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Mesophilic and Psychrotrophic Bacterial Populations on Hot-Boned and Conventionally Processed Beef[1]

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ABSTRACT

Mesophilic and psychrotrophic bacterial counts of hot-boned and conventionally treated cuts from 15 steers were low [Log 0-2 colony forming units (CFU)/cm²] at 0 time; and after 14 days of vacuum-packaged storage (2.2 C), hot-boned cuts had higher counts than conventionally-treated cuts. In the first experiment involving 10 steers, the mesophilic and psychrotrophic counts for hot-boned cuts were Log 5.26 CFU/cm² and Log 5.15 CFU/cm², respectively, and for conventionally treated cuts, log 4.64 CFU/cm² and Log 4.43 CFU/cm², respectively. In the second experiment involving 5 steers, the mesophilic and psychrotrophic counts were Log 6.62 CFU/cm² and Log 6.61 CFU/cm², respectively, for hot-boned cuts; and Log 5.93 CFU/cm² and Log 4.91 CFU/cm², respectively, for conventionally treated cuts. Some hot-boned cuts had low levels (Log 0-3 CFU/cm²) of coliforms, fecal coliforms, *Clostridium perfringens*, coagulase-positive *Staphylococcus aureus* and fecal streptococci. No *Salmonella* were recovered from any cuts. Temperature-decline data indicated that hot-boned cuts had longer (several hours) periods of rapid bacterial growth (above 21 C) than conventionally-treated cuts. The longer rapid growth period for hot-boned cuts may have contributed to higher microbial loads and subsequently to more growth of bacteria in cold storage. Slower chilling of hot-boned samples stemmed from vacuum-packaging and boxing soon after cutting. Temperature control of hot-boned meat during the first several hours of chilling is critical, particularly if hot cuts are vacuum-packaged and boxed before chilling. Some temperature decline guidelines, based on bacterial counts, are presented for hot-boned, vacuum-packaged boxed cuts. Most hot-boned cuts processed and stored under our experimental conditions were bacteriologically acceptable.

The potential advantages of hot-boning beef include facilitating centralized processing, reduced cooler space and reduced energy (refrigeration) input, with no reduction in cut yield (*4*). Those potential economic advantages have prompted an increased interest in hot-boning to produce subprimal beef cuts that can be vacuum-packaged and stored in boxes soon after cutting. To help insure that the process is safe, the microbiology of hot-boning (the number and kinds of microorganisms involved during processing and storage) must be understood and controlled, particularly for vacuum-packaged, hot-boned cuts.

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Microbiological work on hot-boned beef and lamb (*1,6,9,10,11*) has shown counts of Log 3-6 CFU/cm², g, or ml after 1 to 14 days of storage at 1 to 15 C, and that the cuts were generally bacteriologically acceptable.

The purpose of this study was to ascertain mesophilic and psychrotrophic bacterial populations of hot-boned vacuum-packaged beef using conventionally chilled meat as a comparison. Occurrence of indicator organisms and potential pathogens in some meat samples was also monitored.

MATERIALS AND METHODS

Meat processing

Fifteen steers were used in this study. Ten were slaughtered on two successive days (five per day) at hourly intervals in experiment one (September, 1978) and five were slaughtered at hourly intervals in experiment two (March, 1979). Half of each carcass was hot-boned within 2 h postmortem, and the other half of the same carcass was conventionally chilled and cut in the same manner at 48 h postmortem. Samples (32.26 cm²) were removed aseptically from the plate region of the hanging carcass (*12*) at 2 h postmortem and after chilling 48 h at 2.2 C for "0" time samples representing hot-boned and conventionally-processed products, respectively. Thin samples (ca. 2.5 × 15 × 22 cm) from the plate region (cut from the carcass exterior to the underlying connective tissue septa) were excised aseptically immediately adjacent to where "0" time samples had been removed. Then the thin samples were vacuum-packaged in very low gas and H_2O transmission bags (Cryovac SR823), boxed (24 × 56 × 32 cm box) and stored at 2.2 C for 14 days. Subsamples (32.26 cm²) were removed aseptically later for the 14-day analyses from the hot-boned and conventionally processed sides. In the first experiment, two samples (32.26 cm²) adjacent to each other were removed per sampling time, and one sample was removed for each period in the second experiment. After 14 days of storage, all vacuum packages maintained a vacuum within the range of 23.0 to 26.8 inches of Hg. Inches of Hg were determined by the vacuum required to initiate separation of the packaging material from the meat sample.

