	0.86**	0.90**
All samples	29,000	100.0	0.86**	0.86**
		After preliminary incubation		
≤ 25,000	4,500	32.4	0.54**	0.34**
26,000- 300,000	140,000	19.6	0.41**	0.43**
310,000- 990,000	570,000	17.9	0.55**	0.35**
1,000,000-9,900,000	4,000,000	20.1	0.85**	0.94**
>9,900,000	26,000,000	10.0	0.86**	0.93**
Producer samples	4,000,000	58.6	0.94**	0.96**
Commingled samples	3,200,000	41.4	0.91**	0.84**
All samples	3,500,000	100.0	0.92**	0.95**

[a]Data represent 105 producer (June and July, 1970) and 74 commingled (November, 1969 to April, 1970) samples.
[b]PBC = Psychrotrophic Bacteria Count; PEN = Penicillin Count; CVT - Crystal Violet Count.
*Significant ($P<0.05$).
**Significant ($P<0.01$).

coupled with the highly significant correlations between test results on both producer and commingled samples after PI indicate that the shorter PEN and CVT plating procedures could be useful in testing high count milk or milk after PI; however, reliability of these procedures in reflecting the level of psychrotrophic bacteria in low count milk would be extremely limited. Additional research should be devoted to development of a test employing selective inhibitors and media that would be a reliable index of the level of psychrotrophic bacteria present in low count milk. Use of combinations of the various selective inhibitors has not received adequate attention.

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27TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR QUALITY CONTROL

The 27th annual meeting of the American Society for Quality Control will be held at the Sheraton-Cleveland Hotel, Cleveland, Ohio May 21-23, 1973. Quality control experts representing various industrial groups will be in attendance. The Food and Allied Industries Division has several speakers who will deal with current trends and requirements for adequate control in an all day session on Tuesday, May 22, 1973. Personnel will include technical speakers from regulatory agencies, foundations and trade associations as well as leading industrial control managers.

Ample opportunity will be provided to talk with leaders in the field. This is an excellent opportunity for management as well as those charged with quality control to "update" ideas and programs.

Non-members may request a copy of the program and arrange to attend the meeting by writing to the American Society for Quality Control, 161 West Wisconsin Avenue, Milwaukee, Wisconsin 53203.

EFFECT OF PLATE INCUBATION TEMPERATURE ON BACTERIAL COUNTS OF GRADE A RAW MILK[1]

H. E. Randolph, B. K. Chakraborty[2], Otto Hampton[3], and D. L. Bogart[4]
Department of Animal Science
Texas A&M University, College Station 77843

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Abstract

Bacterial counts on 155 raw milk samples obtained with plate incubation temperatures of 27 and 32 C were closely correlated ($r = 0.96$). Correlation coefficients between counts obtained at both 27 and 32 C and psychrotrophic (7 C-10 days) counts for all samples were relatively low, but statistically significant ($P < 0.01$). The correlation to psychrotrophic counts was especially low and in some instances lacking in statistical significance in the sample groups with counts (27 and 32 C) <100,000/ml. Eighty-four of the samples had higher counts at 32 C and 62 samples had higher counts at 27 C. The mean psychrotrophic count of the samples with higher counts at 27 C was higher than the mean psychrotrophic count of the other samples. However, the correlation coefficients were higher for samples with counts higher at 32 C. Incubation at 27 C does not appear to offer significant advantages over the 32 C incubation temperature used in the Standard Plate Count.

The microbial population of raw milk provides an indication of the sanitary conditions employed in its production and handling. Therefore, bacterial counts are used to evaluate milk for compliance with quality standards and regulatory grade classifications *(5, 9)*. The Standard Plate Count (SPC) *(1)* is the most widely used procedure to enumerate the microbial population of raw milk. It is the only microbiological procedure used to determine compliance of Grade A raw milk with health authority standards *(9)*. Enumeration of the maximum number of microorganisms in milk is generally considered desirable *(3, 4)*. Other investigators *(2, 3, 4, 6)* have shown that counts obtained on Standard Methods Agar *(1)* are influenced by the temperature of plate incubation. The 12th (and 13th) edition of *Standard Methods (1)* specifies incubation at 32 ± 1 C for the SPC, but there is some controversy over whether or not this is the best incubation temperature. Higher incubation temperatures have been shown to yield lower counts *(2, 3, 4, 6)*. Nelson and Baker *(6)* noted that the temperature-storage history of milk influences the effect that plate incubation temperature has on bacterial counts. Johns *(5)* indicated that incubation at 32 C is too high for growth of many psychrotrophs. Babel et al. *(2)* found that the mean counts obtained with plate incubation temperatures of 32 and 26 C were essentially the same after 48 hr, but slightly higher counts were obtained at 26 C when plates were incubated for 72 hr. Huhtanen *(4)* found no statistical difference between mean counts obtained after 48 hr with plate incubation at 32 and 27 C but indicated that 32 C appeared to be close to the maximum limit for psychrotrophic microorganisms.

The purpose of this study was to compare counts obtained on Grade A raw milk samples with plates incubated at 32 and 27 C and to relate counts obtained at these temperatures to psychrotrophic counts.

Materials and Methods

Grade A raw milk samples were obtained from Associated Milk Producers, Inc. (AMPI) in Houston and Bryan, Texas. The samples were taken from bulk tanks of individual producers by AMPI representatives and stored in commercially sterile plastic sample bags (Whirl-Pak). Samples were held in ice water until tested. All microbiological tests were initiated within 48 hr after sampling.

Bacterial counts were determined by the agar plate method, using Standard Methods Agar *(1)* and plate incubation at 7 ± 1 C-10 days (PBC), 27 ± 1 C-48 hr, and 32 ± 1 C-48 hr (SPC). Data were subjected to computer analysis to obtain the distribution of samples according to microbial counts, mean counts, and correlation coefficients between the counts obtained at different incubation temperatures.

Results and Discussion

Bacterial counts obtained on 155 individual producer milk samples and the correlation coefficients between counts obtained with plate incubation temperatures of 7, 27, and 32 C are in Table 1. Based on data obtained at 27 C, approximately 62% of the samples had counts <20,000/ml, 24.5% had counts between 21,000-100,000/ml and < 14% of the samples had counts >100,000/ml. The mean counts obtained at 27 and 32 C for the samples in the various count ranges were quite similar. When samples had counts of <100,000/ml, the mean counts obtained by incubation at 32 C were slightly higher than the mean counts obtained at 27 C. However, slightly higher mean counts were obtained at 27 C for samp-

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[1]Technical paper 10053 of the Texas Agricultural Experiment Station, College Station 77843.

[2]Present address: Bio-Engineering Program, Texas A&M University, College Station 77843.

[3]Present address: Pet Incorporated, Johnson City, Tennessee 37601.

[4]Present address: U.S. Army Vet. Det. (MEDDAC) NCID, Patton USAR Center, Bell, California 90201.

TABLE 1. DISTRIBUTION OF SAMPLES ON THE BASIS OF COUNTS OBTAINED AT 27 C AND CORRELATION COEFFICIENTS BETWEEN COUNTS OBTAINED AT 7, 27, AND 32 C.

Range based on 27 C count	Sample distribution	Mean count/ml			Correlation coefficients		
		7 C	27 C	32 C	32 vs. 7	27 vs. 7	32 vs. 27
(count/ml)	(%)						
<5,000	25.2	250	2,800	3,500	0.41**	0.35**	0.83**
5,100- 20,000	36.6	2,000	11,000	12,000	0.20	0.28*	0.95**
21,000-100,000	24.5	9,400	41,000	47,000	0.28	0.24	0.83**
110,000-500,000	11.8	120,000	230,000	220,000	0.73**	0.79**	0.88**
>500,000	1.9	67,000	560,000	510,000	0.67	0.72	0.03
All samples	100.0	18,000	52,000	52,000	0.41**	0.40**	0.96**

[a]Data represent 155 individual producer milk samples.
*Significant ($P < 0.05$).
**Significant ($P < 0.01$).

TABLE 2. GROUPING OF SAMPLES ON THE BASIS OF MAXIMUM COUNT AND CORRELATION COEFFICIENTS BETWEEN COUNTS OBTAINED AT 7, 27 AND 32 C.

Sample group	No. of samples	Mean count/ml			Correlation coefficients		
		7 C	27 C	32 C	32 vs. 7	27 vs. 7	32 vs. 27
Samples with counts higher at 32 C than at 27 C	84	15,000	35,000	46,000	0.83**	0.85**	0.99**
Samples with counts higher at 27 C than at 32 C	62	22,000	77,000	63,000	0.59**	0.61**	0.98**

**Significant ($P < 0.01$).

les with counts >100,000/ml. The mean counts obtained for all the samples at the two incubation temperatures were identical.

Counts obtained at 27 and 32 C for samples at all count ranges were highly correlated with the exception of the sample group with counts >500,000/ml. This apparent lack of correlation may be attributed to the small number of observations included in this group. Correlation coefficients between the 27 and 32 C counts for the other groups of samples ranged from 0.83 to 0.95 and the correlation value for all samples was 0.96. Counts obtained at 27 and 32 C were not highly correlated to the psychrotrophic counts for the samples with counts <100,000/ml, but were more highly correlated when samples had counts >100,000/ml. The correlation coefficients between counts obtained at both temperatures and psychrotrophic counts for the entire group of samples were relatively low, but highly significant ($P<0.01$).

The similarity of counts obtained with plate incubation at 27 and 32 C is consistent with the results of Babel et al. *(2)* and Huhtanen *(4)*. However, the results are somewhat different from those of Hartley et al. *(3)*, who reported higher counts with plate incubation at 28 C than at 32 C. These differences could result from variations in the length of the incubation periods. Hartley et al. *(3)* incubated plates at 28 C for 4 days, compared to the 27 C for 48 hr used in the present study. Babel et al. *(2)* observed similar mean counts at 32 and 26 C after two days incubation for 48 hr, but the 26 C counts were slightly higher than the 32 C counts when plates were incubated for 72 hr. In addition to variations in incubation conditions, differences in microflora, refrigerated storage, and population levels could contribute to differences in counts obtained at the various incubation temperatures *(4, 5, 6, 7)*.

The correlation coefficients between counts obtained at 32 C and the psychrotrophic counts are lower than those reported by previous investigators *(3, 7, 8)*. Tatini et al. *(8)* reported a correlation coefficient of 0.56 between counts obtained at 7 and 32 C on over 600 bulk tank milk samples from Grade A producers. Hartley et al. *(3)* compared the counts obtained at 7 and 32 C for 30 Grade A producers and reported a correlation coefficient of 0.80 between the counts obtained at the two incubation temperatures. The relatively low correlation between the 32 C and psychrotrophic counts as observed in the present study could be related to the low initial counts of a majority of the samples (approximately 62% with counts <20,000/ml) or to variations in the microflora. Low correlations between psychrotrophic and other microbiological counts in milk samples with low initial microbial populations were reported in another paper *(7)*.

Additional comparisons of counts obtained at the different incubation temperatures were made by dividing the samples into two groups based upon the maximum count at 27 or 32 C. One group consisted of 84 samples which had higher counts at 32 than at 27 C while the other group, representing 62 samples, had higher counts at 27 C. The counts obtained for the two groups of samples and the correlation co-

efficients between the various counts are in Table 2. In general, the mean counts obtained on the 84 samples in the group with higher counts at 32 C were lower than for those with higher counts at 27 C. Counts obtained at the different incubation temperatures for both groups of samples were highly correlated ($P<0.01$), but the highest degree of correlation was still between the counts at 27 and 32 C.

Considerably higher correlations were obtained between both the 27 and 32 C counts and the psychrotrophic counts for the group of samples which had higher counts at 32 C. The reason for this is not readily apparent. Johns *(5)* indicated that incubation at 32 C might be too high for growth of psychrotrophs. Presumably the 27 C incubation temperature would be more favorable for growth of psychrotrophs. Therefore, counts obtained at 27 C would be expected to be more closely correlated to psychrotrophic counts than would the 32 C counts. It is possible that individual sample variations between the two groups could be responsible for the observed differences.

Randolph et al. *(7)* reported in another paper that a small number of samples with relatively high counts could exert a marked influence on the correlation coefficients between various microbiological counts. However, no attempt was made to determine the reason for the higher correlations observed between the psychrotrophic counts and the counts obtained at 27 and 32 C for the group of samples that had higher counts at 32 C. Correlation coefficients between the counts obtained at both incubation temperatures and the psychrotrophic counts for both groups of samples were considerably higher than obtained for all of the samples combined (Table 1). Correlations obtained on the two groups of samples are within the ranges of those reported by Hartley et al. *(3)* and Tatini et al. *(8)*.

Several investigators *(3, 4, 5)* have suggested lowering the incubation temperature of the SPC procedure *(1)* to make conditions more suitable for enumeration of psychrotrophic bacteria. However, our results do not indicate that counts obtained by incubation at 27 C for 48 hr provide a more reliable index of the microbiological quality of milk than counts obtained by incubation at 32 C for 48 hr. Counts at 27 and 32 C were highly correlated and the correlation coefficients between the counts obtained at both temperatures and psychrotrophic counts were similar. Samples with counts <100,000/ml showed higher counts at 32 C while those with counts >100,000/ml showed greater growth at 27 C. Thus, it is possible that plate incubation at 32 C for 48 hr might be more effective than at 27 C for 48 hr for Grade A raw milk, since the bacterial population encountered should be <100,000/ml *(9)*. Our results indicate that additional studies should be conducted before consideration is given to lowering the incubation temperature of the SPC method. Due consideration should be given in future studies to the effect of incubation time on counts obtained with different incubation temperatures.

ACKNOWLEDGMENT

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POLYCHLORINATED BIPHENYLS IN MAN'S FOOD–A REVIEW[1, 2]

R. L. Bradley, Jr.
Department of Food Science
University of Wisconsin, Madison, Wisconsin 53706

Abstract

Among environmental contaminants, polychlorinated biphenyls (PCBs) are similar to DDT in persistence, biological magnification through food chains, chemical inertness, and hydrophobic character. While toxicity to lower vertibrates and invertibrates is less than that of DDT, mammals are much more susceptible to the toxic effects. PCBs have been implicated in egg shell thinning in some predacious birds, failure to reproduce and deaths in mink, and economic losses in both the dairy and poultry industries. A wide variety of pathologic changes have been observed–teratogenesis, edema, damage to liver and kidneys, and retarded growth and development of sex characteristics. Growth retardation was temporary in chicks fed $<$ 40 ppm PCBs in a ration and permanently impaired above this concentration. A by-product, tetrachloroparadibenzofuran found in trace quantities in PCBs of European manufacture, is more toxic than PCBs but has similar biological effects. Considerable national research emphasis is being directed toward full resolution of PCB-associated problems.

To extremists, the present amount and increasing rate of pollution in our environment will be terminal for all humans. It is like playing musical chairs on the Titanic. In so many words, if the pesticides don't get you, then the carbon monoxide will! At the other extreme are the apathetic, and the ill advised.

The vast majority of people occupy the middle category. These people care about our environment, wish to preserve it for future generations, and are willing to go to some economic expense to accomplish it. This group is generally aware that a total cleanup is impossible, impractical, unnecessary, and that we can live compatibly with certain amounts of contaminants. This is strengthened by the fact that, in general, the birth weight of babies is not decreasing, there is no apparent increase in congenital defects, rate of growth of children over a number of years is unchanged, and our life span continues to lengthen. To this group the decline of certain wild populations of mammals and birds, serves as a warning sign indicating that we could be in the same predicament if the misuse of the pollutant in question continued. An example of such an environmental contaminant is the subject of this paper.

Another chemical, useful to several industries and known to be inert, insoluble in water, thermally quite stable, and resistant to both acid and alkalis has presented itself as a primary environmental pollutant.

Polychlorinated biphenyls or PCBs are a complex heterogeneous group of chemicals and have been manufactured for 40 years. In 1970, Monsanto sold 36,500 tons of these materials and 18,800 tons in 1971. This is shown by domestic sales in Fig. 1 and by type of PCB in Fig. 2. Voluntary restriction of sales by Monsanto was used in an effort to curb contamination of foods and pollution of the environment *(27)*. This represents the difference in sales between 1970 and 1971 and includes curtailment of sales for plasticizers, surface coatings and sealers, adhesives, printing inks, pesticide extenders, microencapsulation of dyes, and wax modifiers *(16, 21, 27)*. Also, PCBs used as a heat transfer medium, which could lead to contamination of a food supply, was stopped. Uses that continue to be supplied with PCBs are hydraulic and dielectric fluids *(27)*.

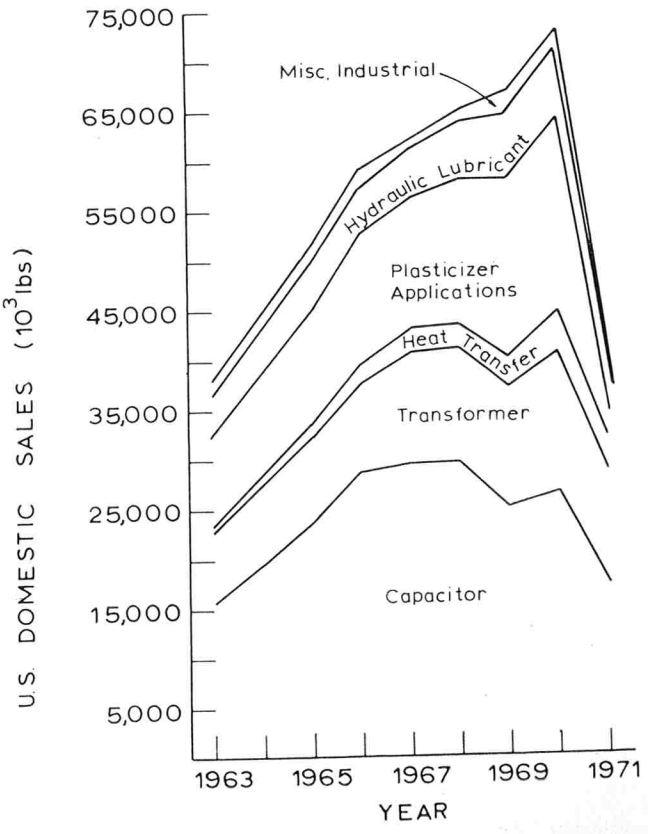


Figure 1. United States domestic sales of PCBs by use. *(27)*

[1]Contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706.

[2]Presented at the 59th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972.

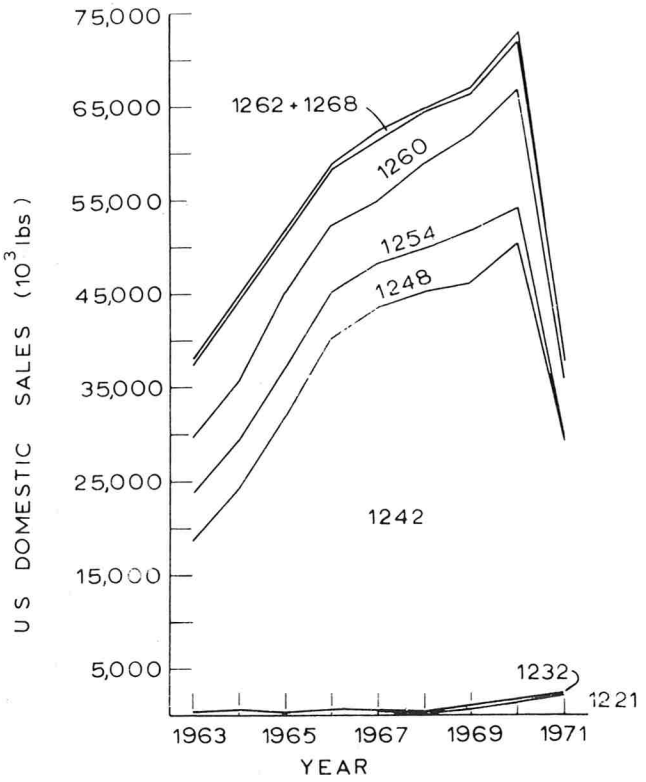


Figure 2. United States domestic sales of PCBs by type. *(27)*

SOME CHEMISTRY OF PCB'S

Structurally PCBs are a heterogeneous group of chemicals with various numbers of chlorine atoms substituted on the diphenyl radical *(11, 29)*. The diagram (Fig. 3) indicates the number of possible isomers and that a total of 210 different isomers is possible by chlorination of any or all of the 10 available positions. It is not known which or how many of these are involved in contamination of food.