Temperature measurements

In the first experiment, temperature of the hot-boned and conventionally processed meat was monitored at 2, 4, 6, 8 and 24 h postmortem under an ambient temperature of 2.2 C. Temperature measurements of semimembranosus and longissimus muscles were used as indicators of the relative chill rates between treatments. Metal dial thermometers were inserted 5 cm into the muscles at constant muscle locations whether muscles were intact on the carcass or excised. In the second experiment, metal dial thermometers were inserted 5 cm into the muscles of the carcass to measure temperature decline of conventionally chilled meat. To simulate commercial conditions, hot-boned vacuum-packaged plate samples were placed in the center of the box, sandwiched between hot meat masses from other parts of the carcass. Temperature was recorded at the center (adjacent to the surface of the plate samples) and 5 cm inward from the exterior of the

meat mass at 0, 1, 2, 3, 4, 6, 20, 22 and 24 h after boxing to obtain internal and external temperatures of hot-boned meat.

Microbiological procedures

Meat samples (32.26 cm^2) were put into Mason jars containing 100 ml of sterile rinse solution (buffer solution for Standard Plate Count, *8*), and transferred to the microbiology laboratory for analysis. The meat was in the rinse solution for 1 h before being shaken vigorously 100 times with an amplitude of 2 ft (procedure for experiment one) or placed in solution for 20 min, blended for 5 sec and then shaken 50 times (procedure for experiment two). Blending the meat for microbiological studies increases recovery of microorganisms (*7*), but excessive blending results in meat slurries that are difficult to pipette.

Liquid samples were withdrawn from the jars for viable cell counts by standard methods (*8*). One set of plates was incubated for 48 h at 32 C for the mesophilic count; another set, for 10 days at 7 C for the psychrotrophic count. The counts were reported as Log_{10} colony forming units (CFU)/cm^2. When averages were reported, arithmetic means were calculated before converting to the Log_{10} number.

Indicator organisms and potential pathogens

In the second experiment, in addition to mesophilic and psychrotrophic counts, we monitored coliforms, fecal coliforms, *Clostridium perfringens*, *Salmonella*, coagulase-positive *Staphylococcus aureus*, and fecal streptococci. Bacteriological procedures are summarized in Table 1. For the 0 time samples, only one dilution of the 3 tube MPN was used to detect the presence of coliforms, fecal coliforms, coagulase-positive *S. aureus*, and *C. perfringens*. For the 14 day stored samples, the 2 dilution, 3 tube MPN method was used to enumerate number of organisms in the meat samples.

RESULTS AND DISCUSSION

The average mesophilic and psychrotrophic counts of hot-boned and conventionally-processed meat are presented in Table 2. The 0-time mesophilic counts for both hot-boned and conventionally-treated cuts were low, ranging between Log 0-2 CFU/cm^2, and indicating good bacterial quality at the onset of the experiments. At 0-time no psychrotrophs were recovered in experiment one; however, low levels of psychrotrophs colonies were detected in experiment two. This is probably due to more psychrotrophs occurring on the surfaces of animals in the winter months. The 14-day sample data indicated that hot-boned meat had higher mesophilic and psychrotrophic counts than conventionally-processed meat, with approximately 1-2 log unit differences between comparable samples. Statistical analyses were not performed because the counts obtained varied widely (Log 0-5 CFU/cm^2 in the first experiment and Log 3-7 CFU/cm^2 in the second experiment). Instead, bacterial counts were tabulated (Table 3) by frequency of ocurrence in four bacterial count ranges: Log 0-2 CFU/cm^2 (low count), Log 3-4 CFU/cm^2 (intermediate count), Log 5-6 CFU/cm^2 (high count), and Log 7 CFU/cm^2 (very high count). Most samples (Table 3) had low or intermediate counts, indicating that most of the meat was microbiologically acceptable. Samples from hot-boned cuts provided more than twice as many high and very high counts as did the conventionally processed cuts. Hot-boned meat from animal C (see Table 4) in the second experiment had log 7 CFU/cm^2 in mesophilic and psychrotrophic counts. Data in Table 4 give the