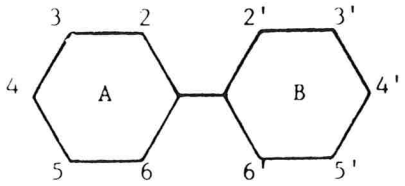
The concentration of PCBs can be approximated using the gas chromatograph and an electron capture detector, techniques employed to separate and identify organochlorine pesticides *(12, 14)*, and a separation technique using silicic acid *(7)*. Known amounts of individual PCBs must be passed through silicic acid to quantitate against unknowns *(21)*. Typical chromatograms show the complex nature of Aroclor, the Monsanto product, 1221, and 1232 (Fig. 4). These are biphenyls containing 21% and 32% chlorine, respectively, as indicated by the last two digits of the identifying number. Moreover, in each of these figures similar peaks are evident and the same compounds appear in nearly all of these Aroclors. In Fig. 5, typical chromatograms for Aroclor 1242 and 1248 are shown. Notice that as the percentage of chlorine increases so does the number of constituents appearing later in the chromatogram or those having more chlorine and longer retention times. In Fig. 6, the chromatograms are typical for Aroclor 1254, 1260, and 1262 *(11)*.

If another benzene ring is added to the biphenyl structure at either the *ortho*, *meta*, or *para* position, a terphenyl results. Instead of 10 possible positions for chlorine there now are 14. A typical example containing 42% chlorine is Aroclor 5442 (Fig. 7). Some material appears early on the chromatogram similar to Aroclor 1242 and 1248, however the bulk appears late after 35 min have elapsed. It has been suggested that biological concentration could make this Aroclor appear as a PCB *(11)*. The terphenyls require special methods to isolate them, and these are usually not employed in residue chemistry laboratories.

Monsanto is the only manufacturer of PCBs in the U. S.; however, PCBs are produced in other countries; i.e., Japan (Kanechlor), Germany (Clophen), France (Phenoclor), Russia, Great Britain, and Italy. The total annual production is guesstimated at 110,000 tons *(27)*. Unfortunately an extremely hazardous and toxic material is a manufacturing by-product in some European PCB mixtures, a polychlorodibenzofuran (Fig. 8) *(42, 44)*. It may contain up to 8 chlorines with the tri and tetra substituted products being most common. This differs by one oxygen atom from tetrachlorodibenzo-p-dioxin identified previously as the causative agent in chick edema factor *(44)*.

POLLUTION

Pollution of our environment and of direct concern



Chlorines on A ring

Chlorines on B ring	0	1	2	3	4	5
0	1	3	6	6	3	1
1		6	18	18	9	3
2			21	36	18	6
3				21	18	6
4					6	3
5						1

Figure 3. The structural configuration for biphenyl and possible sites for chlorination *(11)*.

Table 1. Gross estimates of rates of input and accumulation of PCBs in North America in 1970 (27)

Category of input	Rate tons/year	PCB grade
Vaporization of plasticizers	$1\text{-}2 \times 10^3$	Mostly 1248-1260
Vaporization during open burning	4×10^2	Mostly 1242
Leaks and disposal of industrial fluids	$4\text{-}5 \times 10^3$	1242-1260
Destroyed by incineration and open burning	3×10^3	Mostly 1242
Disposal in dumps and landfills	1.8×10^4	1242-1260
Accumulation in service	7×10^3	1242-1254
Reservoir	**Accumulation, tons**	
Soil excluding dumps	1.5×10^4	
Oceans adjacent to North America	1.5×10^4	
Fresh water (dissolved or in suspension)	1×10^2	
Fresh water sediment	2×10^4	
Biota	$<1 \times 10^3$	

to us, food, has resulted in many ways. Available data (Table 1) are for 1970 (27). It is difficult to quantitate these losses; however, calculations show that the total rate of loss of PCBs is approximately 25,000 tons/year. This accounts for first, loss from manufacturing sites through combustion of waste materials and vaporization. This aerial contamination is then precipitated within a few days since, as with DDT, the PCBs are bound most usually to particulate material. The smaller particles will require rain to precipitate them which may occur in some remote area. Evaporation losses from products in which the PCBs were used to facilitate manufacture or application, such as, plastics, epoxy resins, rubber goods, varnishes, waxes, lacquers, dyes and adhesives are included in this estimate. Estimated emissions are 1,500 to 2,000 tons annually with the vast majority concentrated in urban areas. Exposure in an incinerator for 2 sec at 2000 F is not a significant source and represents the most commonly used and accepted method of destruction as opposed to open burning.

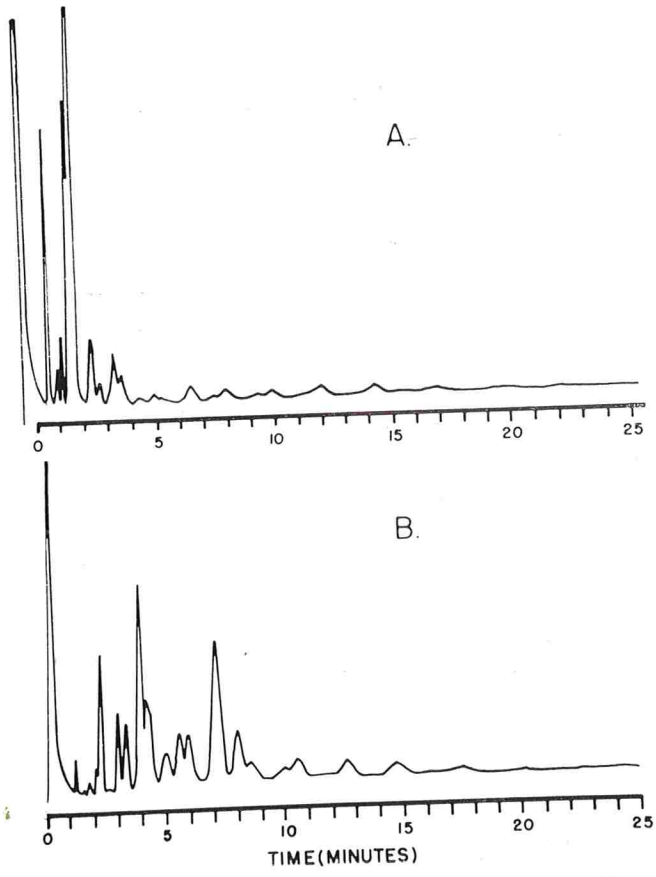


Figure 4. Gas chromatograms of Aroclor 1221 (A) and Aroclor 1232 (B) (11).

Second, loss from manufacturing sites directly into sewers and therefore rivers and lakes is another category and includes both direct dumping of waste fluids containing PCB and PCBs themselves. Because of their insoluble character, PCBs lost by this route usually are quickly bound in some organic entity, such as, river and lake sediment, algae, and protozoa. The amount of PCBs transported to the ocean and fresh water in 1970 was estimated at 4,000 to 5,000 tons. An estimated 18,000 tons were deposited in sanitary landfills, dumps, and incinerators. Another 7,000 tons accumulated in such units as heat exchangers and transformers. Third, losses through leaks in heat exchangers, transformers and capacitors represent another source. This loss has been combined with disposal of hydraulic fluid, heat transfer, and transformer oils to yield an estimated 4,000 to 5,000 tons. The last of the data represent estimations of amounts of PCBs existing at present in various segments of our environment. As a result of these annual losses, food has been contaminated whenever the situation has been correct.

Contamination of Food

It is necessary to understand biological concentration where a chemical constituent like PCBs, which is lipid-soluble, is concentrated through a food chain

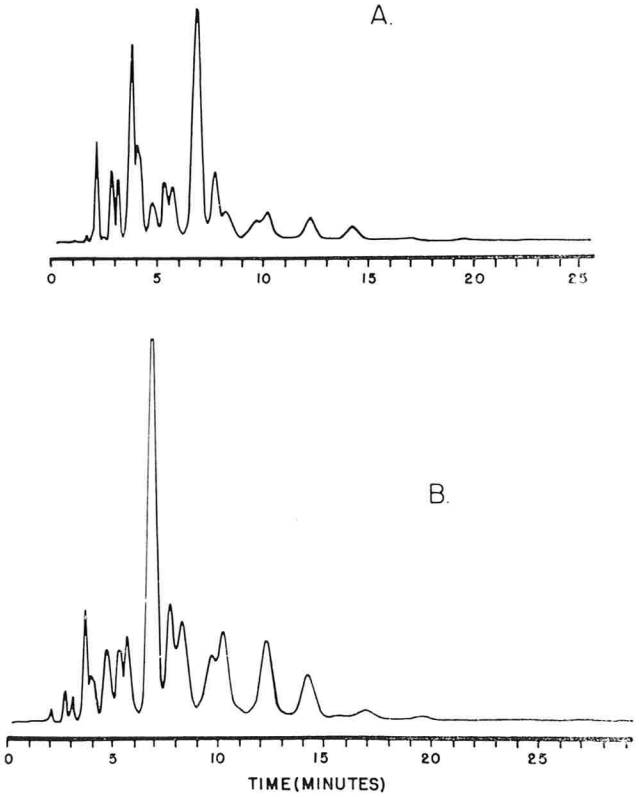


Figure 5. Gas chromatograms of Aroclor 1242 (A) and Aroclor 1248 (B) *(11)*.

(29). This is well documented and values show a magnification of 10- to 100-fold through each predator in a marine system, for example, ultimately reaching a magnitude of 10^6 to 10^7 times higher than in water *(27)*. The larger and older the fish in such an environment, the higher the level. The debacle of the supposed Coho salmon bonanza to the Great Lakes is an excellent example. No trout or salmon longer than 21 inches taken in Lake Michigan are salable presently because of PCB levels above 5 ppm on an edible parts basis *(26)*. This is the tolerance established by the Food and Drug Administration (FDA) for PCBs in fish *(6)*.

Milk has been contaminated because cows were fed silage stored in epoxy coated silos *(15, 38, 39)*. The area of concern is in Ohio, Indiana, and Kentucky. Silo walls were sealed with an epoxy paint that apparently had Aroclor 1254 as a solvent. Research showed migration of the PCBs into corn silage with the greatest concentration in that silage within 3 cm of the wall. It was suggested that the PCBs were dissolved in the silage liquor which with time descended in the silo thereby spreading the contamination. Moreover, automatic unloaders in the silo contributed to the problem by mixing the contaminated with the noncontaminated silage. Natural decontamination of cows is slow and similar to that observed with organochlorine pesticides *(15)*. Some evidence on the fate of PCBs in milk during processing showed that no change occurred in the distribution or content in finished dairy products except in skimmilk that was heated to 70 C for 10 min *(31)*. Also, these data show that, proportionately, PCBs in skimmilk and whey were higher than the concentration in milk from which these products were manufactured. This fact has been observed by others *(17, 24)* and involves the lipoprotein constituents of the milk fat globule membrane. FDA has a temporary guideline of 2.5 ppm PCBs in milk fat *(5, 6)*.

One of the most recent discoveries is that PCBs as a contaminant in paper packaging do migrate to the food product contained inside *(39)*. After investigating the problem, the American Paper Institute pointed to recycled fibers in paperboard as the source of contamination. These fibers proved to be contaminated from carbonless carbon paper *(22, 41)* and printer's inks *(41)*. Currently, because of swift action on the part of paper manufacturers, leaders in the paper industry stated that PCB concentrations presently in packaging materials are not hazardous. The Food and Drug Administration also is concerned about the amount of PCB material found in paper

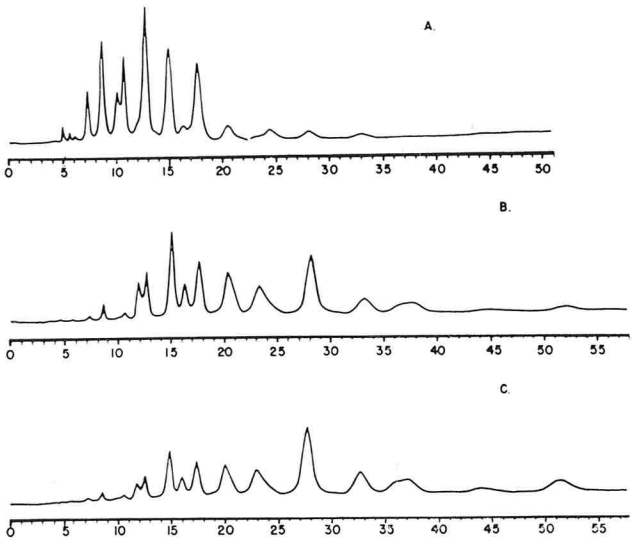


Figure 6. Gas chromatograms of Aroclor 1254 (A), Aroclor 1260 (B) and Aroclor 1262 (C) *(11)*.

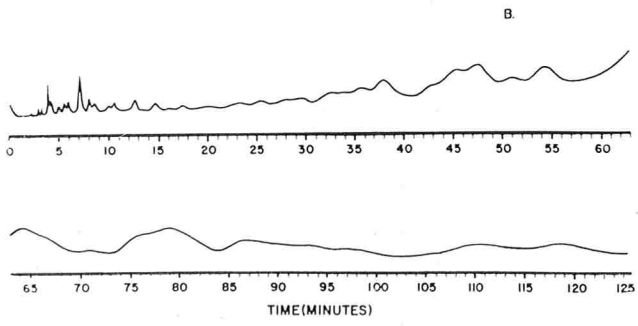


Figure 7. A gas chromatogram of Aroclor 5442 *(11)*.

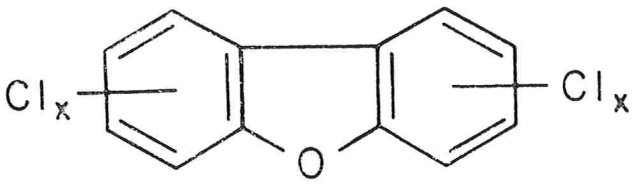


Figure 8. The structural configuration of polychlorodibenzofuran.

and paperboard. They have found PCBs in virgin paper, regular paperboard, and grey board. Presumably the PCB residues from virgin paper are attributable to environmental contamination and traces acquired during manufacturing *(6, 39)*.

Food Chemical News stated that the FDA was considering amending the law to read—no more than 0.1 ppm PCBs in pulp from reclaimed fiber *(3)*. This considered tolerance is at method sensitivity. In the interim, a "temporary guideline" of 5 ppm in food packaging materials has been established *(2, 6)*. In a survey FDA *(5)* found that although 67% of complete food packaging tested contained PCBs at levels as high as 338 ppm, only 19% of the foods in these packages contained PCBs at an average concentration of 0.1 ppm. The maximum level found in these food products was 5 ppm. About 75% of packaged infant cereals contained an average of 0.3 ppm PCBs with a high of 1 ppm. Of the recycled paper samples recently tested, 95% contained < 5 ppm PCBs but during 1970-1971 only 18% were < 5 ppm. FDA stated that progress is being made and this would in effect substantiate the statement from the leaders in the paper industry *(5)*. Of all paperboard, grey board presents the highest contamination in food packaging. Such food items as, crackers, cookies, ready-to-eat breakfast cereals, pretzels, chips, macaroni and noodle products, dry infant cereals, and dry prepared mixes have been contaminated from packaging materials *(20)*.

Hazelton Laboratories in a study supported by the American Paper Institute has examined PCB migration from food packaging *(4)*. Only materials of low gas permeability were checked. PVDC (polyvinylidinechloride or saran) coated paper was best and waxed glassine paper was second best in preventing migration of Aroclor 1242. Polyethylene films were ineffective over a long period. Foil laminates, copolymer films and paper laminates were not tested.

ANIMALS ARE AFFECTED BY PCBs

Of the animals raised by man and contaminated with PCBs, mink appear to be the most susceptible to the chemicals. When Lake Michigan Coho salmon containing high levels of PCBs was incorporated in a mink ration at the 30% level, reproduction either failed or the kits died within 24 hr of whelping. Death of adult mink resulted after 3 months on this diet *(8)*.

In another study *(1)*, Aroclor 1254 contaminated beef was added to a mink ration. All mink fed the diet containing 3.57 ppm PCBs on a whole ration basis died with no reproduction during a 105-day study. At the 0.64 ppm level only one of 12 mink produced kits and these died within 24 hr of whelp-

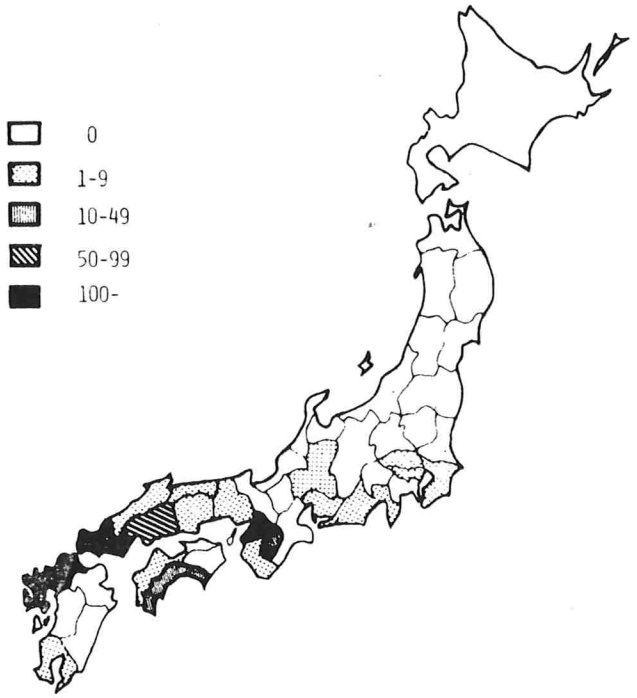


Figure 9. Number of patients with Yoshu by prefecture (23).

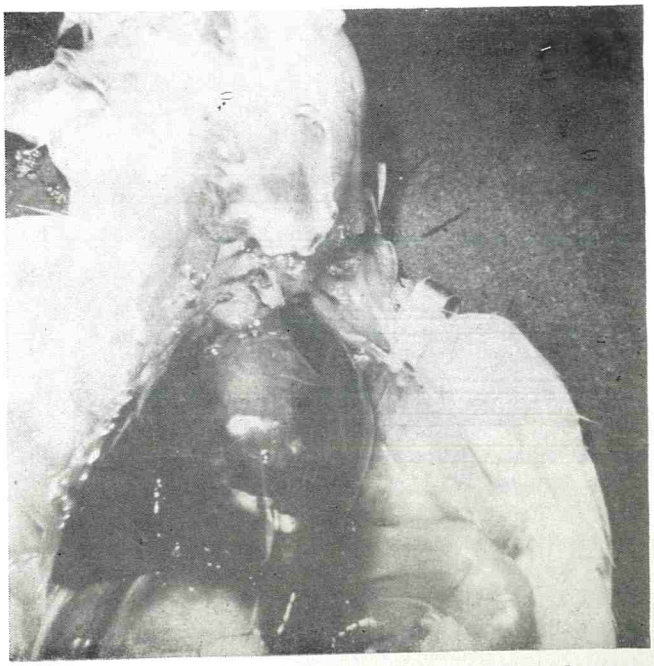


Figure 10. Hydropericardium in a chick fed a diet containing 50 ppm Aroclor 1248 *(33)*.

ing. Similar results have been obtained with laboratory rats, however, dietary concentrations of PCBs used to produce reproductive failures were 100 ppm Aroclor 1254 *(13)*.

Poultry has been contaminated mostly through leaks in heat exchangers used to prepare feed or through contaminated fish meal *(39)*. Moreover, some poultry rations contain rejected bread, crackers, and cookies which were added to the feed with their packaging material. Such feed has resulted in contaminated poultry and eggs. As of March, 1972, 1,086,908 chickens were destroyed because of contamination *(5)*. FDA has established a temporary tolerance of 5 ppm PCBs in the fat of poultry and 0.5 ppm in eggs *(6)*.

The inspection staff of the U. S. Department of Agriculture (USDA) was alerted for evidence of leaks in heat processing equipment that uses toxic materials where food or feed might be contaminated *(5)*. Subsequently, FDA has in effect banned equipment using PCBs *(5)*.

PCBs In Humans

PCB contamination in humans *(32, 45)* has been found in quantities greater than 1 ppm in 31.1% of 637 adipose samples examined, while 19.6% had between trace and 1 ppm. The samples were collected in 18 states and the District of Columbia. Positive samples were obtained in all the states. Apparently humans like other mammals can degrade the PCB isomers that contain a few chlorine atoms; therefore chromatograms initially will show small peaks and later peaks indicative of high concentrations. In a study done in collaboration with the Michigan Department of Public Health, even house dust sweepings in homes of occupationally exposed population in Southwest Michigan contained up to 180 ppm PCBs.

Some Japanese people reportedly have been contaminated with PCBs in rice oil *(13, 23)*. The disease, called "Yusho" or oil disease, was first observed in October of 1968. The map of Japan (Fig. 9) shows the frequency of the disease by prefecture. The highest incidence occurred in Fukuoka-Ken. From a symptomatic standpoint, of the 136 initial cases 38.3% of the patients showed swelling of the upper eye lids and increased eye discharge and 33.1% exhibited acne types of skin eruptions. Others showed dark brown pigmentation of the nails and skin, accentuation of hair follicles, numbness and swelling, or edema of the limbs. To date 1,057 cases of Yusho have been reported. Most of the patients apparently used a particular brand of canned rice oil which contained between 2,000 and 3,000 ppm of Kanechlor 400, a PCB with 48% chlorine. The oil became contaminated through a leak in a vacuum deodorizer used for processing. This is the only case where significant contamination of humans has resulted from one source of food. A follow-up study has been conducted to show the current state of the patients and growth effects on children. Of 23 boys and 19 girls affected with Yusho during 1967-1969, the weight and height gains of boys decreased significantly while no effect was observed with the girls when compared to their classmates. About 50% of adult patients have shown improvement over original clinical symptoms, while about 10% showed worsening effects. Such effects as persistent headache, numbness in limbs, weight loss, and fatigue are clear indications that the effects of Yusho are extremely difficult to overcome.

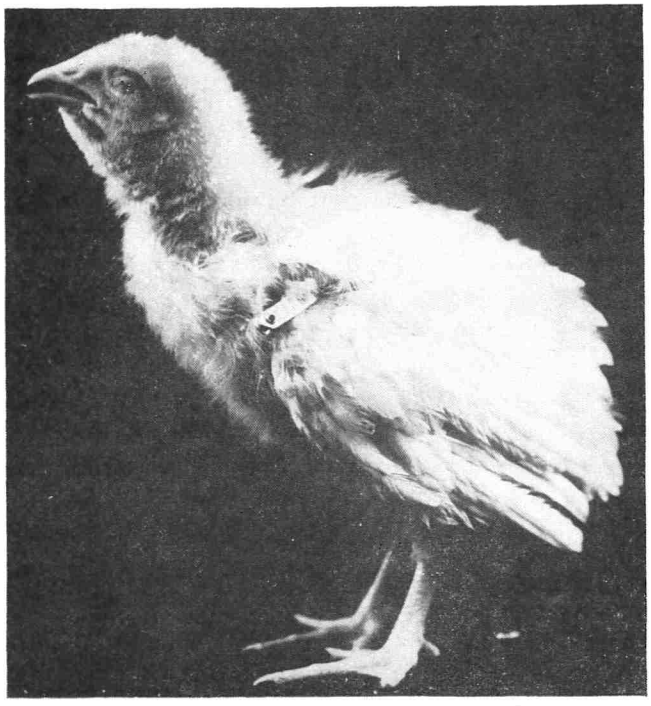


Figure 11. External symptoms of edematous condition. Note gasping *(33)*.

Figure 12. Metabolic product from 2, 2, 5, 5′-tetrachlorobiphenyl fed to rats and pigeons *(19)*.

Experiments With Animals

A study conducted at the University of Wisconsin and using rats has shown hypertrophy of the liver, lack of weight gain, and proliferations of the endoplasmic reticulum of the liver *(28)*. Concurrent with increased liver size was an increase in hepatic enzyme activity. The evidence of hepatic enzyme activity has been observed by others *(12, 29, 36, 40)* Moreover, PCBs have been shown to be antihormonal in

hepatic microsomes *(9, 25, 30, 36)*. This can be correlated with poor reproduction in animals fed PCBs as well as retarded development of secondary sex characteristics in chicks *(34)*. The edema observed in the Yusho incident has also been noted in chicks. Evidence from research with one-day old chicks indicates general edema and provides a vivid example of hydropericardium (Fig. 10) *(33)*. The chick was fed a diet containing 50 ppm Aroclor 1248. The heart sac contained 7 ml of fluid. Also, results of edema can be seen externally in chicks with the gasping symptom shown in Fig. 11. Acnegenic reaction has been vividly shown by Vos and Beems using rabbits *(42, 43)*.

STABILITY OF PCBs

PCBs do not persist *ad infinitum*. Research has shown that 4-chlorobiphenyl fed to rabbits was excreted in the urine as 4-(p-chlorophenyl) phenol and 4-chlorobiphenyl glucosiduronide *(10)*. Subsequently, this metabolic hydroxylation of lower PCB homologs (Fig. 12) was identified in rats and pigeons as one route of excretion as a water soluble product *(19)*. Also, exposure of PCBs to ultraviolet energy at 310 nm causes polymerization, formation of hydroxylated and carboxylated products, and reductive dechlorination *(18, 37)*.

SUMMARY

The mechanism of toxicity of PCBs in humans and animals is still unclear. There are numerous areas where no substantive information exists. On the one hand we find extreme sensitivity with mink and on the other lack of sensitivity in some birds and mammals. PCBs appear to cause no mutagenic effects; are teratogenic when fed to chicks but not to rats *(13)*. Two instances of bladder cancer have been identified in rats *(13)*. Of all the factors studied, reproduction appears to be the most vulnerable function in birds, fish, and mammals *(40)*.

One of the most perplexing problems yet unresolved is the involvement of the various homologs and isomers, i.e., which are toxic, which are not; how is toxicity affected by the position of chlorine substituents, and is there a synergistic effect among them.

There are five distinct pathological patterns for humans—acnegenic, liver damage, porphyria, edema, and immunosuppression *(23, 42)*. However, none of the underlying reactions in the human body have been studied or reported in detail. It is obvious from a search of the literature that human contamination is ubiquitous much as with DDT and its analogs and the toxicity to humans is moderate to high especially when PCBs are inhaled as vapors. Again, no studies have outlined the fate of PCBs when ingested. We do know that, like organochlorine pesticides, PCBs are lipophilic and therefore stored in adipose tissue. When the total picture of the cases of Yusho in Japan is examined, perhaps a fuller understanding will be available of the toxicity, long-term effects, and rate and route of discharge of PCBs from the human body.

Finally it is apparent that Monsanto has limited its sales and controlled the uses of PCBs. Perhaps foreign manufacturers will follow this example. The concentration of PCBs in the environment is great and widespread. It will remain for some time *(35)*. How long it will be here appears impossible to estimate.

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THE EFFECTS OF WARM-UP OF MILK IN SIMULATION OF HOME CONDITIONS ON THE SUBSEQUENT COOLING RATE AND FLAVOR ACCEPTABILITY OF THE PRODUCT[1]

A. C. Smith, H. Patricia MacLeod, and L. R. Glazier
Department of Animal Industries
University of Connecticut, Storrs 06268

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Abstract

Milk in half-gallon cartons was exposed for up to 2 hr at 90 F to simulate carry-home conditions and/or up to 1 hr at room temperature at intervals simulating time elapsed during meals for a 2-day duration. Milk of superior bacteriological quality at time of purchase exposed to the above conditions remained acceptable in flavor. Poor quality milk resulted in excessive flavor deterioration.

The temperature rise of milk during distribution and home use and its effect on keeping quality is of concern to the dairy industry and the consumer. There is little information in the literature regarding the effects of such temperature changes, particularly those occurring during meals when the milk may be held at room temperature for various time intervals of up to 1 hr or more. Temperature rises above 47 F during filling have a detrimental effect on the keeping quality of milk in paper cartons *(3)*. A note recently published indicated that temperature rises induced by incubation at 120 F for 30, 60, and 120 min increased bacterial numbers and decreased flavor scores. Differences in taste were detected between control and incubated samples after only 1 day of storage *(2)*.

The objective of this study was to ascertain if the acceptability of the product was affected by increases in the temperature of milk during time periods simulating take-home and mealtime intervals and subsequent slow cooling in the home refrigerator.

Experimental Procedure

Half-gallon cartons of homogenized milk representative of four brands were obtained from the supply storage of local supermarkets. Six cartons of each brand were obtained from a single case to assure duplicate samples. The cartons were transported to the laboratory in styrofoam packers and then placed in storage at 45 F. The milks had been pasteurized at 172 to 177 F by the high temperature-short time system and the temperatures at pick-up were between 44 and 46 F. The time of pasteurization was established by a call to the production manager at each of the four plants. This enabled storage of samples at 45 F for an elapsed time of 4 days from pasteurization to simulate assumed normal time lapse between pasteurization and sale to the consumer. Following the storage period, samples were removed for the warm-up trials and a sample of each brand was judged for flavor and examined bacteriologically by standard procedures *(1)* for standard plate and coliform counts. Flavor scores were obtained by presenting coded samples in random fashion to a trained panel of two and averaging the scores. In flavor acceptability trials, a duplicate set of samples was used to obtain temperature data of the milk in the cartons during warm-up and subsequent cooling. Temperatures were recorded by means of copper-constantan thermocouples located in the center of the cartons with a Speedomax Type G, 16 lead recorder having a range of −200 to 40 C, an accuracy of ±0.1 C and recording charts with lines at 2 C intervals.

In the first warm-up study, the experimental milk samples were transferred from 45 F storage to thermostatically controlled incubators for 1 or 2 hr at 70 and 90 F. Subsequently, the samples were placed in the 40 F refrigerator in which the control sample had been placed immediately after the 45 F 4-day storage period. Sufficient milk was removed daily from all samples during 40 F storage for flavor evaluation. This was continued until the panel's average score for each sample was below 36.

The second study entailed treatment of the milk samples after removal from 45 F 4-day storage as follows: *(a)* 1 hr at 90 F in an incubator on the AM of the first day to simulate carry-home warm-up, *(b)* room temperature at 4-hr intervals for 0.5 or 1 hr at M and PM on the first day and AM, M, and PM on the second day to simulate mealtime warm-up, *(c)* combination of (*a*) and (*b*) above, and (*d*) a control sample refrigerated at 40 F. The temperature of the room during simulated mealtime warm-ups was 81.6 F (±1) and 83.5 F (±1.5) for the 0.5- and 1-hr periods, respectively. The samples were placed in a 40 F refrigerator after each warm-up period. Ten ounces of milk were removed from all cartons before each warm-up period. The aliquots removed from cartons containing thermocouples were discarded; the others were used for flavor judgments. Results are expressed as the number of meals at which the milk would be acceptable (flavor score of ≥ 36). An assumption was made that the milk when purchased would be opened the same day and consumed within 2 days.

Results and Discussion

The rate of cooling milk (Fig. 1) in half-gallon cartons during storage at 40 F subsequent to warm-up for 1 or 2 hr at 70 and 90 F follows generally recognized curves. The graphs in the figures have been separated for clarity only, and thus are directly comparable. Placement of the samples in the refrigerator caused a rise in temperature of the storage facility

[1]Scientific contribution No. 531, Agricultural Experiment Station, University of Connecticut, Storrs.

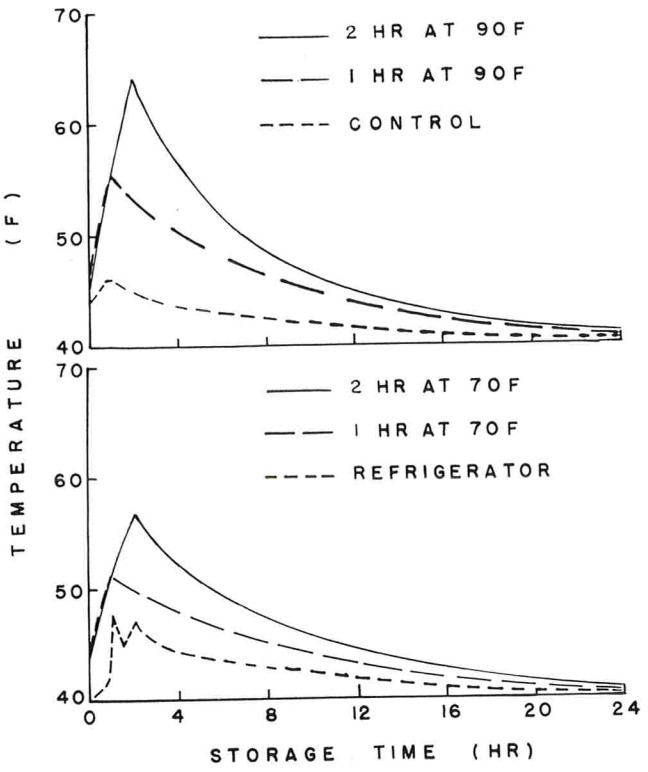

Figure 1. The cooling rate of milk in half-gallon cartons during storage at 40 F following warm-ups simulating transportation from store to home.

and a simultaneous rise in temperature of the control sample of milk. The lengths of time these samples were above 50 F, particularly for the 2-hr warm-up, would be expected to have an adverse effect on the keeping quality of the milk. There was a small decrease (Table 1) in the mean values for keeping quality (expressed as days to reach a flavor score of <36) for the experimental samples from those of the control samples. These mean differences varied from 0.3 to 1.3 days and are probably of minor practical significance since they occur approximately 2 weeks after pasteurization. However, this warm-up may be of considerable importance if subsequent to warm-up the milk is allowed to remain on the table during family meals. Experiments involving three meal-time warm-up periods will be discussed later. The quality of the product at time of pick-up by the customer appears to determine keeping quality (Table 1). Sample four with its higher initial standard plate count had a poorer keeping quality. The discrepancy in the shelf life of sample 2 for 1 hr at 90 F may be caused by recontamination of the milk in that particular carton.

Typical cooling rate curves for milk in half-gallon cartons during storage at 40 F following warm-ups simulating hypothetical home conditions are presented in Fig. 2. The "C" milk as depicted in Fig. 2 representing temperature rise during carry-home time in the first peak and rises during meals in the remaining five peaks was at relatively warm temperatures for extended periods of time. The milk was above 50 F for approximately 15 and 13 hr during the 1st and 2nd day, respectively. The shorter time on the 2nd day probably resulted from quicker cooling of the much smaller volume of milk remaining in the carton (at this late stage in the trial). The "B" milk (Fig. 2) which was not subjected to the warm-up simulating carry-home time did not reach as high temperatures during the 1st day, but on the 2nd day the results were very similar to the "C" milk. The "A" milk depicting carry-home time (Fig. 2) remained above 50 F longer than the milk depicted in Fig. 1 for 1 hr at 90 F because the refrigerator was reacting to the in-and-out movement of samples. The temperature of the refrigerator is not presented in the graph because of its erratic movement as samples were removed or placed therein, but except for the start of the trial when its temperature was 40 F, it approximated 1.5 F below the temperature of the control "D" milk (Fig. 2). Graphs for the milks held at room temperature for 0.5 hr for five periods during 2 days are not presented because they closely resembled the results in Fig. 2 except that the peaks were 3-4 F lower.

Warm-up of milk samples at intervals for 2 days did not affect acceptability unless the product at simulated time of purchase was of an inferior quality (Table 2). For example, in brands 4, 6, and 8 repre-

TABLE 1. EFFECT OF WARM-UP AND SUBSEQUENT COLD STORAGE ON THE ACCEPTABILITY OF PASTEURIZED MILK IN HALF-GALLON CARTONS

Sample number	Quality prior to warm-up			Warm-up				
	SPC/ml	Coliform/ml	Flavor (Score, Criticism)	Control	1 hr 70F	2 hr 70F	1 hr 90F	2 hr 90F
				(Days[a] to a flavor score <36)				
1	3,000	8	38.5 cooked & feed	12	11	9	10	9
2	1,200	<1	38.0 cooked & feed	12	12	10	7	12
3	680	<1	39.0 cooked & feed	13	14	15	15	14
4	38,000	<1	38.5 cooked & feed	8	7	8	8	7
Mean				11.3	11.0	10.5	10.0	10.5

[a]Values do not include 4 days storage before warm-up.

TABLE 2. EFFECT OF WARM-UPS SIMULATING HOME CONDITIONS ON THE ACCEPTABILITY OF PASTEURIZED MILK IN HALF-GALLON CARTONS

Brand number	Quality before warm-up		Treatments			
	SPC/ml	Coliform/ml	A[1]	B[1] (Meals[2] to a flavor score <36)	C[1]	D[1]
				½-hr warm-up		
1	730	<1	>6	>6	>6	>6
2	2,000	<1	>6	>6	>6	>6
3	300	<1	>6	>6	>6	>6
4	1,000,000	177	6	6	5	>6
				1-hr warm-up		
5	2,200	43	>6	>6	>6	>6
6	230,000	<1	6	6	6	>6
7	680	<1	>6	>6	>6	>6
8	100,600	62	>6	>6	6	>6

[1]A. 1 hr at 90 F on AM of 1st day
B. Room temperature for ½ or 1 hr at M and PM of 1st day any AM, M, and PM of 2nd day
C. Combination of C and B
D. Control
[2]Values do not include 4 days storage prior to warm-up

senting milks with poor bacteriological quality, most of the warmed-up samples were acceptable for fewer meals than their controls. Whereas, in the brands 1, 2, 3, and 7 with superior bacteriological quality, differences between the warmed-up and control samples were not noticeable nor did differences appear after 3 days of storage at 40 F (data not shown) following the 2-day warm-up period. Flavor acceptability variances among the three types of warm-up were negligible except for possibly brands 4 and 8. A comparison between the 0.5- and 1-hr warm-ups should not be made since facilities available precluded doing these two-warm-ups at the same time. However, 0.5-hr warm-ups appear as detrimental as 1 hr when poor quality milk is purchased.

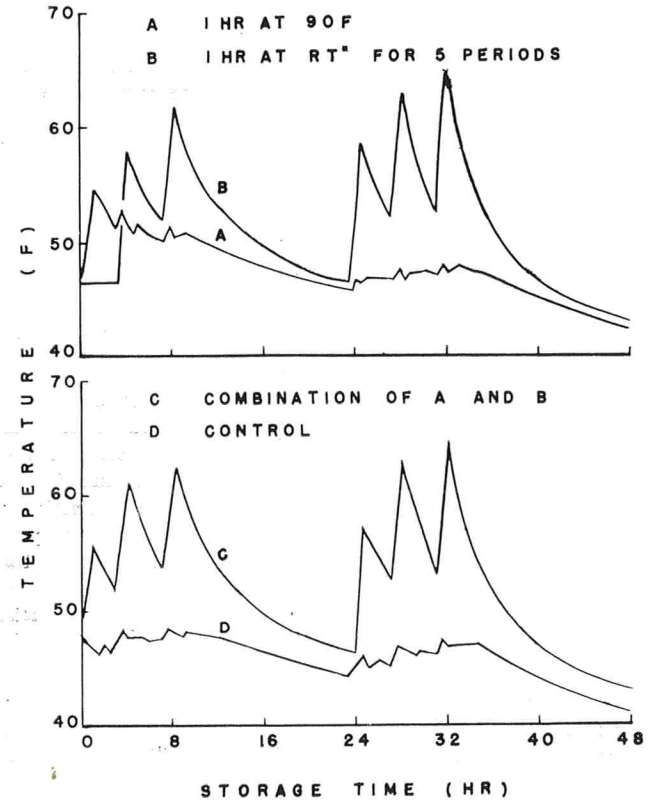

Figure 2. The cooling rate of milk in half-gallon cartons during storage at 40 F following 1 hr warm-ups simulating home conditions (* room temperature).