TABLE 1. *Summary of bacteriological determinations for each meat sample.*[a]

Bacteria monitored	Procedure references	Enumeration procedures	Incubation temperature and time
Quantitative determinations			
Total mesophile	Standard Methods (*8*)	Pour plate	32 C, 48 h
Total psychrotroph	Standard Methods (*8*)	Pour plate	7 C, 10 d
Coliforms	BAM (*2*)	2 dilution, 3 tube MPN (*3*)	37 C, 48 h presumptive 37 C, 48 h confirmative
Fecal coliforms	Klein and Fung (*5*)	Subculture of positive coliform tubes	44.5 C, 24 h confirmative
Coagulase positive *Staphylococcus aureus*	BAM (*2*)	2 dilution, 3 tube MPN (*3*)	37 C, 24 h enrichment 37 C, 24 h plating 37 C, 24 h liquid culture 37 C, 4 h coagulase test gram reaction
Clostridium perfringens	BAM (*2*)	2 dilution, 3 tube MPN (*3*)	46 C, 4 h preenrichment 37 C, 24 h enrichment 37 C, 24 h anaerobic plating 37 C, 24 h biochemical tests gram reaction
Fecal streptococci	Difco KF agar	Pour plate	37 C, 48 h
Qualitative determination *Salmonella*	BAM (*2*)	2 portions of 10 ml of blended meat solution	37 C, 24 h preenrichment 37 C, 24 h enrichment 37 C, 24 h plating 37 C, 24 h biochemical tests agglutination test gram reaction

[a]Each meat sample of the second experiment was blended for 5 sec and shaken 50 times. From this liquid sample, 0.1 ml, 1 ml, or 10 ml was aseptically withdrawn to perform all the above tests. Dilutions were made as needed.

TABLE 2. *Mesophilic and psychrotrophic counts on hot-boned and conventionally processed beef samples.*

	Days stored	Mesophile counts Hot-boned	Conv	Psychrotroph counts Hot-boned	Conv
A	0	1.57[1]	1.94	NR[2]	NR
	14	5.26	4.64	5.15	4.43
B	0	2.34	1.96	1.70	1.43
	14	6.62	5.93	6.61	4.91

A - Experiment 1, 10 steers.
B - Experiment 2, 5 steers.
Hot-boned - hot-boned cuts excised at approximately 2 h postmortem.
Conv - conventional cuts excised at 48 hr postmortem.
[1]Average count in Log Colony Forming Units/cm².
[2]NR - not recovered in 0 dilution samples.

occurrence and numbers of indicator organisms and potential pathogens from cuts in the second experiment. At 0-time, tests for coliforms, fecal coliforms and *C. perfringens* were positive for hot-boned samples from animal C and for *S. aureus* from conventionally boned sample from animal B. All samples had very low (Log 0-1 CFU/cm²) or nonrecoverable levels of fecal streptococci; so contamination with potential pathogens was low at the onset of the experiment.

In the 14-day samples, indicator organisms and potential pathogens were found more frequently on hot-boned than on conventionally processed meat. Hot-boned meat from animal C harbored all the organisms for which tests were conducted (except *Salmonella*) although in low numbers (Log 0-3 CFU/cm²). Conventionally-processed cuts from animal C also had coliforms, fecal coliforms, and coagulase-positive *S. aureus* in low numbers. Apparently animal C was contaminated more during slaughter than any of the other four animals in experiment 2. Zero-time and 14-day mesophilic and psychrotrophic counts for cuts from animal C were highest among all animals studied.