TABLE 3. EFFECT OF WARM-UPS SIMULATING HOME CONDITIONS ON THE FLAVOR SCORE[1] OF PASTEURIZED MILK IN HALF-GALLON CARTONS

Storage (hr)	Treatments			
	A[2]	B[2]	C[2]	D[2]
		½-hr warm-up		
0	38.5	38.5	38.5	38.5
24	38.1	37.9	38.2	38.1
48	37.1	36.3	36.3	37.5
		1-hr warm-up		
0	39.0	39.0	39.0	39.0
24	38.9	39.0	38.9	38.9
48	37.8	37.0	36.6	38.4

[1]Average of 4 samples.
[2]A. 1 hr at 90 F on AM of 1st day.
B. Room temperature for ½ or 1 hr at M and PM of 1st day and AM, M and PM of 2nd day.
C. Combination of A and B.
D. Control.

The average flavor score of the samples during the 2-day warm-up period which simulated home conditions is presented in Table 3. The flavor scores of the warmed-up samples were lower than those of the controls after storage for 48 hr. The greatest difference from the controls occurred in the samples warmed up for 0.5- or 1-hr periods to simulate home conditions during meals. The predominant factor in the low flavor scores for the warmed up samples after 48 hr of storage (Table 3) was the poor bacteriological quality of brands 4, 6, and 8 (Table 2) at simu-

lated time of purchase.

Poor quality milk at time of purchase will deteriorate more quickly than good quality milk with warm-up by the consumer and thus may result in consumer complaints. Such complaints may be avoided if the original quality of the milk is excellent, even if the consumer is lax in maintaining proper storage temperatures. Efforts should be made to improve the bacteriological quality of both raw and pasteurized milk and thus prolong shelf-life.

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REPORT OF THE COMMITTEE ON DAIRY FARM METHODS: 1971-1972

A. K. SAUNDERS, *Chairman*
A. E. PARKER, *Western Asst. Chairman*
J. B. SMATHERS, *Eastern Asst. Chairman*

In 1971-1972 we have had ten task committees working diligently to present members of the International Association of Milk, Food and Environmental Sanitarians' membership with useful material.

ANTIBIOTICS, PESTICIDES, AND OTHER ADULTERANTS

M. W. JEFFERSON, *Chairman*

Adulteration of milk supplies by antibiotics continues to concern the dairy industry and public health authorities. Control and use of antibiotics varies between areas of the country. Use of antibiotics to treat mastitis is under close supervision when used by a veterinarian, and is usually well supervised by the dairyman when used for such treatment. There have been reports where antibiotics have been used to treat milking dairy animals for problems other than mastitis, which has caused some milk adulteration. This has been particularly true in the treatment of cattle for breeding problems.

Education continues to be a tool which would be emphasized and used, to control and direct proper use of antibiotics. The Task Committee recommends that:

(a) Educational programs be continued and strengthened on use of antibiotics on dairy cattle. Such programs should be directed to the dairyman, practicing veterinarian, extension service personnel, and other people working directly with milk producers.

(b) Use of antibiotics should be carried out as directed by the label, and all antibiotics should be properly labeled, giving clear and precise directions for use. Antibiotics, whether formulated by a veterinarian or by a pharmacist, should carry a clear and precise label as to the product and directions for use.

(c) Controls on a nationwide basis to limit the dose rate of antibiotics, the different types of antibiotics, and their use by dairy farmers.

Pesticides continue to be a problem in certain areas of the country; but indications are that controls placed on use and time of application have decreased the incidence of pesticide contamination in milk supplies. Surveillance programs continue to play an important part in eliminating adulteration of milk supplies by pesticides. Substances identified as polychlorinated biphenyls (PCBs) have been of concern to the dairy industry in many areas of the country. These materials are not classified as a pesticide, but are somewhat similar to DDT. Surveys have been conducted by the Food and Drug Administration and many state regulatory agencies to determine the incidence of PCBs in the milk supplies. Indications are that PCBs have been in our environment for many years, and it will take considerable effort to eliminate PCBs from our environment.

Label directions continue to be very important in the use of pesticides. The Task Committee recommends: *(a)* educational programs concerning the use of pesticides be continued and strengthened; *(b)* directions on labels of all pesticides be plain, simple, and distinct; *(c)* commercial applicators be licensed and controlled; and *(d)* chlorinated hydrocarbon pesticides as well as PCBs be prevented from use where residues cannot be controlled.

Use of automated equipment to clean and sanitize milk contact equipment on dairy farms and in receiving and transfer stations as well as plants increases the possibility of adulteration of milk by sanitizers and detergents. Continued vigilance should be carried out in establishing and maintaining control of the operation of such equipment to prevent adulteration of milk. The Task Committee recommends that research be directed toward development of suitable tests for protection against chemicals in milk supplies.

Excess water in milk continues to exist in most areas of the country. Use of automated equipment to handle milk could contribute to adulteration of milk with excess water. Use of plate and tubular coolers where the cooling medium is at a greater pressure than the milk could cause adulteration if leakage occurred. Indications are that tubular and plate coolers will be used to a much greater extent in the future because of cooling requirements, and use of bulk holding and cooling tanks not designed for present use. Adulteration from excess water, chemicals, and microorganisms is possible unless proper controls are maintained.

It is recommended that: *(a)* serious attention be given to use of tubular and plate coolers on dairy farms where they are used as a partial or complete cooling facility; and *(b)* that the dairy producer be educated as to the use of external type cooling facilities in conjunction with farm bulk milk cooling tanks.

(Continued on Page 171)

COOLING RATES OF FOODS

R. W. Dickerson, Jr. and R. B. Read, Jr.
U. S. Department of Health, Education, and Welfare
Public Health Service, Food and Drug Administration
Bureau of Foods, Cincinnati, Ohio 45226

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Abstract

Rapid cooling is essential to prevent multiplication of microorganisms in potentially hazardous foods. This requirement is frequently not met with viscous foods in large containers. The time required to cool an 8-gal container of white sauce from 105 to 57 F was 25 hr. Similarly, a 14-gal container of beef stew required 84 hr to cool from 115 to 50 F. Under some conditions, cooling times are directly proportional to the square of the shortest dimension of the food sample. For example, if the shortest dimension is doubled, cooling times are increased by a factor of four.

The effect of most plastic containers on cooling rates of foods is generally insignificant. Thermal properties of polyethylene, nylon, and Teflon are similar to thermal properties of foods, and a 1/8-inch-thick container will have about the same effect as an additional 1/8-inch thickness of the food. Polystyrene, however, has a very low thermal conductivity and will significantly delay cooling of most foods.

Bacteria are responsible for 90% of food-borne illnesses *(24)*, and many outbreaks are the result of inadvertent holding of food at improper temperatures for an extended period of time. When hot foods are placed in a refrigerator, temperatures must pass through the range that supports rapid growth of food poisoning bacteria and this should be accomplished as rapidly as possible. For large volumes of foods, rapid cooling depends in part on the cooling capacity of the refrigerator but, to a greater extent, it depends on the geometry and thermal properties of the foods. The objective of this work was to review the effect of geometry and thermal properties on the cooling rates of foods in the refrigerator.

Previous Methods and Recent Developments

For all solid foods and most stews, sauces, custards, and puddings, heat is transferred from the food to the refrigerator by conduction heat transfer through the food, which is a slow process. For example, the time required to cool an 8-gal container of white sauce from 105 to 57 F was 25 hr *(12)*. Because of their poor heat transfer characteristics, these classes of foods have received considerable attention, and the cooling rates of some of these foods during refrigeration have been measured *(12, 13, 17, 18, 19)*.

In analyzing these data, the usual approach is to plot a heat penetration curve (Fig. 1), and the f-value *(2)* obtained from the slope of the curve identifies the cooling rate. With the f-value, temperatures can be predicted for any time during cooling. Much additional information can be obtained, however, if the data are plotted as a function of $\alpha\tau/s^2$ (Fig. 2), where α = thermal diffusivity of the food, τ = time, and s = half-thickness or radius of the food sample *(3, 9)*. By use of Fig. 2, temperatures may be predicted not only as a function of time (as in Fig. 1) but also as a function of geometry, thermal diffusivity of the food, initial temperature of the food, and ambient temperature in the refrigerator. Figure 2 was developed from data in the original article (s = 4.3 inch, T_o = 105 F, T_R = 47 F) *(12)* except for thermal diffusivity, which was not available. When data are lacking for foods of high water content, thermal diffusivity may be taken as 10% below the value for water at a comparable temperature *(7)*. This yields

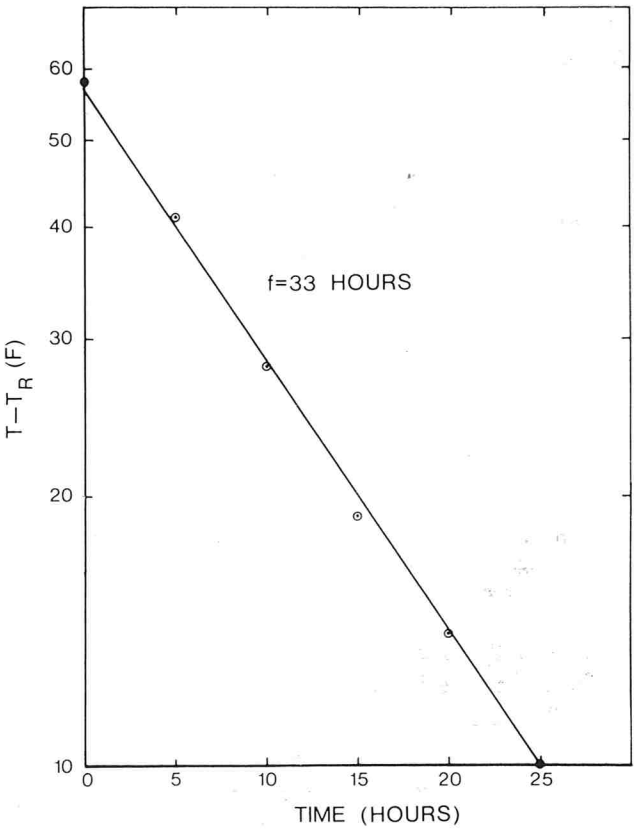

Figure 1. Cooling 8 gal of white sauce in a refrigerator. From Longree and White *(12)*. The heat penetration curve.

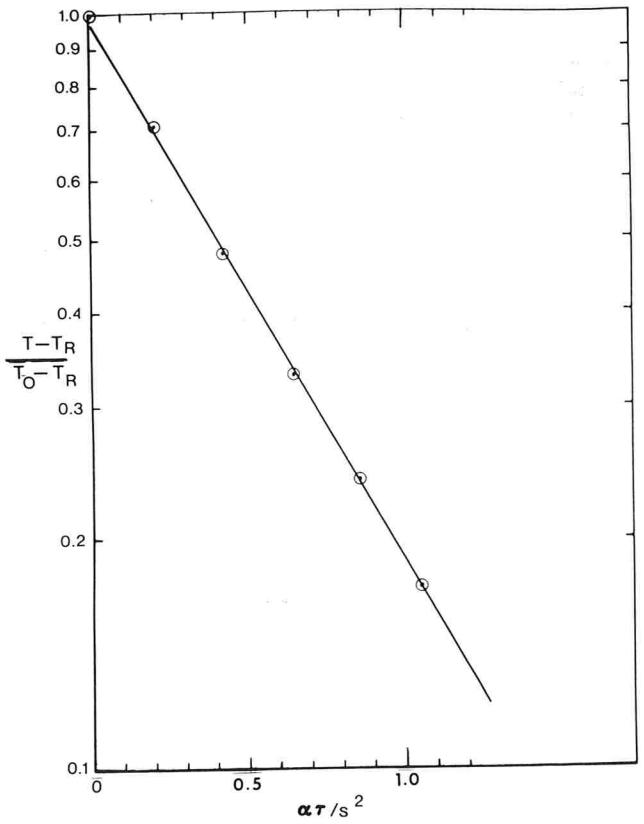

Figure 2. Cooling 8 gal of white sauce in a refrigerator. From Longree and White *(12)*. Cooling rate shown as a function of $\alpha\,\tau/s^2$.

a value of 0.013 inch²/min.

The technique is not new; it was first developed in 1878 *(8)*. Thompson *(23)* recommended it for foods in 1919, but the technique was not used because the state of the art would not permit reliable measurements of thermal diffusivity; values were in error by as much as 100% between replicate tests *(23)*. Reliable methods of measuring thermal properties of foods are now available *(5, 6, 11, 15, 20)*, and tabulations of thermal properties have been published *(7)*. A recent review of thermal conductivity of foods is also available *(21)*. This has led to an increased use of the term $\alpha\,\tau/s^2$ in correlating heat transfer data and predicting cooling times of foods *(4, 6, 22)*. It shows that cooling times are a function of both geometry (s^2) and thermal properties (α) of the food.

Cooling times are also a function of environmental conditions at the food surface. A refrigerator with an internal fan will cool foods faster than one without a fan. Furthermore, there is a complicated relationship between geometry, thermal properties, and environmental conditions at the food surface. A technique is available to predict cooling rates of foods, however, it is somewhat specialized and requires a background in heat transfer and mathematics.

The need for the specialized knowledge can be eliminated by neglecting the relationship of environmental conditions at the food surface with geometry and thermal properties. Of course, accuracy is also sacrificed, but a rough approximation of cooling times is an improvement over the information now available to the Health Officer. The reader interested in the more precise and detailed technique of predicting heat transfer rates is referred to the applicable references *(3, 4, 6, 9, 22)*.

GEOMETRY

When the relationship between food geometry and environmental conditions at the food surface is neglected $\alpha\,\tau/s^2$ has constant value for a given level of cooling. If the food of Fig. 2 is cooled from 140 to 50 F in a refrigerator at 32 F, then $(T - T_R)/(T_O - T_R) = (50\text{-}32)\,(140\text{-}32) = 0.17$, and $\alpha\,\tau/s^2 = 1.05$. Therefore, for two different tests in the same refrigerator, with the same air flow and the same degree of loading the refrigerator,

$$\alpha_2\ \tau_2/s_2^2 = \alpha_1\ \tau_1/s_1^2 \qquad (1)$$

where subscripts 1 and 2 refer to the two different tests. For the same food, $\alpha_2 = \alpha_1$ and equation 1 reduces to

$$\tau_2 = \tau_1\ (s_2/s_1)^2 \qquad (2)$$

For rectangular samples, cooling times vary as half-thickness squared. For cylindrical and spherical samples, cooling times vary as radius squared. If the radius of a sample is doubled, the cooling time (140 to 50 F) will be approximately 4 times greater. If the radius of the sample is tripled, the cooling time will be approximately 9 times greater.

The same geometry ratios apply to any level of cooling. If the time required to cool a sample of 4-inch radius from 115 to 50 F is 5 hr, the time required to cool the same sample of 12-inch radius, through the same temperature zone would be approximately 45 hr. To use equation 2, it is necessary to know the cooling time, τ, for one heat transfer condition, and the cooling times of different geometries of foods are estimated for the same heat transfer condition.

In using equation 2, *the dimension* s *must be the shortest dimension* of the food sample. If a sample is 4 inches thick and 12 inches in radius, doubling the radius will have no effect on cooling time; the 4-inch dimension is the controlling factor. Regardless of the size or configuration of a food sample, the shortest dimension between surface and center governs heat transfer rates. A 4 × 8 × 12-inch tray of chicken a la king has a cooling rate that is within 5% of the rate for a 4-inch plate infinitely wide and long *(6)*.

Equation 2 is free of error only when the original assumption is operative; that is, the relationship be-

tween food geometry and environmental conditions at the food surface is negligible. In steam autoclaves and vigorously agitated water baths, the condition is satisfied and results from equation 2 are accurate. For most refrigerators, results from equation 2 are useful approximations, and the following example illustrates the magnitude of error.

Assume the time required to cool a sample of food of 4-inch radius from 140 to 50 F is known to be 33 hr. Using equation 2 the predicted cooling time for a food sample of 8-inch radius is 132 hr. Using the more detailed technique *(6)* which accounts for the relationship between food geometry and environmental conditions at the food surface, the predicted cooling time is 94 hr. In this instance, the error involved in the use of equation 2 is 40%.

The cooling time for the white sauce of Fig. 1 was 25 hr, during which a significant amount of bacterial growth occurred; bacterial counts increased from 100/ml at the start of cooling to 10^8/ml during the first 6 hr of cooling *(12)*, demonstrating a significant hazard to consumer health. Furthermore, the 8-gal batches used by Longree and White *(12)* are not considered large; 15-gal containers are common.

Since use of equation 2 on these larger containers yielded a predicted cooling time in excess of 100 hr, we measured the cooling time of a 15-gal container filled with 14 gal of beef stew.

The stainless-steel container was 16 inches in diameter, 18 inches high, and filled to a depth of 16 inches with beef stew. The recipe was 36 lb of stew beef, 12 lb of carrots, 8 medium Spanish onions, 20 lb of potatoes, 72 oz of green peas, 4 oz of beef flavor soup base, 1 qt of cream corn starch, 6 lb, 9 oz of tomato puree, 35 oz of flour, 2-3/4 lb of instant potatoes, and 24.5 qt of water. One thermocouple was placed at the geometric center of the food, and 8 additional thermocouples were placed radially 1 inch apart between the center and the container wall. With the food at 150 F, the container was placed in a walk-in refrigerator held at 40 F. Ambient air temperature in the refrigerator was measured with a thermocouple and was 40 F during the test, indicating the refrigerator had ample capacity for the load.

The time required to cool the center of the food through the temperature range of 115 to 50 F was 84 hr (Fig. 3). From a practical standpoint, a container of food is not left in the refrigerator that long; routine storage would be about 24 hr. During the first 24 hr, the central temperature remained above 120 F; this leads to the observation that, for large containers, the hazardous location occurs not at the center, but near the container wall for the first 24 hr, where temperatures dropped from 150 F to 115 F

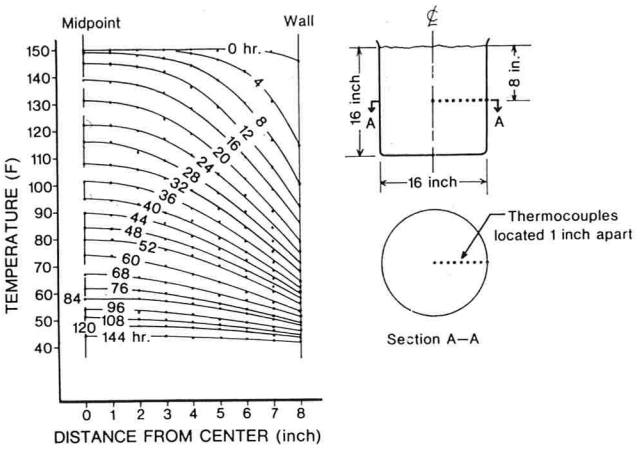

Figure 3. Radial temperature profiles during refrigeration of a container filled with 14 gal of beef stew.

during the first 4 hr and remained between 115 F and 75 F for the remaining 20-hr period. Consequently, warm viscous and solid foods refrigerated in large containers are potentially hazardous to consumer health.

Continuous agitation reduces cooling times by a factor of 3 *(13)* and is effective for containers up to a capacity of about 6 gal. For the large container (Fig. 3), however, agitation is not effective. The factor of 3 improvement from Longree et al. *(13)* would reduce the cooling time from 84 to 28 hr; however, 28 hr also is unacceptable. Furthermore, some foods are damaged by mechanical stirring. When agitation cannot be used Longree et al. *(13)* recommend cooling in shallow pans.

Thermal Properties of Foods

For heating and cooling of foods, the important thermal properties are thermal conductivity, k; density, ρ; and specific heat, C_P. They can be combined into a single relation

$$\alpha = 2.4\ k/\rho\ C_P \qquad (3)$$

where 2.4 is the factor required to convert α from ft²/hr to inch²/min. Thermal diffusivity, α, is a measure of the quantity of heat absorbed ($\rho\ C_P$) by a material for a given temperature change, and further indicates the ability of the material to conduct heat (k) to adjacent molecules.

Like geometry, thermal diffusivity of the food also has an effect on cooling time. When the relationship between thermal properties and environmental conditions at the food surface is negligible, and geometry is identical, equation 1 reduces to

$$\tau_2 = \tau_1\ (\alpha_1/\alpha_2) \qquad (4)$$

For equal geometries, cooling times vary inversely as thermal diffusivity. For a given heat transfer test, if the food is changed to one with a thermal diffusi-

vity one-half that of the previous sample, cooling times will be increased by a factor of 2. To use equation 4, the cooling time, τ, must be known for one heat transfer condition, and the cooling times for different samples of foods are obtained for the same heat transfer condition. Equation 4 is subject to the same restriction as equation 2 and yields only an approximation of cooling time. Use of equation 4 can be demonstrated by considering the problem of storing foods in plastic containers.

Most plastic containers are insulators of heat and the question arises whether these containers will delay the cooling of foods. Thermal properties of some foods and containers are shown in Table 1. Thermal diffusivities of stainless steel and glass are significantly greater than those of foods, and their cooling times (from equation 4) are much shorter than those of foods. Consequently, these two materials will not delay the cooling of foods. Thermal diffusivities of polyethylene, nylon, and polytetraflouroethylene (Teflon)[a] are considerably lower than those of glass or stainless steel; however, they are about the same as that of potato salad. A container fabricated from 1/8-inch-thick polyethylene will have the same effect on cooling rates as an additional 1/8-inch thickness of the food. For conventional container thicknesses of about 1/8 inch, nylon, polyethylene, and Teflon will not significantly delay the cooling of foods.

Thermal diffusivity of polystyrene insulation is 15 times greater than that of foods, and will cool 15 times faster than food of a similar size (equation 4). This does not mean, however, that polystyrene is a better conductor of heat. Conversely, the polystyrene insulation will conduct heat only 1/12 as fast as beef (see thermal conductivities in Table 1). This lower thermal conductivity, however, is compensated by a much lower heat-absorbing capacity. The heat absorbing capacity (ρ C_P) of polystyrene is almost 200 times less than beef; therefore, for a given temperature decrease, the heat to be removed per ft^3 of polystyrene is only 1/200 of that required for beef.

This leads to the conclusion that very light foods consisting chiefly of air, such as meringue, will cool much faster than ordinary heavier foods. Even if the meringue is placed in a polystyrene insulating container, it will not significantly increase cooling times because both the container and the food have about the same thermal diffusivity and thermal conductivity. The only effect will be the increased geometry (equation 2) caused by the container.

When a heavy food such as potato salad is placed in a polystyrene-insulated container, a different situation exists, and cooling times will be increased significantly. Because of its large heat-absorbing capacity, the potato salad must release a large amount of heat for a given amount of cooling, and all of the heat must pass through the insulated container which restricts the large flow of heat by its low thermal conductivity (Table 1).

Therefore, polystyrene containers will not affect cooling rates whenever thermal diffusivity of the polystyrene is greater than that of the food; *provided* thermal conductivity of the polystyrene is not significantly less than that of the food. This condition is met for very light foods consisting chiefly of air. When thermal conductivity of the polystyrene is significantly less than that of the food, as it is for most foods, polystyrene will delay the cooling of the food.

SUMMARY

Of all the factors influencing cooling rates of foods, geometry of the food sample has the most pronounced effect. For foods stored in bulk, the food itself is responsible for the delay in cooling the innermost portions of the food. Equations 2 and 4 yield reasonable estimates of cooling time. Clearly, they are not intended for precision work, but they are useful tools in evaluating field procedures for refrigerating and storing foods.

function of α τ/s^2.

[a]Mention of company or product names implies neither endorsement nor criticism by the Food and Drug Administration.

TABLE 1. THERMAL PROPERTIES OF SOME FOODS AND CONTAINER MATERIALS BETWEEN 32 AND 90 F

Food or material	Thermal diffusivity	Thermal conductivity	Apparent density	Specific heat	Reference
	($inch^2$/min)	(Btu/hr ft F)	(Lb/ft^3)	(Btu/lb F)	
Stainless steel	0.38	9.2	490	0.12	*(14)*
Glass (borosilicate)	0.056	0.65	140	0.20	*(10)*
Polyethylene (low density)	0.014	0.19	57	0.55	*(16)*
Nylon	0.012	0.14	68	0.40	*(16)*
Polytetraflouroethylene (Teflon)	0.010	0.15	140	0.25	*(16)*
Potato salad	0.013	0.28	63	0.79	*(6)*
Beef, lean sirloin	0.012	0.29	72	0.84	*(7)*
Cod fish	0.013	0.31	62	0.88	*(7)*
Polystyrene insulation	0.11	0.024	1.6	0.32	*(1, 16)*
Water	0.014	0.34	62.4	1.00	*(7)*

NOMENCLATURE

C_p = specific heat, Btu/lb F.
f = slope of the heat penetration curve, hour.
k = thermal conductivity, Btu/hr ft F.
s = characteristic dimension, radius of the sphere, radius of the cylinder, half-thickness of the plate, inch.
T = temperature, F.
T_o = temperature of food before start of refrigeration, F.
T_R = ambient temperature in refrigerator, F.
α = thermal diffusivity, inch^2/min.
τ = time, min.
ρ = apparent density, lb/ft^3.

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REPORT OF COMMITTEE ON DAIRY FARM METHODS 1971-1972

(Continued from Page 166)

The Task Committee feels that clarification is needed among Federal agencies in responsibility for pesticides, antibiotics, and other adulterants of milk. Changes have taken place on the Federal level which have eliminated certain programs within one department and placed similar responsibility in other departments. The pesticide program, which formerly was a part of the U. S. Department of Agriculture and the Food and Drug Administration, is now supervised by the Environmental Protection Agency. The water supply and pollution program which existed in the Department of Interior is now a program in the Environmental Protection Agency. Waste problems which were in the U.S. Public Health Service are now in the Environmental Protection Agency. In view of these changes it is recommended that the Farm Methods Committee consider including someone from the Environmental Protection Agency as a member.

CLEANING AND SANITIZING OF FARM MILK EQUIPMENT

JAMES WELCH, *Chairman*

This Task Committee finds many changes taking place on our present dairy farms. As an example, we all see some dairy farms using larger milk lines (3 inches), pre-coolers, and dairy plant type storage tanks. Many of these installations resemble a small dairy plant. Therefore, the committee feels that the following items need further evaluation and consideration: *(a)* study by pipeline manufacturers regarding gallonage requirements, *(b)* when air injection systems are used, slugs per minute requirements should be established, *(c)* establish criteria for water heating capacities, and *(d)* maintenance of water temperature during wash cycles.

The following are the recommendations of this committee:

A. *Temperature recording devices*:

1. When enclosed tanks (1,000 gallons or over) are to be cleaned in place, a temperature recording device should be installed.

2. Pipelines over 2 inches in size are being cleaned in con-

junction with a pre-cooler—consideration should be given to the installation of a temperature recorder.

3. Consideration should be given to require fittings in the system to accommodate easy installation of recording devices for either a checking device or a permanent installation.

B. Automatic cleaning equipment

1. When pre-rinses are programmed into an automatic cleaning device, initial rinse temperature should be no higher than tepid.

2. A complete education program should be conducted at time of installation relative to the operation of the unit.

3. Establish a preventive maintenance program for automatic equipment.

4. Intensify requirements for water supplies to be of adequate volume and pressure.

C. Vacuum systems

1. The vacuum pump or reservoir be installed in a location so the operator has easy access for maintenance. This includes servicing the unit, inspection, and cleaning. Area should have floor drain facilities.

EDUCATION

VERNON D. NICKEL, *Chairman*

The Educational Task Committee has the continual assignment of gathering new material which is sent to Dr. Elmer H. Marth, Editor of the *Journal of Milk and Food Technology,* for publication.

During the past year new material has been listed and its source has been published in the *Journal.* Additional material has been evaluated for publication.

The Task Committee is in the process of adding slide collection information on various phases of the dairy farm operations to its Committee function. Many valuable slide collections are available. The cost and availability of these slides will be furnished for publication in the *Journal.*

PLASTICS

BERNARD M. SAFFIAN, *Chairman*

In 1972, a survey on replacements for polyphosphates was continued with particular emphasis on effects that polyphosphate replacement would have on plastics used on dairy farms.

If polyphosphates are replaced by more alkaline materials and/or materials which require higher cleaning temperature, the life and efficient use of plastic components will be reduced. Polyethylene and polypropylene are semi-rigid, acrylic and polycarbonate are rigid, and polyvinyl chloride is generally used as a flexible material. These are the most commonly used plastics in dairy farm equipment, with a minor amount of nylon and Teflon used as seals and gaskets.

Polyethylene, acrylic, and polycarbonate stress-crack more readily as alkalinity and/or temperature increase. Polypropylene is quite resistant to stress-cracking. Flexible polyvinyl chloride hardens at the exposed surface and will eventually stress-crack with increasing alkalinity (pH). Increased temperature hastens stress-cracking, accelerates temporary opacity or "fogging", and causes temporary softening and permanent distortion at bends and fittings. Use of a warm air dryer prevents the temporary water "fogging."

Higher cleaning temperature generally causes more problems (fogging and hardening) with plastics than does higher alkalinity (hardening only). If phosphate replacements were developed, their efficient use could possibly require relatively low cleaning temperatures or such materials could cause increased damage to some plastics if higher temperatures were needed. Therefore, a survey of state regulations was made to determine whether cleaning temperatures and/or times are specified which may restrict changes in cleaner chemistry.

In the survey of 50 states plus Puerto Rico and the Virgin Islands, we received a 90% response to our inquiries. Only one state had specific regulations over and above the *Grade A Pasteurized Milk Ordinance 1965 Recommendations* of the United States Public Health Service. This regulation requires that the cleaner be circulated at 130-140 F for at least 15 min. Four states made recommendations, but these are not regulations. The balance of the states follow the USPHS recommendations which do not specify cleaning conditions, only that the system be cleaned after every milking and the milk product must meet bacteria count limits.

Many replies mentioned that criteria are not any specific set of cleaning conditions, but more importantly the quality of the milk product regardless of cleaning procedures used. Several replies stated that it would not be wise to specify cleaning conditions because these would vary depending on milking equipment and water conditions. So it appears that possible modification of cleaners with questionable need of temperature—time changes, will not be restricted by present state regulations.

The following questions were asked of leading dairy cleaner manufacturers:

(a) Has a replacement for polyphosphates been developed? *Answer*: One company reports that a material has been developed and is available. Others have experimental products undergoing field testing in which much lower concentrations of polyphosphates are present or polyphosphate is not used at all.

(b) Will replacement for polyphosphates require a higher or lower temperature and/or time of cleaning? *Answer*: Normally the same time and temperatures presently used will be sufficient.

(c) Will modified cleaners increase cleaning cost and for what reason?
Answers: There are indications that cost will be increased 15-30% because of raw materials. Also, it is possible that water conditioners would be required on most farms and it may be necessary to use acid rinses. At present, there are areas with water conditions that do not require acid rinse.

(d) What is the current state or present or proposed legislation regarding use of polyphosphates in cleaners?
Answers: Some current and much pending legislation is limiting the concentration of polyphosphates. However, it appears that detergents for use in machine dishwashers, dairy equipment, beverage equipment, food processing equipment, or industrial cleaning equipment may be exempt.

It appears that banning or severely limiting the concentration of polyphosphates in cleaners for many applications could create a public health hazard because of decreased cleaning ability.

Several questions have been asked in the past in regard to items pertinent to plastics in past reports. These are covered in the following issues of the *Journal of Milk and Food Technology*: 1965-66 - Vol. 30, No. 1; 1966-67 - Vol. 31, No. 1, No. 2, No. 3; 1967-68 - Vol. 32, No. 7; and 1968-69 - Vol. 33, No. 5.

TESTING FOR CLEANLINESS OF MILK PRODUCTION

M. H. ROMAN, *Chairman*

The previous report of this task committee included re-

commendations on: *(a)* significance of the mixed sample sediment test, *(b)* procedures for clean milk production, *(c)* adoption of sediment testing as one of the monitoring tests for cleanliness of milk production, and *(d)* use of a producer's sediment record as means for selecting dairy farms where milking time inspections and sediment tests should be conducted.

Since the previous report, a study was undertaken on a national basis to determine the incidence of unclean milk. Reports were received from five task committee members. Results are as follows:

Acceptable	78.2%	$-<$ 1.5 mg/gal. basis
Probationary	15.2	-1.5 to $<$ 3.0 mg
Unsatisfactory	6.6	$-<$ 2.5 or 3.0 mg

The study showed that there is a need for a uniform classification system in rating sediment tests for degree of cleanliness. Some areas use No. 1, 2, 3; others use No. 1, 2, 3, 4; and other use terms such as 1.5 mg, 3.0 mg etc. per gallon basis. Some note 2.5 mg and others 3.0 mg as reject grade. Further, it was reported that milk cleanliness testing is not compulsory nor is it being practiced in some areas.

A 4 oz. milk sample tested through a filter area 0.2-inch in diameter maintains the standard volume-area relationship. With the advent of the 4 oz. universal milk sample procured at each farm bulk milk collection, an ad-hoc committee made a study to determine if 0.2-inch diameter tests could be uniformly graded with a photo standard of this same size. Such a photo standard was prepared using two sediment levels, one being at a probationary 1.5 mg level and the other at an unsatisfactory or 3.0 mg level. A group of 0.2-inch sediment tests in a relatively narrow sediment intensity range were selected for grading. Seven graders rated the sediment tests independently using the photo standard. Gradings were alike in 223 out of 273 ratings or an 82% agreement in the gradings. From this study, it can be concluded that: *(a)* The 4 oz. - 0.2-inch diameter test has merit in determining care used by the milk producer and thus the 4 oz. universal sample can be used in screening the producer's milk for cleanliness. *(b)* Grading a large number of disks is made easier and more uniform by arranging the group of disks in either ascending or descending order of sediment intensity as visually observed. Following this arrangement, the grader has only to determine the cut-off point for each sediment grade group.

RECOMMENDED PROCEDURES FOR THE WELDING OF STAINLESS STEEL MILK LINES ON DAIRY FARMS

KENNETH HARRINGTON, *Chairman*

This report is the consensus of this task committee, and is submitted as guidelines for "Recommended Procedures for the Welding of Stainless Steel Milk Lines on Dairy Farms."

I. Welder-installer recommendations

A. Farm pipeline welder-installers should be approved by official regulatory agencies.

B. Farm pipeline welder-installer's name and address should be submitted as part of the CIP installation application. However, it is not recommended that the installation be started until tentative approval of the application has been received.

C. It is recommended that the welder-installer notify the enforcement agency when the installation is to be started.

D. The farm pipeline installer should certify to the producer upon completion of the installation, in writing, that the installation meets sanitation standards and 3-A Standards.

II. Regulatory agency inspection proceedings

A sanitarian need not be present when the installation is made, but the installer should make a sample weld at the start of each day's operation to be sure the welding device is operating properly. These welds should be cut off and labeled as to the approximate location of the welds for that particular day's operation. These samples should be tied together and left at site of installation for the supervising sanitarian's inspection.

III. Machine or hand welding

Both are satisfactory methods of installation. The entire milk line is to be welded; however, an optional standard milk line union could be installed in the milk house to provide quick disassembly for inspection. There should be no restrictions in the pipeline which will impede the velocity. The receiving receptacle should not be welded into the pipeline and the milk pump should be so mounted that it can be easily inspected.

IV. Unsatisfactory installation

Unsatisfactory installation from the standpoint of appearance as well as noncompliance with 3-A Standards and sanitation standards should be cut out and corrected by the installer. It is further recommended that the installation be inspected as soon as possible after completion by the supervising sanitarian.

V. The following are considered unacceptable workmanship:

A. Grinding of welds
B. Welds sagging
C. Pin holes
D. Scorching or discoloration
E. Welds showing ridges which would impede or restrict solution flow.

VI. Borescope examination

Most sanitarians feel that a Borescope examination is too costly and should not be necessary if the above procedures are enforced. Its use should be optional as a tool for regulatory officials.

CLEANING AND SANITIZING OF FARM PICKUP TANKERS

BOYD M. COOK, *Chairman*

The farm bulk pickup tanker, the backbone of the milk collection system in the United States, is the vital link in the quality chain from producer to processor. This tank must be cleaned, sanitized, and maintained in a condition where it cannot cause any product deterioration during pumping or transporting. The responsibility for these actions must be clearly defined to reach this objective.

The Task Committee attempted to determine the preferred procedures and methods to clean and sanitize the pickup tanker. The driver - receiver has the direct responsibility to be certain the pump, tank, and other milk contact surfaces are cleaned and sanitized before pumping milk into the truck. The Task Committee is aware that numerous opportunities for failure are present due to use of relief drivers, deliveries at various times of day and night, and various cleaning facilities provided at different dairies. Sanitarians have found that the following procedures have worked.

I. Driver responsibility before loading

A. The driver, regardless of who drove the truck the day before, must be certain the tank interior, manhole gaskets, pump, hose, and valve are cleaned before departure for pickup. He must also determine that the tanker has been sanitized before departure.

B. If the unit is found to be deficient in cleaning, remedial action should be taken to clean and sanitize the unit prior to loading.

C. At times, spare units are not used for several days. Before use the unit should be checked for cleanliness and odor. If found to be clean and odor-free, the unit should be sanitized before use. If it has been out of use for over 2 days, it should be rewashed and sanitized before use.

D. To aid in consumer acceptance of our industry, the driver should keep the outside of the unit as clean as practical at all times. Routine washing is a must.

II. Responsibility of driver and/or plant personnel following receipt of the load

A. The Task Committee finds that most successful programs clearly define the responsibility for the cleanliness of the tank interior, manhole area, pump, hose, and pump compartments. Plant personnel normally are assigned responsibility for the interior of the tank and the manhole area including gaskets, breathers, and lids. (1) Plant personnel may clean the interior of the tank by hand washing or CIP cleaning with spray heads, nozzles, or tear drop heads inserted through the manholes or CIP ports installed in the tank. Care must be given to proper sizing of the pumps, solution volumes, and piping to do an adequate cleaning job on tanks of various sizes and dimensions. Built-in nozzles or spray heads in the tankers should be installed to assure proper distribution of the cleaning solution within each tank. (2) Plant personnel may be required to hand wash the CIP ports, manhole covers, gaskets, and other manhole accessories. (3) The health authority should establish a tagging procedure calling for the plant employee to sign that he has cleaned and sanitized his area of the truck.

B. The driver operator should be required to be certain the pump compartment, hoses, and pump are cleaned and sanitized following every delivery. (1) Proper wrenches, brushes, mat, or soft container necessary to disassemble and wash the pump, valves, hoses, and other accessories should be provided by the trucker so that he can be assured that they are available at each delivery location. The mat or soft container is to hold the parts of the pump to prevent denting, scarring or other damages to the parts. (2) It is suggested that the plant should provide a method of cleaning the pump hose by recirculation within a tank containing a hot cleaning solution of proper strength. This method of cleaning assures internal and external cleanliness of the hose and tends to increase the life of the hose. (3) Following cleaning, the pump parts should be assembled and sanitized by spray or circulation of a sanitizing solution. The responsibility for seeing that this is accomplished rests with the driver even though the requirement to do the work in some markets may be assigned to plant personnel. (4) The driver should be required to sign the truck cleaning tag indicating that he has cleaned the truck and equipment in accordance with local regulation.

III. Responsibility of the dairy plant reload station, transfer station, or receiving station

A. The station should furnish the necessary hot water, detergents, sanitizers, and facilities to allow plant personnel and/or drivers to accomplish proper cleaning and sanitizing of all tankers.