TABLE 3. *Frequency of occurrence of mesophilic and psychrotrophic bacteria on stored hot-boned and conventionally processed beef.*

	Mesophilic counts A		Mesophilic counts B		Psychrotrophic counts A		Psychrotrophic counts B		
Bacterial range	Hot-boned	Conv	Hot-boned	Conv	Hot-boned	Conv	Hot-boned	Conv	Remarks[2]
Log 0-2 CFU/cm²	6.7[1]	23.3	0	0	3.3	23.4	0	0	Low count
Log 3-4 CFU/cm²	16.7	6.7	3.3	10.0	20.0	6.7	3.3	10.0	Intermediate count
Log 5-6 CFU/cm²	10.0	3.3	10.0	6.7	10.0	3.3	10.0	6.7	High count
Log 7 CFU/cm²	0	0	3.3	0	0	0	3.3	0	Very high count
Total percentage	100%				100%				

A - Experiment 1, 10 steers.
B - Experiment 2, 5 steers.
Hot-boned - hot-boned cuts stored for 14 days.
Conv - conventional cuts stored for 14 days.
[1]Frequency of occurrence of samples in each bacteria range expressed in % of total number of samples (30 samples of each for mesophilic count and psychrotrophic count).
[2]Arbitrary designation for convenience of discussion.

TABLE 4. *Indicator and potential pathogenic organisms on hot-boned and conventionally processed beef samples.*[1]

	Coliform		Fecal coliform		*Clostridium perfringens*		*Salmonella*		*Staphylococcus aureus*		Fecal streptococci	
Animal	H-B	Conv	H-B	Conv	H-B	Conv	H-B	Conv	H-B	Conv	H-B	Conv
A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.60	1.36
B	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	0.48
C	D	ND	D	ND	D	ND	ND	ND	ND	ND	1.28	0.66
D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.78	1.04
E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.49	0.66
A	0.67[2]	ND	0.67	ND	0.45	ND	ND	ND	ND	ND	ND	ND
B	0.97	ND	0.70	ND	1.95	ND	ND	ND	ND	ND	2.15	ND
C	1.53	1.89	1.53	1.15	0.28	ND	ND	ND	0.08	0.57	3.26	ND
D	1.15	ND	0.45	ND	ND	ND	ND	ND	ND	ND	1.15	ND
E	0.45	ND	ND	ND	ND	ND	ND	ND	0.08	ND	2.53	ND

D = Detected.
ND = Not detected.
[1]Experiment 2.
[2]Log organisms or CFU/cm².
H-B = Hot-boned cuts.
Conv = Conventional cuts.

Excluding data from animal C would significantly reduce total counts and frequency of detecting pathogenic organisms. Even so, these data (including animal C) do not show that hot-boned meat is a health hazard because potential pathogens, when found, were in low numbers. No *Salmonella* were detected.

Hot-boned cuts chilled more slowly than conventional cuts for the first 24 h of chilling. Conventionally chilled semimembranosus muscles (intact on the carcass) reached 21 C in 11 h compared with 14.4 h for the packaged and boxed counterparts (data not presented). In the conventional treatment, it took 4.6-6 h for the longissimus muscle to reach 21 C while it took 7.4-7.8 h for the hot-boned counterparts to reach 21 C.

In the boxed samples (Fig. 1), it took 6.4 h for the interior and 3 h for the exterior of hot-boned cuts to reach 21 C while it took 1.7 h for the chilled carcasses to reach 21 C. The 0-time temperatures (Fig. 1) were taken after sample preparation which was approximately 3 h after slaughter. Expressed in C-h (degree C above 21 C and time to reach 21 C), microorganisms had 26.1 C-h and 7.5 C-h to grow in the interior and exterior of boxed hot-boned cuts compared with 2.1 C-h in the carcass. The second experiment resembles possible practices in a hot-boning operation designed to produce subprimal cuts used for steak and roast production.