B. The station should establish a routine quality control check to assure that the cleaning solutions are in compliance with the manufacturer's recommendations and that plant personnel and/or drivers are completing the required work. Recording thermometers on CIP lines can be used to compare cleaning cycles to the load receiving reports as a means of determining compliance with plant cleaning procedures. Routine laboratory checks of detergent and sanitizer strength should be a requirement.

IV. Responsibilities of the health authority

A. The health authorities should meet with local plant personnel, operators, and haulers to establish equipment criteria and procedures that will minimize the problems in the market, such as: (1) Standardizing CIP systems in the market area to be compatible with all truck units in the market and adequate to handle the largest units in operation (2) Establish location requirements for truck CIP ports so that the equipment at various plants will properly wash all truck units. (3) Establish a spot check procedure to determine compliance by all personnel involved. (4) Meet with plant personnel and truck operators to amend procedures to meet new problems with minimum inconvenience and expense to the industry. (5) Work with all segments of the industry to establish solution temperatures, solution concentrations, and facility requirements to make the best use of today's technology.

The Task Committee recommends that CIP cleaning systems include the following: *(a)* valve sizes should be standardized, small valves restrict solution flow; *(b)* proper size CIP pumps for adequate coverage of tanker walls with cleaning solution, manufacturer's recommendations should be followed to insure proper volumes and pressures in the cleaning system; *(c)* initial rinse water should be discarded down the drain; *(d)* proper positioning of the spray ball or tear drop spray head within the tank is critical for complete coverage; *(e)* burst wash and rinse cycles may be needed to allow the return pump to keep the flooding of the long tankers to a minimum.

SAMPLING OF BULK TANK MILK

HELENE UHLMAN, *Chairman*

The Task Committee on Sampling of Bulk Tank Milk has the responsibility of insuring that there is no breakdown in the handling of Grade A milk at a point where it can be most vulnerable. This interim report contains pertinent recommendations needed now; and will be finalized at the next IAMFES conference.

Length of agitation time is, in the consensus of the committee, a subject that needs priority in recommendations. Since tank sizes, agitators, and speeds are such variables, each tank should carry on the Identification Plate and the calibration chart, the recommended agitation time for a homogeneous sample. Length of time the milk has been quiescent and level of the milk in the tank are important variables to be considered.

Additional recommendations of the task committee are: *(a)* State Regulations on time of agitation should be reviewed as to their adequacy for modern tanks now being installed. *(b)* Bulk tank manufacturers should design tanks and provide supporting data to assure a homogeneous sample. A two speed agitator might be helpful. This includes a slow speed for the cooling process and higher speed for sampling with a timer on the higher speed. *(c)* Interval times for agitators are recommended on farm tanks for quality control and the obtaining of homogeneous samples. *(d)* Study is needed on agitation and sampling of milk in over-the-road tankers. *(e)* Study is needed on inspection and sampling facilities for silo and other large capacity tanks. *(f)* Manufacturers should specify foundation requirements to support large capacity tanks to avoid movement of the tank. *(g)* Sample carrying cases on tank trucks should be considered for a field type of study. The study should result in recommendations for de-

sign and materials for temperature control and durability.

Over-the-road tankers with long hauling distances involved is another area that should be studied. A split-sample might be considered. This sample to be taken by a licensed sampler at the time of tank origin and sealing; maintaining high quality control on the cooling phase during transit, and sending sample along with the tanker to its destination.

It is the consensus of the Task Committee that maintaining close liaison with interested groups such as USPHS, IMS Laboratory committee, industry representatives, and regulatory personnel will insure success in reaching our goal of the highest quality control of Grade A milk.

WATER TREATMENT AND PROTECTION

DALE TERMUNDE, *Chairman*

Water treatment and protection are becoming increasingly important to the dairy industry. The continued trend toward consolidation of dairying is closely correlated with installation of highly complex, automated CIP dairy farm equipment with additional demands for consistently high quality water.

A review of water treatment and protection procedures, as prepared by this task committee, is furnished as suggested guidelines to help improve total water quality standards for dairy farm usage.

Bacteriological testing of dairy farm water supplies is established in the U. S. Public Health Service publication #229, *Grade "A" Pasteurized Milk Ordinance, 1965 Recommendations,* requiring such testing for new permits or upon any repair of a water supply. Additional bacteriological testing of water supplies at a specific designated time should be considered.

Closely related to the problem of bacterial contamination of the water supply is the presence of water impurities, i.e., chemical contamination. Knowledge of pH, degree of hardness, total mineral content, and similar factors is necessary to determine proper germicidal treatment; is essential for specialized conditioning of the water to provide efficient and economical cleaning and compatibility with sanitizers, and is needed to reduce corrosion and to improve taste and odor.

Metering devices should supply water without having a submerged inlet. An air space between the inlet and level of tank overflow has proven the most satisfactory. If an air space is not practical, an approved anti-siphon valve should be installed to insure against cross contamination of water supplies.

Removal of hardness minerals through the ion exchange process (water conditioning) can improve the effectiveness of detergents and bactericides by eliminating mineral precipitation. Water conditioning equipment should be thoroughly disinfected before use to insure against contamination of the water supply.

Iron filtration may be feasible in water supplies with high iron content (over 3 ppm) to eliminate the oxidation of iron caused by hypochlorite sanitizing solutions. Iron filtration units should be disinfected before use to insure against contamination of the water supply.

Neutralization of acid water supplies (below pH 6.5) with chemical feed pumps or neutralizing filters has proven beneficial in reducing detergent consumption and increasing the life expectancy of water piping and plumbing fixtures.

Sand filtration is important to eliminate clogging of water inlet screens, for effective cleaning ability of equipment, and to prevent the abrasive action on milk contact surfaces. Coarse sand removal may normally be accomplished through the centrifugal process. However, colloidal sand particles require cartridge or media filtration.

The most common method of controlling bacterial contamination of farm water supplies is chlorination. Many factors must be considered if this system is to operate successfully: *(a)* Chlorine demand in raw water supplies may vary greatly, resulting in increased levels of chlorine residual which may be objectionable to taste and odor. *(b)* Sufficient contact time is necessary for maximum killing efficiency. The capacity of retention tank is important. *(c)* Chlorine residual must be checked and equipment inspected and serviced on a regular basis. *(d)* Highly alkaline and extremely cold water supplies may require increased levels of chlorine residual. *(e)* Hard water supplies may require additional maintenance to prevent clogging of injection fittings and check valves.

Super chlorination followed by dechlorination (on drinking water) is the most widely recommended on farm use treatment. Additional research is suggested to determine if iodine residuals may prove practical for germicidal treatment of water supplies.

Ultra-violet sterilization of farm water supplies is gaining in consumer acceptance. However, there are many limiting factors determining its total effectiveness. The USPHS has written criteria for specific installation and operation. Turbidity, iron content, and the water color are important factors in determining effectiveness of this unit. Periodic disinfection of the water distribution system may be required, since ultra-violet treated systems have no residual action.

Present pasteurization systems for bacterially contaminated water supplies have proven uneconomical for on-farm application.

High quality water, available in quantity, has become a requirement for installation of highly automated CIP dairy farm equipment. Water treatment and protection of farm water supplies, therefore, greatly increases in importance.

The National Academy of Sciences is presently preparing a report, *Quality Criteria for Water To Be Used For Agricultural Purposes.* This report is to be presented to the Environmental Protection Agency late in 1972. Additional research to better define the relationship between quality water supplies and milk supplies is required so that this new information may be brought to the attention of the dairy industry.

The Task Committee on Water Treatment and Protection further suggests that additional reference material be reviewed by interested members.

1. *Water Quality Criteria,* 1968, Report of the National Technical Advisory Committee to the Secretary of the Interior—Federal Water Pollution Control Administration, Section 4, *Agricultural Uses.*

2. *Manual of Individual Water Supply Systems,* U. S. Public Health Service publication #24.

3. *Water Supply and Plumbing Cross Connection,* U. S. Department of Health, Education and Welfare, Public Health Service.

4. *Rural Water Supplies, circular* #145, University of Vermont.

5. *Evaluating Water Treatment Methods,* H. V. Atherton, University of Vermont.

ANIMAL WASTE DISPOSAL

RUSSEL LOCK, *Chairman*

The Animal Waste Disposal Task Committee this year was mainly involved in accumulating information and attempting to ascertain our role in the animal waste disposal problem. Through assistance from the National Milk Producer's Federation, information was distributed to the task committee on: (a) Model State Feedlot Environmental Control Act,

(b) questionnaires from the Environmental Protection Agency, (c) Uniform Terminology for Rural Waste Management, and (d) Copies of all available State Regulations.

It is obvious in reviewing current and pending state and Federal legislation that the governmental agencies charged with the responsibility for regulating animal waste disposal will not be the regulatory officials who administer the pasteurized milk ordinances. Pollution control boards and water quality commissions are being created and given the authority for regulating waste disposal.

Our responsibility as an association representing professional people with long experience in protecting public health should be to establish regular lines of communication with these pollution control boards and commissions. This will enable coordination of recommendations to minimize the possibility of conflicting provisions and unnecessary burdens on the dairy farmer.

We recommend close cooperation with these agencies in establishing good livestock waste management and pollution abatement policies that are in the public interest.

We recommend that animal waste regulations must be made on a regional basis related directly to topography, soil, and climatic conditions.

We urge that cattle manure be considered as a resource in the food production cycle and not as a waste by-product. Regulations should be promulgated to facilitate utilization of this resource wherever it can be accomplished without environmental insult.

FARM METHOD COMMITTEE MEMBERS

A. K. Saunders, *Chairman,* Manager Detergent Division, The De Laval Separator Company, 5724 North Pulaski Road, Chicago, Illinois 60646.

A. E. Parker, *Western Assistant Chairman,* Multnomah Co. Milk Sanitation Section, 104 S. W. Fifth Avenue, Portland, Oregon 97204.

James B. Smathers, *Eastern Assistant Chairman,* Director of Field Services, Maryland & Virginia Milk Prod. Assn., Inc., 1530 Wilson Boulevard, Arlington, Virginia 22209.

J. C. Flake, *Farm Methods Report Editor,* Director of Sanitary Standards, Evaporated Milk Association, 910 Seventeenth Street, N. W., Washington, D. C. 20006.

John Adams, National Milk Producers Federation, 30 F Street Northwest, Washington, D. C. 20001.

R. L. Appleby, Manager Milker Equipment, The De Laval Separator Company, Duchess Turnpike, Poughkeepsie, New York 12602.

William L. Arledge, Director of Quality Control, Suite No. 506, Portland Federal Building, Louisville, Kentucky 40202.

Henry V. Atherton, Department of Animal Sciences, University of Vermont, College of Agriculture, Burlington, Vermont 05401.

Richard L. Ayres, Chief Sanitarian, Los Angeles Co. Health Dept., Branch Office, Tulare, California 93274.

Sidney E. Barnard, Extension Dairy Specialist, Pennsylvania State University, 213 Borland Laboratory, University Park, Pennsylvania 16802.

R. A. Belknap, Sampling Surveillance Officer, Food and Drug Administration, Department of Health, Education and Welfare, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

Phillip Bergner, Milk Control Specialist, Alameda County Health Department, 15001 Foothill Boulevard, San Leandro, California 94377.

James A. Black, Milk Sanitarian, Oregon State Dept. of Agriculture, 635 Capital Street, Salem, Oregon 97310.

A. Richard Brazis, Chief, Laboratory Development Section, Department of Health, Education and Welfare, Food Microbiology Branch, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

Glenn R. Briody, Milk Plant Sanitarian, Multnomah County Milk Sanitation Section, 104 S. W. Fifth Avenue, Portland, Oregon 97204.

James Burkett, Sanitarian, Northwest Iowa Milk Sanitation Unit, 3340 Stone Park Blvd., Sioux City, Iowa 51104.

Glenn Cavin, Cedar Valley Cooperative Milk Association, 1936 Hawthorne, Waterloo, Iowa 50704.

Boyd M. Cook, Head Fieldman, Maryland Cooperative Milk Producers Assn., Inc., 1717 Gwynn Avenue, Baltimore, Maryland 21207.

Floyd M. Copenhaver, Chief, Dairy Section, Kansas City Health Dept., 10th Floor, City Hall, Kansas City, Missouri 64106.

Robert Dawson, Product Manager, Dairy Farm Equipment, Babson Brothers, 2100 South York Road, Oak Brook, Illinois 60521.

Pat J. Dolan, Bureau of Dairy Service, California Department of Agriculture, 1220 North Street, Sacramento, California 95814.

C. W. Dromgold, Acting Administrative Chief, Milk Control Service, 1220 Carr Lane Avenue, St. Louis, Missouri 63104.

William J. Ernst, Jr., Technical Service Department, Chemical Specialities Division, BASF Wyandotte Corporation, 1532 Biddle Avenue, Wyandotte, Michigan 48192.

Harold L. Faig, Training Officer, Cincinnati Training Facility, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

E. J. Fincher, Technical Specialist, Bureau of Food Inspection, State of Wisconsin, Department of Agriculture, 801 West Badger Road, Madison, Wisconsin 53713.

Clarence C. Gehrman, Dairy and Food Division, Washington State Department of Agriculture, P. O. Box 128, Olympia, Washington 98501.

Harry D. Gleason, Sanitarian, State of Washington, Department of Agriculture, Court House Annex, Box 708, Chehalis, Washington 98532.

Buck Greene, Assistant District Agent, Eastern Area, Louisiana Cooperative Extension Service, Knapp Hall, Louisiana State University, Baton Rouge, Louisiana 70803.

W. J. Harper, Department of Food Science and Nutrition, Ohio State University, 2121 Fyffe Road, Columbus, Ohio 43200.

Kenneth Harrington, Assistant Sales Manager, Babson Bros. Company, 2100 South York Road, Oak Brook, Illinois 60521.

Keith A. Harvey, Public Health Milk Rating Survey Coordinator, Environmental Improvement Division, Idaho Department of Health, Statehouse, Boise I,daho 83707.

A. J. Huff, Dairy Farm Inspector, Virginia Department of Agriculture & Commerce, 3425 Frontier Road N. W., Roanoke, Virginia 24012.

M. W. Jefferson, Chief, Dairy Inspector Service, Commonwealth of Virginia, Division of Animal Health and Dairies, 1444 East Main Street, Richmond, Virginia 23219.

R. E. Lock, Manager, Dairy Products, De Laval Separator Company, 350 Duchess Turnpike, Poughkeepsie, New York 12602.

Ben Luce, Chief, Dairy Inspection Section, Dairy & Food Division, Department of Agriculture, P. O. Box 128, Olympia, Washington 98501.

Clarence Luchterhand, Division of Health, Department of Health and Social Services, State of Wisconsin, P. O. Box 309,

Madison, Wisconsin 53701.

Omer Majerus, Sales Manager, Universal Milking Machine Division, 408 First Avenue South, Albert Lea, Minnesota 56007.

C. M. Mecham, Laboratory Supervisor, Maryland and Virginia Milk Producers Assn., Inc., 1530 Wilson Boulevard, Arlington, Virginia 22209.

David E. Monk, Supervisor, Environmental Health Services, Department of Community Health, 1900 E. 9th Street, Wichita, Kansas 67214.

Melvin Neff, Quality Control Supervisor, Upper Florida Milk Producers Assoc., P. O. Box 6962, 4851 Nolan Street, Jacksonville, Florida 32205.

Vernon D. Nickel, Sanitarian, St. Louis Department of Public Health, 416 Tenth Street, Crystal City, Missouri 63019.

Vernal S. Packard, Jr., Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55101.

Lester E. Peik, Alpenrose Dairy, 6149 S. W. Shattuck Road, Portland, Oregon 97200.

Loren E. Pine, Sr. Milk & Dairy Sanitarian, 1020 West Sixth Street, Ontario, California 91761.

Mark L. Prescott, Fieldman, Safeway Stores, Inc., Milk Department, P. O. Box 275, Clackamas, Oregon 97015.

R. G. Raup, District Sales Manager, The De Laval Separator Company, 5724 N. Pulaski Road, Chicago, Illinois 60646.

Richard Rintelmann, Assistant Vice President, National Accounts, Klenzade Products, Division of Economics Laboratory, Inc., Osborne Building, St. Paul, Minnesota 55102.

M. H. Roman, Supervising Inspector, State of New York York, Department of Agriculture, 18 Eugene Street, Lowville, New York 13367.

Robert J. Ryan, Associate Sanitarian, Bureau of Milk and Food Sanitarian, State of New York Department of Health, 845 Central Avenue, Albany, New York 12206.

Bernard Saffian, Associate Director, Norton Company, P. O. Box 1624, Stow, Ohio 44224.

Bernard J. Scheib, Manager, Dairy Technical Services, West Agro Chemical, Inc., 267 Warren Avenue, Kenmore, New York 14217.

Paul W. Scherschel, Director of Quality Control & Standards, Associated Milk Producer, Inc., Tri-State Region, 8550 West Bryn Mawr Avenue, Chicago, Illinois 60631.

C. Gayle Shrader, Chief, Milk Control, Department of Public Health, Louisville and Jefferson County, 400 East Gray Street, P. O. Box 1704, Louisville, Kentucky 40201.

Stephen B. Spencer, Dairy Specialist, 213 Borland Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802.

Donald K. Summers, Food and Milk Consultant, Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, 513 W. S. Custom House, Denver, Colorado 80202.

Dale Termunde, Babson Brothers, 2100 South York Road, Oak Brook, Illinois 60521.

Alvin E. Tesdal, Dairy Specialist, Dairy & Consumer Division, State Department of Agriculture, Agriculture Building, Salem, Oregon 97310.

Harold E. Thompson, Jr., Chief, Milk Sanitation Branch, Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, Washington, D. C. 20204.

Leon Townsend, Milk Survey Officer, Kentucky State Department of Health, 275 East Main Street, Frankfort, Kentucky 40601.

Helene Uhlman, Milk and Food Co-ordinator, Gary Health Department, 1429 Virginia Street, Gary, Indiana 46407.

R. J. Weaver, Director of Field Operation, Milk Control District No. 1, Associated Suburban Boards of Health, 75 East Lancaster Avenue, Ardmore, Pennsylvania 19003.

James Welch, Assistant Manager, Farm Sales, Klenzade Products, Division of Economics Laboratory, Inc., Osborne Building, St. Paul, Minnesota 55102.

Robert L. West, Dairy Foods Inspector, Bureau of Dairy Service, Department of Agriculture, 2550 Mariposa Street - Room 3080, Fresno, California 93721.

Harvey J. Wilhelm, Director, Producer Services, Mountain Empire Dairymen's Association, Inc., 945 - 11th Street, Denver, Colorado 80204.

TASK COMMITTEES 1971-72 AND 1972-73

The following is the breakdown of the ten task committees and the membership of each:

I. *Antibiotics, Pesticides and Other Adulterants*: M. W. Jeffson, *Chairman,* Richard Brazis, W. J. Harper, D. K. Summers, Leon Townsend, R. L. West.

II. *Cleaning and Sanitizing of Farm Milk Equipment*: James Welch, *Chairman,* James Burkett, Floyd Copenhaver, C. C. Gehrman, Bernie Schieb, Gayle Shrader.

III. *Education*: Vernon D. Nickel, *Chairman,* Sidney Barnard, Ben Luce, Vernal S. Packard, Pat J. Nolan.

IV. *Plastics*: Bernard M. Saffian, *Chairman,* Robert Dawson, Omer L. Majerus, David Monk, R. G. Raup, Richard Rintelmann, Bernie Schieb, Stephen Spencer.

V. *Testing for Cleanliness of Milk Production*: M. H. Roman, *Chairman,* Phillip Bergner, Glenn Cavin, Harry Gleason, Melvin Neff, Gayle Shrader, Harold E. Thompson, Richard Weaver.

VI. *Recommended Procedures for the Welding of Stainless Steel Milk Lines on Dairy Farms*: Kenneth Harrington, *Chairman,* R. L. Appleby, Richard Ayers, Glenn Cavin, Harry Gleason, Clarence Luchterhand, Omer L. Majerus, Lester Peik, R. G. Raup, Robert J. Ryan, Richard Weaver, Harvey Wilhelm.

VII. *Cleaning and Sanitizing of Farm Milk Pickup Tankers*: Boyd M. Cook, *Chairman,* James Black, Glen Briody, W. J. Ernst, Jr., Emmett Fincher, A. J. Huff, Melvin Neff, Loren Pine.

VIII. *Sampling of Bulk Tank Milk*: Helene Uhlman, *Chairman,* William Arledge, Ray Belknap, C. W. Dromgold, Keith Harvey, Ben Luce, Clinton Mecham, Mark Prescott, Harvey Wilhelm.

IX. *Water Protection and Treatment*: Dale Termunde, *Chairman,* Henry Atherton, C. W. Dromgold, Harold Faig, Keith Harvey, Loren Pine, Alvin Tesdal.

X. *Animal Waste Disposal*: Russel Lock, *Chairman,* John Adams, William Arledge, Henry Atherton, Robert Dawson, Buck Greene, Loren Pine, Paul Scherschel.

HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON FEBRUARY 20, 1973

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y-Treas., Dept. of Food Technology, Dairy Industry Bldg., Iowa State University, Ames, Iowa 50010."

0104 Storage Tanks for Milk and Milk Products As Amended

116 Jacob Brenner Company, Inc. (10/ 8/59)
450 Arlington, Fond du Lac, Wisconsin 54935

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St., Little Falls, N. Y. 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets, Chester, Pennsylvania 19013

1 Chicago Stainless Operations (5/ 1/56)
5001 No. Elston Avenue, Chicago, Illinois 60630

2 CP Division, St. Regis (5/ 1/56)
100 C. P. Ave., Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

76 Damrow Company (10/31/57)
196 Western Avenue, Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street, So., Peterborough, Ont., Canada

109 Girton Manufacturing Company (9/30/58)
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
9001 Rayo Avenue, South Gate, California 90280

127 Paul Mueller Company (6/29/60)
P. O. Box 828, Springfield, Missouri 65801

197 Paul Mueller (Canada), Ltd. (9/ 9/67)
84 Wellington St., South, St. Marys, Ont., Canada

213 Sanitary Processing Equipment Corp. (2/18/72)
Butternut Drive, E. Syracuse, N. Y. 13057

233 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, Calif. 90039

21 Technova, Inc. Gosselin Division (9/20/56)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

0203 Pumps for Milk and Milk Products Revised, as Amended

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wis. 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd., Oak Brook, Ill. 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

63R CP Division, St. Regis (4/29/57)
100 C. P. Ave., Lake Mills, Wisconsin 53551

205R Dairy Equipment Co. (5/22/69)
1919 So. Stoughton Road, Madison, Wis. 53716

180R The DeLaval Separator Co. (5/ 5/66)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

65R G & H Products, Inc. (5/22/57)
5718 52nd Street, Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way, Costa Mesa, Calif. 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road, Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive, Pittsburgh, Pa. 15238

241 Purity S.A. (9/12/72)
Alfredo Noble #38, Industrial fte. deVigas
Tlalnepantla, Mexico

148R Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Ave., Springfield, Ohio 45501

163R Sta-Rite Industries, Inc. (5/ 5/65)
P. O. Box 622, Delavan, Wisconsin 53115

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 53rd Street, Kenosha, Wisconsin 53140

219 Tri-Canada Limited (2/15/71)
6500 Northwest Drive, Mississauga, Ont., Canada

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007

52R Viking Pump Div.
Houdaille Industries, Inc. (12/31/56)
406 State Street, Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

0403 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

87 Cherry-Burrell Corporation (12/20/57)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

37 CP Division, St. Regis (10/19/56)
100 C.P. Ave, Lake Mills, Wis. 53538

75 Gaulin, Inc. (9 /26/57)
44 Garden Street, Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
60-Eleventh Ave., N.E., Minneapolis, Minn. 55413

0511 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road, Almont, Michigan 48003

98R Beseler Steel Products, Inc. (3/24/58)
417 East 29th, Marshfield, Wisconsin 54449

70R Jacob Brenner Company (8/ 5/57)
450 Arlington, Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., S. E., Minneapolis, Minn. 55114

118 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

66 Dairy Equipment Company (5/29/57)
1818 So. Stoughton Road, Madison, Wisconsin 53716

123 DeLaval Company, Ltd. (12/31/59)
113 Park Street, South Peterborough, Ont., Canada

45 The Heil Company (10/26/56)
3000 W. Montana Street, Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
West Hill, Ontario, Canada

232 Litewate Transport Equipment Corp. (4/ 4/72)
4220 South 13th Street, Milwaukee, Wis. 53221

80 Paul Mueller (Canada), Ltd. (11/24/57)

84 Wellington Street, So., St. Marys, Ont., Canada

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minn. 56340

144 Portersville Equipment Company (5/16/63)
Portersville, Pennsylvania 16051

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street, Arthur, Illinois 61911

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Ave., North Kansas City, Mo. 64116

189 A. & L. Tougas, Ltée (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

0809 Fittings Used on Milk and Milk Products Equipment, and Used on Sanitary Lines Conducting Milk and Milk Products, Revised

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186

138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada

245 Babson Brothers Company (2/12/73)
2100 S. York Road, Oak Brook, Illinois 60521

82R Cherry-Burrell Corporation (12/11/57)
2400 Sixth Street, S.W. Cedar Rapids, Iowa 52406

124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South Peterborough, Ont., Canada

184R The DeLaval Separator Co. (8/ 9/66)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

67R G & H Products, Inc. (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413

203R Grinnell Company (11/ 7/68)
260 W. Exchange St., Providence, R. I. 02901

218 Highland Equipment Corporation (2/12/71)
74-10 88th St., Glendale, N.Y. 11227

204R Hills McCanna Company (2/10/69)
400 Maple Ave., Carpentersville, Ill. 60110

34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140

239 LUMACO (6/30/72)
Box 688, Teaneck, N. J. 07666

230 ITT Moreland Products, Inc. (3/27/72)
P.O. Box #34, Wrightsville, Pa. 17638

200R Paul Mueller Co. (3/ 5/68)
P. O. Box 828, Springfield, Mo. 65801

244 Pittsburgh Brass Manufacturing Co. (2/10/73)
P.O. Box 387-A, Rd. #6, Irwin, Pa. 15642

149R Q Controls (5/18/64)
Occidental, California 95465

227 Stainless Steel Craft Corporation (1/11/72)
4503 Alger Street, Los Angeles, California

89R Sta-Rite Industries, Inc. (12/23/68)
P. O. Box 622, Delavan, Wis. 53115

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140

191R Tri-Canada Fittings & Equipment, Ltd. (11/23/66)
21 Newbridge Road, Toronto 18, Ontario, Canada

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

0900 Thermometer Fittings and Connections Used on Milk and Milk Products Equipment and Supplement 1, As Amended

32 Taylor Instrument Process Control,
Div. Sybron Corp. (10/ 4/56)
95 Ames Street, Rochester, New York 14601

206 The Foxboro Company (8/11/69)
Neponset Ave., Foxboro, Mass. 02035

1000 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

1103 Plate-Type Heat Exchangers for Milk and Milk Products, As Amended

20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013

38 CP Division, St. Regis (10/19/56)
Fort Atkinson, Wisconsin 53538

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South Peterborough, Ont., Canada

17 The DeLaval Separator Company (8/30/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

1204 Internal Return Tubular Heat Exchangers, for Milk and Milk Products, As Amended

243 Babson Brothers Company (10/31/72)
2100 S. York Road, Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Duchess Turnpike, Poughkeepsie, N. Y. 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pa. 17846

238 Paul Mueller Company (6/28/72)
P. O. Box 828, Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P. O. Box 118, Mora, Minnesota 55051

225 Sanitary Processing Equipment Corporation (11/24/71)
Butternut Drive, East Syracuse, N.Y.

1301 Farm Milk Cooling and Holding Tanks—Revised, As Amended

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road, Oak Brook, Illinois 60521

11R CP Division, St. Regis (7/25/56)
100 C. P. Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park, St. Cloud, Minn. 56301

4R Dairy Equipment Company (6/15/56)
1919 S. Stoughton Road, Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd., El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road, Preston, Ont., Canada

12R Paul Mueller Company (7/31/56)
P. O. Box 828, Springfield, Missouri 65801

58R Schweitzer's Metal Fabricators, Inc. (2/25/57)
806 No. Todd Avenue, Azusa, California 91702

235 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, California 90039

216R Valco Manufacturing Company (10/22/70)
3470 Randolph St., Huntington Pk., Calif. 90256

42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126

55R Superior Metalware Industries,
(Formerly John Wood Company) (1/23/57)
509 Front Avenue, St. Paul, Minnesota 55117

170R The W. C. Wood Co., Ltd. (8/ 9/65)
5 Arthur Street, South, Box 750, Guelph, Ont., Canada

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

1400 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers, As Amended

122R Cherry-Burrell Corporation (12/11/59)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140

78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

1604 Evaporators and Vacuum Pans for Milk and Milk Products, Revised

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207

111R Blaw-Knox Food & Chemical Equip., Inc. (2/12/59)
P. O. Box 1041
Buffalo, N. Y. 14240

164R Mora Industries, Inc. (4/25/65)
999 E. Maple Street, Mora, Minnesota 55051

107R C. E. Rogers Company (8/ 1/58)
P. O. Box 118, Mora, Minnesota 55051

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48010

1700 Fillers and Sealers of Single Service Containers, For Milk and Milk Products, As Amended

221 Bertopack Limited (5/25/71)
75 Ardelt Place, Kitchener, Ontario, Canada

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404

139 Exact Weight Scale Company (4/15/68)
944 W. 5th Ave., Columbus, O. 43212

137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232

220 Haskon, Inc., Package Equipment Division (4/24/71)
2285 University Ave., St. Paul, Minnesota 55114

211 Twinpak, Inc. (2/ 4/70)
2225 Hymus Blvd., Dorval 740, P.Q.

1901 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

141 CP Division, St. Regis (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

2201 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls, N. Y. 13365

154 CP Division, St. Regis (2/10/65)
100 C. P. Ave., Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

181 Damrow Company (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280

155 Paul Mueller Co. (2/10/65)
P. O. Box 828, Springfield, Missouri 65801

195 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

234 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, California 90039

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

2300 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc.
869 S. Knowles Ave., New Richmond, Wis. 54017

222 Maryland Cup Corporation (11/15/71)
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

2400 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365

158 CP Division, St. Regis (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P. O. Box 828, Springfield, Mo. 65601

2500 Non-Coil Type Batch Processors for Milk and Milk Products

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365

159 CP Division, St. Regis (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

167 Paul Mueller Co. (4/26/65)
Box 828, Springfield, Mo. 65801

196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

2600 Sifters for Dry Milk and Dry Milk Products

228 J. H. Day Co. (2/28/72)
4932 Beech Street, Cincinnati, Ohio 45202

171 Entoleter, Inc. (9/ 1/65)
Subsidiary of American Mfg. Co.
251 Welton St., Hamden, Connecticut 06511

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard, Mt. Vernon, N. Y. 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P. O. Box 1041
Buffalo, New York 14240

185 The Orville-Simpson Co. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223

176 Sprout, Waldron & Co., Inc. (1/ 4/66)
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

2800 Flow Meters for Milk and Liquid Milk Products

223 C-E IN-VAL-CO, a division of Combustion Engineering, Inc. (11/15/71)
P. O. Box 556, 3102 Charles Page Blvd., Tulsa, Oklahoma 74101

231 The DeLaval Separator Company (3/27/72)
350 Dutchess Turnpike
Poughkeepsie, New York 12603

226 Fischer & Porter Company (12/ 9/71)
County Line Road, Warminster, Pa. 18974

224 The Foxboro Company (11/16/71)
Foxboro, Massachusetts 02035

LETTER TO THE EDITOR

Sizing holding tubes of high-temperature short-time pasteurizers for Power law food products

DEAR SIR:

Studies on the minimum holding (residence) times of viscous food products in holding tubes of high-temperature short-time pasteurizers have been published in this *(5, 6)* and other journals *(2, 8)*. These studies showed that the flow of food products was laminar and hence the sizing of holding tubes on the basis of either a salt conductivity test with water in turbulent flow or the average linear velocity of the food leads to shorter residence times for the fastest particles.

It was in turn suggested *(2, 8)* that the holding tube length be calculated from the relation

$$L = \frac{8Qt}{\pi D^2} \qquad (1)$$

which is based on the assumption that the food product is a Newtonian fluid in fully developed laminar flow. Considering the expensive equipment and the special skills required for the experimental determination of the holding tubes *(2, 5, 6, 8)*, the utility of methods to compute the lengths of holding tubes is recognized.

Many food products are non-Newtonian fluids and although empirical in nature the simple Power law model

$$\tau = K \gamma_n \qquad (2)$$

has proved to be useful in characterizing many fruit purees *(4)* and in engineering studies on cereal doughs *(3)*. Depending on the magnitude of n, the fluid can be classified variously: $n = 1$ Newtonian, $n < 1$ pseudoplastic, and $n > 1$ dilatant. Methods for determining the index n can be found in the literature *(7)*. For these fluids, from laminar velocity profiles which have been confirmed experimentally *(1)*, the equation for the holding tube length is

$$L = \left(\frac{4Qt}{\pi D^2}\right) \left(\frac{3n + 1}{n + 1}\right) \qquad (3)$$

Equation (3) is an extension of Equation (1) and is applicable to pseudoplastic and dilatant fluids as well.

To determine the error when the residence time is calculated assuming Newtonian flow characteristics rather than Power law fluid characteristics, the residence time of the fastest particle of a Power law product (t_n) is compared to that of a Newtonian fluid (t). The comparison is made assuming equal volumetric flow rates and the same holding tube. From Equations (1) and (3), it can be shown that

$$\frac{t_n}{t} = \frac{2(n + 1)}{(3n + 1)} \qquad (4)$$

Results of calculations based on Equation (4) shown in Table 1 indicate that use of Newtonian fluid data leads to over design for pseudoplastic fluids and under design for dilatant fluids.

In conclusion the non-Newtonian nature of many food products must be taken into consideration for the design of holding tubes. Earlier studies have shown that residence time data with Newtonian fluids in turbulent flow should not be used for viscous food products. The equations and calculations presented here show that using Newtonian laminar flow residence time would lead to holding tube lengths longer than necessary for pseudoplastic fluids and shorter than necessary for dilatant fluids.

TABLE 1. COMPARISON OF RESIDENCE TIMES

Magnitude of n		t_n/t
0.2	Pseudoplastic	1.50
0.6	Pseudoplastic	1.14
1.0	Newtonian	1.00
1.2	Dilatant	0.96
1.6	Dilatant	0.90

M. ANANDHA RAO
Faculdade de Tecnologia de Alimentos
University of Campinas
Caixa Postal 1170
Campinas, S.P., Brazil

(Continued on Page 188)

THE WILLIAMS-STEIGER OCCUPATIONAL SAFETY AND HEALTH ACT OF 1970[1]

EARL D. HEATH
Office of Training and Education
Occupational Safety and Health Administration
U. S. Department of Labor, Washington, D.C. 20210

ABSTRACT

On December 29, 1970, the President signed the Williams-Steiger Occupational Safety and Health Act of 1970, the most comprehensive law ever designed for ensuring safe and healthful working condition. The Act, which applies to virtually the entire Nation's workforce, is the result of more than 60 years of involvement by the Federal and State governments, by employers, and by employees in an effort to assure American workers a safe and healthful job environment. The Congress has presented each of us with both a great potential and a great challenge. The essence of the Act is cooperation, for only by working together can we ensure worker protection and eliminate the needless waste that accompanies occupational deaths, injuries and illnesses. Past accomplishments to safeguard the worker have not been adequate. Each year, more than 14,000 workers continue to die as the result of occupationally-induced injuries. Another 2 million suffer disabling injuries. And, according to an estimate by the Surgeon General of the United States, some 400,000 are stricken by occupationally-related illnesses. Today, when National concern is focused on all phases of the environment, it is only natural that we give special consideration to the job site where American workers spend an estimated one-quarter of their time.

On December 29, 1970, the President signed the Williams-Steiger Occupational Safety and Health Act of 1970 (Public Law 91-596), the most comprehensive law ever designed for insuring safe and healthful conditions in the American workplace. The Act applies to virtually the entire Nation's workforce and to some five million establishments.

Between the early 1800's and now, there have been numerous measures introduced by the various States and by the Federal Government to improve the safety and health of the worker. A chronology of attitudes toward industrial accident prevention efforts throughout this Nation's history makes interesting reading. J. E. Trainer, in an address before the Annual Meeting of the American Society of Safety Engineers in 1949, reported than at one time: " . . . It was not thought unreasonable to expect one fatality for every floor of a building under construction; or that two men might well lose their lives per mile of tunnel driven; or that one employee could be expected to donate his future for every million dollars spent on a general construction project . . ." Happily, these "norms" are well behind us.

The voluntary safety movement in the United States has proven effective to the extent that there were enough volunteers. This was pointed up in the testimony of the National Safety Council before the Congress when the Occupational Safety and Health Act of 1970 was under consideration. A portion of this testimony is quoted here: " . . . In all candor, it is the National Safety Council's judgment that there is no real substitute for the effectiveness of the voluntary safety movement for those who participate. Unfortunately, not all participate . . . "

The Act is a general statute covering almost 60 million employees in about 5 million establishments. Coverage applies to all business in commerce with one or more employees, including those in agriculture, the professions, and retail and service industries. As a matter of policy, houshold domestic employees and immediate family members working on family farms are generally not subject to the provisions of the Act. To the extent that other Federal agencies exercise statutory authority affecting occupational safety and health with respect to specific working conditions, the Act is inapplicable.

The intent of the Act is to provide a vehicle for reducing the high social and economic costs of occupationally related injuries, illnesses, and deaths by a combination of Federal, State, and private efforts. The Act mandates not only Federal standards setting and enforcement activities, but also training for employers and employees, information efforts, the active involvement of the States, loans by the Small Business Administration to small businesses to assist them in coming into compliance, and the conduct of needed research and statistical programs. We in the Department are actively engaged in all of these efforts, though we recognize that we have a long way to go. We also recognize that we cannot hope to achieve the objectives of the Act unless employers and employees work together to voluntarily overcome many of the problems that exist.

The Act is not a totally new concept. Rather, it is the logical result of more than 60 years of involvement by the Federal and State governments, and by employers and employees and their represen-

[1]Presented at the 59th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972.

tatives in an effort to assure American workers a safe and healthful work environment.

Past accomplishments to safeguard the worker have not been adequate. Each year, more than 14,000 workers continue to die as the result of occupationally-induced injuries. Another 2 million suffer disabling injuries. And, according to an estimate by the Surgeon General of the United States, some 400,000 are stricken by occupationally-related illnesses. Today, when National concern is focused on all phases of the environment, it is only natural that we give special consideration to the job site where American workers spend an estimated one-quarter of their time.

A major factor in past failures to create relatively hazard-free workplaces may be that these efforts were fragmented. Never before have all sectors of the working community been united in one major campaign. The Williams-Steiger Occupational Safety and Health Act of 1970 calls on the best resources of the Federal and State governments, and of employers and employees and their representatives. It assigns responsibilities to each group.

In designing this law, the Congress has presented each of us with a great potential and a great challenge. The essence of the Act is cooperation. For only by working together can we ensure worker protection and eliminate the needless waste that accompanies occupational death, injury, or illness. The Occupational Safety and Health Administration (OSHA) which was established to implement the Act is a decentralized organization with 10 Regional, 49 Area, and 2 District Offices. Following President Nixon's approach to all National Government, our structure is based on responding to the needs of citizens at the local level. Thus, the bulk of our personnel are in the field; and regional and area officials help to set National policy.

Operating Philosophy

Secretary of Labor, James D. Hodgson, in a speech at the National Safety Congress on October 27, 1971, made four points regarding OSHA's operating philosophy. These bear repeating.

Principle No. 1

What is done willingly is done well. This means that we, in government, cannot do the job alone. We depend on the willingness of others — the willingness of employees, organized labor, business, and industry.