The greater number of organisms recovered from hot-boned cuts may have stemmed from the time-temperature differences in the rapid microbial growth region (above 21 C) compared with conventionally chilled beef. Consequently, the bacteria had the initial impetus to multiply in storage, resulting in higher counts and a greater recovery of indicator organisms and potential pathogens compared with the conventional cuts after 14 days in vacuum storage. Additionally, surfaces of hot-boned cuts were handled during fabrication, providing for a greater potential for bacterial growth during chilling than if the carcass were left intact.

The mesophilic and psychrotrophic counts (except for those of cuts from one animal) were similar to those reported by others (*1,6,9,10,11*) for hot-boned cuts (Log 3-6 CFU/cm²). Our storage time was generally longer (14 days) than times others used. Indicator and potential pathogens, when detected, were not numerous and mainly from cuts from one animal, with no *Salmonella* detected.

Most hot-boned cuts processed and stored under the experimental conditions we used were bacteriologically acceptable — with Log 7 CFU/cm² as an arbitrary index of spoilage. However, our data indicate that temperature control of hot-boned meat during early hours of chilling is critical. We are evaluating the efficacy of chilling hot-boned meat more rapidly (before or after boxing) to reduce microbial growth without creating tenderness problems stemming from rapid chilling (cold shortening) of pre-rigor muscle.

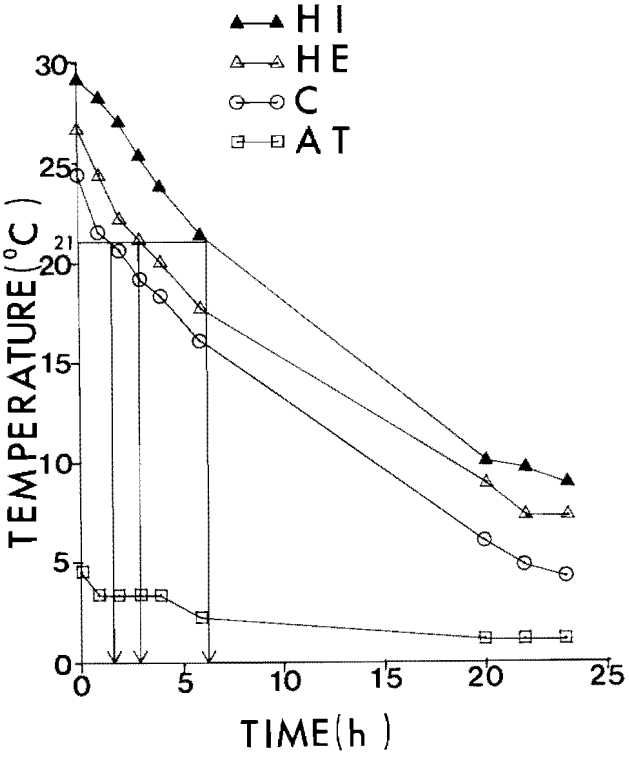

Figure 1. *Temperature decline of boxed, hot-boned and conventionally processed beef samples (Experiment 2). HI = Internal temperature of boxed hot-boned cuts. HE = External temperature of boxed hot-boned cuts. C = Temperature of carcass conventionally chilled. AT = Ambient temperature.*

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REFERENCES

1. Follett, M. J., G. A. Norman, and P. W. Ratcliff. 1974. The ante rigor excision and air cooling of beef semimembranosus muscles at temperatures between − 5 C to + 15 C. J. Technol. 9:509-523.
2. Food and Drug Administration. 1972. Bacteriological analytical manual. USDHEW, Washington, D.C.
3. Fung, D. Y. C., and A. A. Kraft. 1969. Rapid evaluation of viable cell counts using the Microtiter system and MPN technique. J. Milk Food Technol. 32:408-409.
4. Kastner, C. L. 1977. Hot processing — update on potential energy and related economies. Proc. 13th Meat Ind. Res. Conf., March 24-24, 1977. University of Chicago.
5. Klein, H., and D. Y. C. Fung. 1976. Identification and quantification of fecal coliform using violet red bile agar at elevated temperature. J. Milk Food Technol. 39:768-770.
6. Kotula, A. W., and B. S. Emswiler. 1978. Effect of hot-boning in microbial counts of selected primal and ground beef. Am. Soc Animal Sci. Abstract 47:288-289.
7. Lazarus, C. R., A. Abu-Baker, R. L. West, and J. L. Oblinger. 1977. Comparison of microbial counts on beef carcasses by using the moist swab contact method and secondary tissue removal technique. Appl. Environ. Microbiol. 33:217-218.
8. Marth, E. H. (ed.). 1978. Standard methods for the examination of dairy products, 14th ed. Am. Public Health Assoc., Inc., Washington, D.C.
9. McLeod, K., K. V. Gilbert, R. Wyborn, L. M. Wenham, C. L. Davey, and R. H. Locker. 1973. Hot cutting of lamb and mutton. J. Food Technol. 8:71-78.