Principle No. 2

The Department of Labor is charged with enforcing the law. And, we're going to. But, I think it's fair to say that we'll be guided as much by the spirit of the law as by the letter.

Principl No. 3

We are doing all we can to encourage the States to assume their responsibilities under the Act because we believe this is the best way to make the program work.

Principle No. 4

Our whole training and education effort is so important that the OSHA program will stand or fall on it. I can assure you that it's going to stand.

OSHA's Three Phases To Date

During Phase I of OSHA's operation, the major project was the selecting, reviewing, and issuing of the initial group of occupational safety and health standards. Phase II of our operation was the initiation of compliance safety and health visits. OSHA is now in Phase III, which is the involvement of the States in the implementation of the Act.

Occupational Safety and Health Standards

The initial standards package, published in the *Federal Register* on May 29, 1971, incorporated existing Federal standards from the Walsh-Healey Act, the Maritime Safety Act, and others. The package also incorporated selected National consensus standards developed by such organizations as the American National Standards Institute (ANSI), and the National Fire Protection Association (NFPA).

By definition, a standard as applied to this subject is a rule or regulation established in accordance with law or other competent authority, which designates safe and healthful conditions or practices by which work must be performed to prevent injury or illness.

The Department of Labor has been charged with having "invented" these standards. It didn't happen that way. Not one of the initial standards was our "invention". The standards, promulgated on May 29, 1971, had all been previously adopted either through private sector review or Federal administrative procedures. Some of these, we know, contain questionable elements which we are now in the process of modifying, but all are national consensus or established Federal standards as required by the Act.

Issuance of standards is a continuing responsibility. Since promulgation of the initial standards package in May 1971, we have revised and updated existing standards. New standards are contemplated in tunnel construction and agriculture as well as in the health area. In this latter field, we will rely heavily on the National Institute for Occupational Safety and Health, which has primary responsibility for research activity in the health area.

Only in the case of the maritime and construction industries, in which unique workplace conditions exist, have we issued special bodies of standards applicable only to the industry. The Act recognizes that employers may do things in many different ways and be as effective in terms of safety and health as would be compliance with the standards issued. An employer may either petition for a change in the standard or else apply for a variance from its requirements.

Section 5 of the Act states in part: "Each employer shall furnish to each of his employees, employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees. This "general duty clause" shall be used only in cases involving alleged serious violations where *(a)* no standard exists, and *(b)* where the specified and stringent requirements of the general duty definition involving recognized hazards and death or serious physical harm are met.

Enforcement of the Safety and Health Standards

To ensure compliance with the safety and health standards promulgated by the Secretary under the Act, OSHA has a force of compliance safety and health officers in the field who conduct visits to establishments.

Almost from the first it was clear that we would have to establish priorities for workplace inspections, since there are an estimated 5 million such establishments. Thus, we proceeded in accordance with the "worst first" principle mandated by the Act and requiring us to give maximum attention to the most hazardous workplaces.

"Imminent danger" situations must obviously receive first consideration. These are situations in which it can reasonably be expected that death or serious physical harm will result before the danger can be eliminated through normal enforcement proceedings. The Act gives us authority to seek restraining orders and, injunctive relief in U. S. District Courts in these situations, and we have made it clear to our compliance staff that they must respond immediately whenever they receive information suggesting that imminent danger exists. We have inspected several of these situations; fortunately, most were resolved through voluntary compliance. In the case of the Port Huron tunnel disaster in Michigan, we did obtain a temporary restraining order and later a preliminary injunction against permitting employees to work in or near the tunnel until an acceptable plan of safe operations had been put into effect.

Putting aside these emergency situations, our first priority is inspection of workplaces following catastrophies or accidents resulting in fatalities. We conduct prompt inspections, often within hours of the accident, to determine if imminent danger exists and whether citations and penalties should be proposed.

The next inspection priority is response to valid employee complaints. Prompt response to complaints is a key element in protecting the employees' safety and health, and in maintaining confidence in program administration. To date, we have received over 4,000 complaints, and most of them have been investigated.

The third inspection priority is the target industry and target health hazards programs — those industries and toxic substances presenting the greatest risks. In the target industry program we have selected five industries with high injury frequency rates — *marine cargo handling, lumber and wood products, roofing and sheet metal, meat and meat products, and miscellaneous transportation equipment* (mostly mobile home construction and recreational vehicle construction). In the target health hazards program, we chose asbestos, lead, silica, cotton dust, and carbon monoxide — all known toxic substances covered by specific standards and affecting large numbers of employees.

Our last inspection priority is for a random cross section of all industry. We interpret the Act as covering all employers with one or more employees, whether engaged in profit or non-profit enterprises.

An actual inspection consists of showing credentials to the employer, discussing with him the purpose and scope of the inspection; giving him informational materials; the walk-around, in which both employer and employee representatives may participate; interviewing employees as necessary; a closing conference with the employer in which apparent violations and what would be involved in abatement are discussed; and, if violations are noted, appropriate citations are then prepared with proposed penalties, if any, and abatement periods. In proposing penalties, the size of the establishment, gravity of the violations, the good faith of the employer, and the history of past violations are taken into consideration.

We conduct follow-up inspections to determine if abatement requirements are being met. We have made follow-up inspection mandatory in cases of willful, repeated, and serious violations or in cases in which the employer leads us to believe abatement will not take place. In other cases, follow-up inspections are discretionary with our Area Directors.

First Instance Citations

First instance citations are specifically provided for

in the Act. Past experience in State programs amply demonstrates that initial warnings alone just do not do the job. Seldom is any corrective action taken. Moreover, the prospect of receiving merely an initial warning is slight inducement for an employer to take corrective action until after an inspection is made. Thus, Congress provided for citations and, where appropriate, penalties when violations even in the first instance are found to exist.

REVISED COMPLIANCE MANUAL

Experience soon convinced us of the need to modify the first compliance operations manual. We issued a comprehensive new edition in January 1972, containing much detailed material on inspection procedures, compliants, imminent dangers, violations, citations, and proposed penalties. We also decided to make the new manual available to the public, even though much of the material was exempt from disclosure. Our decision to publish the revised manual was taken as part of our policy of maximum disclosure. Under this policy we have disclosed all citations, proposed penalties, and similar documents, and even pertinent portions of investigative files when law enforcement action based on those files no longer appeared likely. We welcome public scrutiny and try to be responsive to legitimate public comment.

STATE PARTICIPATION

The Act encourages the States to assume the "fullest responsibility for the administration and enforcement of their occupational safety and health laws . . ." It makes clear that they are to be true partners with the Federal Government through developing plans for carrying out their own programs which will eventually replace Federal standards setting and enforcement efforts in areas covered by their plans. All States and territories except two have received grant funds to help develop State plans. Many States require comprehensive enabling legislation to mount an effective program. Thirteen have already passed such legislation, and at least an additional 25 have bills pending. The Department of Labor has moved to assist the States to participate by adopting a policy of funding a full 50% of the cost of approved State plans without resort to arbitrary formulaes for allocation of funds.

RECORDKEEPING

Before the Act, the only data on occupational injuries was based on a voluntary system involving about 150,000 employers. Occupational illnesses were "guesstimated" from time to time, with little or no hard data. To allow effective program direction as well as to highlight for employers and employees what is really happening on the worksite, new recordkeeping and reporting systems were mandated by the Act. The recordkeeping system requires that a log of occupational injuries and illnesses be maintained by employers. A supplementary detailed record is required for each injury. However, most injuries are now covered by workmen's compensation and those reports usually contain all of the information for the supplementary record, with no separate form needing to be prepared. A summary of the injuries and illnesses must be prepared annually and made available to employees. The records are kept at the establishment, and are not submitted to the Government, except on specific request.

The Bureau of Labor Statistics conducts an annual survey of injuries and illnesses. To do this, a random sample of firms is selected and asked to report their actual experience based on the records that they are required to keep. For the next survey to be conducted in January 1973, about 2% of the establishments with fewer than 20 employees will be asked to report. We have initiated proceedings to exempt employers of <8 employees from all regular recordkeeping requirements.

CONSULTATIVE SERVICES

We would like to provide greater consultative services to employers than we have been able to do under the Act. However, whenever a Department official goes into a employer's premises for any purpose under the Act, except for an inspection in connection with the issuance of a variance under Section 6, he is required to note any violations and citations must be issued as appropriate. Under Section 9 of the Act, if, upon inspection, a compliance officer believes that an employer has violated a requirement of the Act, an appropriate citation and proposed penalty shall be issued to the employer. Since Section 8 makes it clear that any entering upon the employer's premises is regarded as an inspection provided for in Section 9, we have been legally precluded from providing any consultation visits on an employer's premises. Assistant Secretary George C. Guenther, in a statement before the Subcommittee on Environmental Problems Affecting Small Business on Occupational Safety and Health on June 22, 1972, said: "We would support an amendment to provide for workplace visits, upon request, for specific consultative purposes by Department of Labor advisors. Voluntary compliance, which we consider essential to the success of the whole occupational safety and health program would be greatly enhanced by such an amendment."

It will not be possible for OSHA to achieve the

purposes of the Act through compliance visits alone. We will continue to rely heavily upon voluntary compliance by employers. We assume that the great majority of employers want to comply with the safety and health standards. However, to do this, employers and employees must know what is expected of them and must know how to achieve these goals and objectives. Here is where training can play a major role.

TRAINING AND EDUCATION

Section 21 of the Act places responsibilities for training upon both the Department of Health, Education and Welfare, and the Department of Labor. HEW's responsibilities relate to educational programs to provide an adequate supply of qualified manpower to carry out the purposes of the Act, and informational programs on the importance of and proper use of safety and health equipment.

The Secretary of Labor's responsibilities for training under the Act, in addition to training of his own staff to implement the legislation, require him to: *(a)* provide for establishment and supervision of programs for the education and training of employers and employees in the recognition, avoidance and prevention of unsafe or unhealthful working conditions in employments covered by the Act, and *(b)* consult with and advise employers and employees, and organizations representing employers and employees as to effective means for preventing occupational injuries and illnesses.

In developing its strategy for developing training for, and delivering training programs to, the target populations, OSHA recognized that it could not impact directly upon this Nation's total workforce. But, OSHA can and is working with field populations which can impact upon the ultimate target populations. Examples of the field populations with which OSHA is currently working are: *(a)* the vocational education community, *(b)* training of cadres of instructors, *(c)* training of employee representatives, *(d)* training of employer representatives, *(e)* training of compliance safety and health officers, and *(f)* training of OSHA personnel at the National Office and in the field.

Among the many training efforts initiated by OSHA in support of the Act's goals and objectives are those intended to improve the level of understanding of employer and employee rights and responsibilities under the Act and their understanding of how factors which may lead to injuries or illnesses can be eliminated or otherwise controlled. One of these is a programmed instruction text and an administrator's manual which, upon completion sometime this fall, will be made available through the U.S. Government Printing Office. Another project is development, in cooperation with NIOSH, of a rigorous home study course in occupational safety and health. Through efforts of this type, we expect to reach large numbers of employers and employees. Moreover, many employees, as the result of having taken this type of training, can then become more effective workmen and supervisors. Many will be able to perform effectively on occupational safety and health committees within their establishments, and some may be able to obtain positions as collateral duty safety and health personnel within their establishments.

What resources are available to assist the training specialist to help both employers and employees increase their understanding of the Act and how it affects them? *First*, there are the occupational safety and health standards promulgated by the Secretary under the Act. These are available in the *Code of Federal Regulations.* The principal parts are 1910 and 1926. *Next*, there is the *Compliance Operations Manual*, which is the "bible" of the compliance safety and health officer. It is available at $2.00 a copy from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Armed with the standards and the *Compliance Operations Manual*, it should be possible for an employer to learn thoroughly what is expected of him under the Act and how the compliance safety and health officer performs his tasks when conducting a compliance visit. Another useful publication is the *Inspection Survey Guide: A Handbook of Guides and References to Safety and Health Standards for Federal Contracts Programs.* This publication is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 for $2.25 per copy.

Now, moving from publications to services, there are the 49 Area Directors of the Occupational Safety and Health Administration located in as many cities. These individuals are prepared to provide detailed information to employers, to employees, and to representatives of employers and employees, and to others who have questions pertaining to the Act. Each Area Director and each Regional Administrator, through his staff, is prepared to provide orientations on the Act for employer audiences and for employee audiences. OSHA has prepared packaged programs, complete with training aids, that are used in the field to acquaint employers and employees and their representatives and other interested individuals with the Act and how it is being implemented.

CONCLUSION

We are developing and carrying out a balanced

program effort, looking not only at today's statutory requirements but also at the improvements necessary over the next four years and beyond. We are dedicated to building a broad program that will eliminate occupational hazards from the American workplace. We believe we have made a substantial and responsible start toward this goal.

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LETTER TO THE EDITOR

(Continued from Page 181)

NOTATION

D = diameter of tube
K = constant, Eq. (2)
L = holding tube length
n = Power law index
Q = volumetric flow rate of fluid
t = residence time of the fastest particle
γ = shear rate
Subscript
n = for a Power law fluid

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LETTER TO THE EDITOR

Do proposed standards make a medicine out of milk?

DEAR SIR:

The Federal Food and Drug Administration's proposed standards for milk and milk products *require* that Vitamin D be added to all milk and Vitamins A and D to all low fat and skim milk products. Then, they state that all of the products shall be labeled as special dietary products. I question the legality of such a requirement. However, if Food and Drug is able to make this mandatory, as published in the *Federal Register*, a most important basic food, nature's most nearly perfect food, will be relegated to the category of a medicine.

We realize that milk is an ideal carrier for additional vitamins and that Vitamin D is more readily assimilated by the body in the presence of milk, with its abundance of calcium. Milk is our most inspected, tested, and analyzed food all the way from the producer through the processing plant, to the consumer. A single Vitamin D assay costs upwards of $40.00.

As a member of the National Labeling Committee, I am aware of the work, time, and expense to both industry and state regulatory agencies on changing our milk and fluid milk products labeling the last two years to comply with the

Federal Fair Packaging and Labeling Act. This has just been completed. Now, if Food and Drug gets its proposal through, it would require *again* changing all our carton, bottle cap, and package labeling all over the United States. Furthermore, it is absolutely impossible to get all this new required labeling on a bottle cap.

Consumers are now used to Vitamin D Milk or Fortified Milk being so labeled, with the amount of each vitamin added specified on the label in compliance with the Fair Packaging and Labeling Act. Now we would have to change all this to read "Milk, Vitamin D Added" or "Vitamin A and D Added" as the case might be. This would require a considerable amount of time, work, and expense to both industry and state regulatory agencies and would accomplish nothing except to increase the cost of fluid milk to consumers and to ultimately increase taxes of consumers to pay for this ridiculous change!

Is this the kind of "consumer protection" the consumer wants and needs?!!

BEN LUCE
Dairy Inspection Section
Dairy and Food Division
Washington Department
of Agriculture
Olympia, Washington

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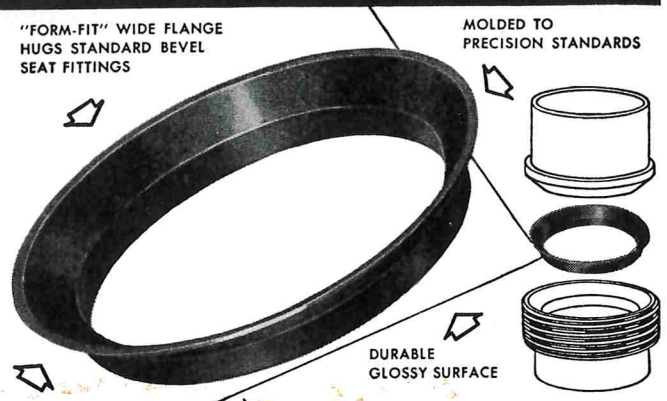

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4180 Lorain Avenue • Cleveland 13, Ohio

Haynes SELF-CENTERING SNAP-TITE® Gaskets

SIZES 1" - 1½"
2" - 2½" - 3"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

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*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

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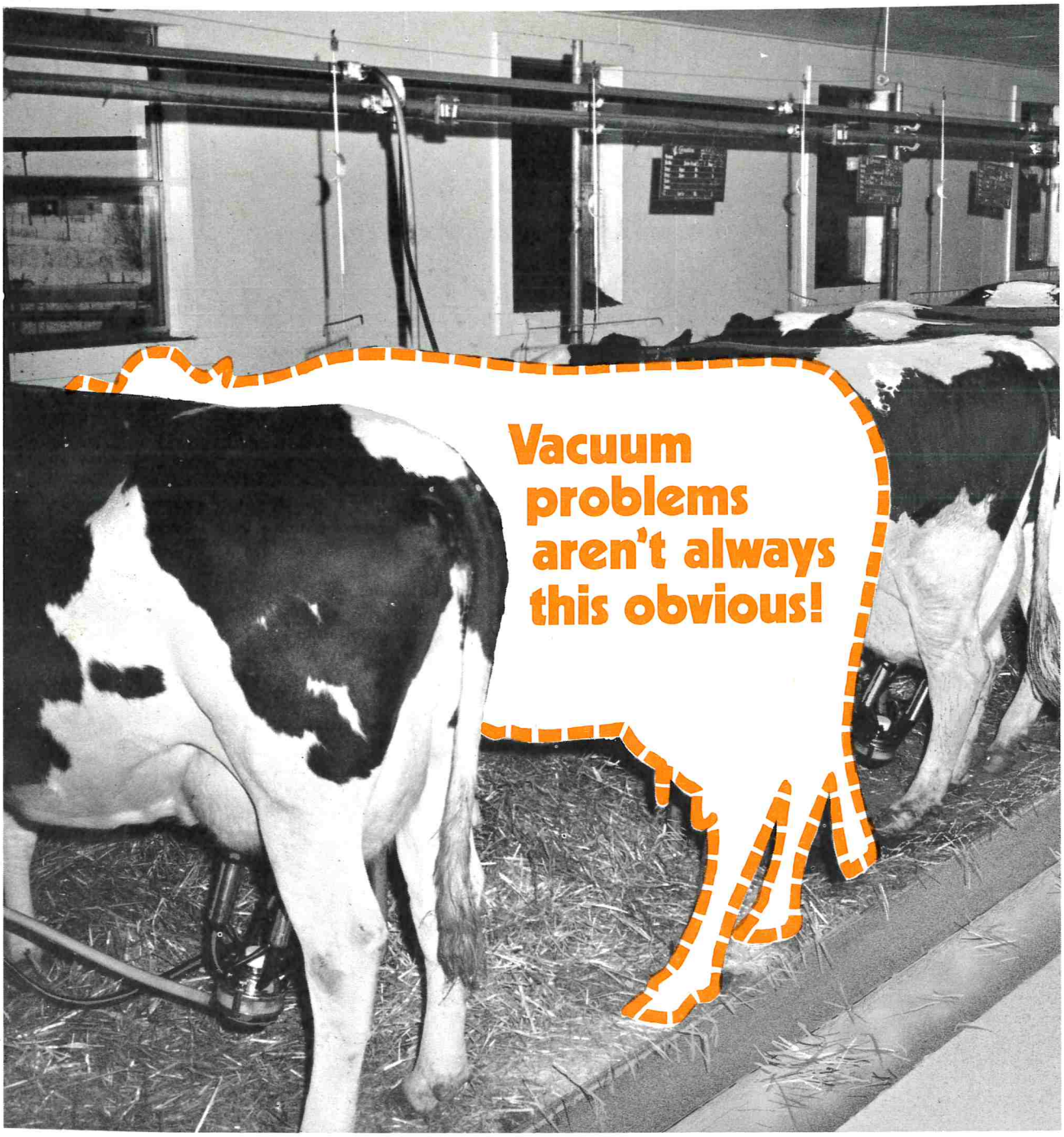

Vacuum system problems can take a top cow out of your milking herd years before you'd normally cull her. The reason?

An improperly functioning vacuum system can cause teat and udder irritation—opening the door to mastitis. If mastitis is already present, vacuum problems can aggravate the condition.

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