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tubing life, as evidence by this study. However, after the initial large losses, little difference was obvious in the rate of PAE extraction by any of the fluids used.

REFERENCES

1. Calley, D. J., J. Autian, and W. L. Guess. 1966. Toxicology of a series of phthalate esters. J. Pharm. Sci. 55:159.
2. Carpenter, C. P., C. S. Weil, and H. F. Smyth, Jr. 1953. Chronic oral toxicity of di-2-ethylhexyl phthalate in rats, guinea pigs, and dogs. Arch. Ind. Hyg. 8:219.
3. Cerbulis, J., and J. S. Ard. 1967. Method for isolation and detection of dioctyl phthalate from milk lipids. J. Assoc. Offic. Anal. Chem. 50:646.
4. Dillingham, E. O., and J. Autian. 1973. Teratogenicity, mutagenicity and cellular toxicity of phthalate esters. Environ. Health Perspectives 3:81-90.
5. Epstein, S. S., and H. Schafner. 1968. Chemical mutagens in the human environment. Nature 219:385.
6. Gesler, R. M. 1973. Toxicology of di-2-ethylhexyl and other phthalic acid ester plasticizers. Environ. Health Perspectives 3:73-80.
7. Graham, P. R. 1973. Phthalate ester plasticizers --why and how they are used. Environ. Health Perspectives 3:3-12.
8. Guess, W. L. 1967. Characterization of subtle toxicity of certain plastic components used in the manufacture of polyvinyls. Am. J. Hospital Pharm. 24:495.
9. Guess, W. L., and S. Haberman. 1968. Toxicity profiles of vinyl and polyolefinic plastics and their additives. J. Biomed. Mater. Res. 2:313.
10. Harris, R. S. 1956. Chronic oral toxicity of DEHP in rats and dogs. Arch. Ind. Health 14:259.
11. Hites, R. A. 1973. Phthalates in the Charles and Merrimack Rivers. Environ. Health Perspectives 3:17-22.
12. Hodge, H. C. 1943. Acute toxicity for rats and mice of 2-ethylhexanol and 2-ethylhexyl phthalate. Proc. Soc. Exp. Biol. Med. 53:20.
13. Horwitz, W. (ed.). 1975. Official methods of analysis of the Association of Official Analytical Chemists, 12th ed. AOAC, Washington, D.C.
14. Jaeger, R. J., and R. J. Rubin. 1970. Contamination of blood stored in plastic bags. Lancet 2:151.
15. Jaeger, R. J., and R. J. Rubin. 1973. Phthalate ester metabolism in the isolated, perfused rat liver system. Environ. Health Perspectives 3:49-51.
16. Jaeger, R. J., and R. J. Rubin. 1973. Extraction, localization and metabolism of di-2-ethylhexyl phthalate from PVC plastic medical devices. Environ. Health Perspectives 3:95-102.
17. Krauskopf, L. G. 1973. Studies on the toxicity of phthalates via ingestion. Environ. Health Perspectives 3:61-72.
18. Lawrence, W. H., M. Malik, J. E. Turner, A. R. Singh, and J. Autian. 1975. A toxicological investigation of some acute short term, and chronic effects of administering di-2-ethylhexyl phthalate (DEHP) and other phthalate esters. Environ. Res. 9:1.
19. Marcel, Y. L., and S. P. Noel. 1970. Contamination of blood stored in plastic packs. Lancet 1:35.
20. Marx, J. L. 1972. Phthalic acid esters: biological impact uncertain. Science 178:46.
21. Mayer, T. L., Jr., and H. O. Sanders. 1973. Toxicology of phthalic acid esters in aquatic organisms. Environ. Health Perspectives. 3:153-158.
22. Mayer, T. L., Jr., D. L. Stalling, and J. L. Johnson. 1972. Phthalate esters as environmental contaminants. Nature 238:411.
23. Metcalf, R. L., G. M. Booth, C. K. Schuth, D. J. Hansen, and P.-Y. Lee. 1973. Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. Environ. Health Perspectives 4:27-34.
24. Nematollhi, J., W. L. Guess, and J. Autian. 1967. Plasticizers in medical application. J. Pharm. Sci. 56:1446.
25. Ogner, G., and M. Schnitzer. 1970. Humic substances: fulvic acid dialkyl phthalate complexes and their role in pollution. Science 170:317.
26. Peters, J. W., and R. M. Cook. 1973. Effect of phthalate esters on reproduction in rats. Environ. Health Perspectives 3:91-94.
27. Rubin, R. J., and R. J. Jaeger. 1973. Some pharmacologic and toxicologic effects of di-2-ethylhexyl phthalate (DEHP) and other plastizicers. Environ. Health Perspectives 3:53-60.
28. Schaffer, C. B., C. P. Carpenter, and H. F. Smyth, Jr. 1945. Acute and subacute toxicity of di-2-ethylhexyl phthalate with a note upon its metabolism. J. Ind. Hyg. Toxicol. 27:130.
29. Schulz, C. O., and R. J. Rubin. 1973. Distribution, metabolism, and excretion of di-2-ethylhexyl phthalate in the rat. Environ. Health Perspectives 3:123-130.
30. Singh, A. R., W. H. Lawrence, and J. Autian. 1972. Teratogenicity of phthalate esters in rats. J. Pharm. Sci. 61:51.
31. Wildbrett, G. 1973. Diffusion of phthalic acid esters from PVC milk tubing. Environ. Health Perspectives 3:29-36.

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Royal Soc. Health J. (U.K.) 92:121-130.
17. Malton, R. 1976. Refrigerated retail display for fresh meat. Inst. Meat Bull. (U.K.) 91:17-19.
18. Michener, H. D., and R. P. Elliott. 1964. Minimum growth temperatures for food poisoning, fecal-indicator and psychrophilic microorganisms. Adv. Food Res. 13:340-396.
19. Pivnick, H., I. E. Erdman, D. Collins-Thompson, G. Roberts, M. A. Johnston, D. R. Conley, G. Lachapelle, U. T. Purvis, R. Foster, and M. Milling. 1976. Proposed microbiological standards for ground beef based on a Canadian survey. J. Milk Food Technol. 39:408-412.
20. Snedecor, G. W., and W. G. Cochran. 1972. Statistical methods (6th ed.). The Iowa State University Press, Ames, Iowa.
21. Taylor, A. D. 1973. Gases in fresh meat packaging. Inst. Meat Res. Bull. (Bristol) 79:26-32.
22. Tompkin, R. B. 1973. Refrigeration temperature as an environmental factor influencing the microbial quality of food - a review. Food Technol. 27:54-58.
23. Witter, L. D. 1961. Psychrotrophilic bacteria - a review. J. Dairy Sci. 44:983-1015.

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10. McLeod, K., K. V. Gilbert, S. J. Fairbairn, and R. H. Locker. 1974. Further experiments on the hot-cutting of lamb. J. Food Technol. 9:179-184.
11. Schmidt, G. R., and K. V. Gilbert. 1970. The effect of muscle excision before the onset of rigor mortis on palatability of beef. J. Food Technol. 5:331-338.
12. Thomas, J. D., D. M. Allen, M. C. Hunt, and C. L. Kastner. 1977. Nutritional regime, post-slaughter conditioning temperature, and vacuum packaging effects on bacteriology of beef carcasses and retail meat cuts. J. Food Prot. 40:678-682.