HACCP
Supporting Documentation

[redacted]

Letter of Guaranty: Processing Aids

The USDA Food Safety and Inspection Service, FSIS Directive 5000.1, *"Verifying an Establishment's Food Safety System Handbook"* requires an establishment to develop and employ sanitation or processing procedures that meet USDA regulatory sanitation performance objectives.
The products listed below have ingredients which are either: (1) approved by the Department of Health and Human Services of the Food and Drug Administration (FDA) as secondary direct food additives as noted in 21 CFR Part 173; (2) GRAS substances listed in 21 CFR Parts 182, 184; or (3) are permitted by the USDA Food Safety and Inspection Service as noted in 9 CFR Parts 318, 381 or 424.

The product is safe and effective and will not adulterate food product when used as a processing aid under the intended conditions for use as described on the product label, catalog sheet, or specified in a Standard Operating Procedure (SOP).

[redacted]

This letter is only applicable for products made in the USA as indicated on the product label. Please contact your Ecolab representative for inquiries and letter of guaranty requests.

Sincerely,

[redacted]

Updated: May 17, 2013

The Letter of Guaranty (LOG) status is reviewed each time a formula change is considered. This letter remains in effect as long as the formula does not significantly change.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service

Food and Drug Administration
College Park, MD 20740

December 11, 2012

[redacted]

Re: Food Contact Substance Notification (FCN) 001236
Acknowledgment Letter

Dear Mr. [redacted]

This letter acknowledges receipt of your notification, FCN 001236, on October 15, 2012, submitted on behalf of [redacted] for the food contact substance and use described as follows:

FCS:
[redacted]

Notifier:
[redacted]

Manufacturer/Supplier:
[redacted]

Intended Use:

As an antimicrobial agent on meat carcasses, parts, trims and organs. The FCS will be added as a spray, rinse, dip, chiller water or scald water.

Limitations/Specifications:

The FCS will be used in accordance with current industry practice where the process solution will not exceed the following component concentrations: [redacted]

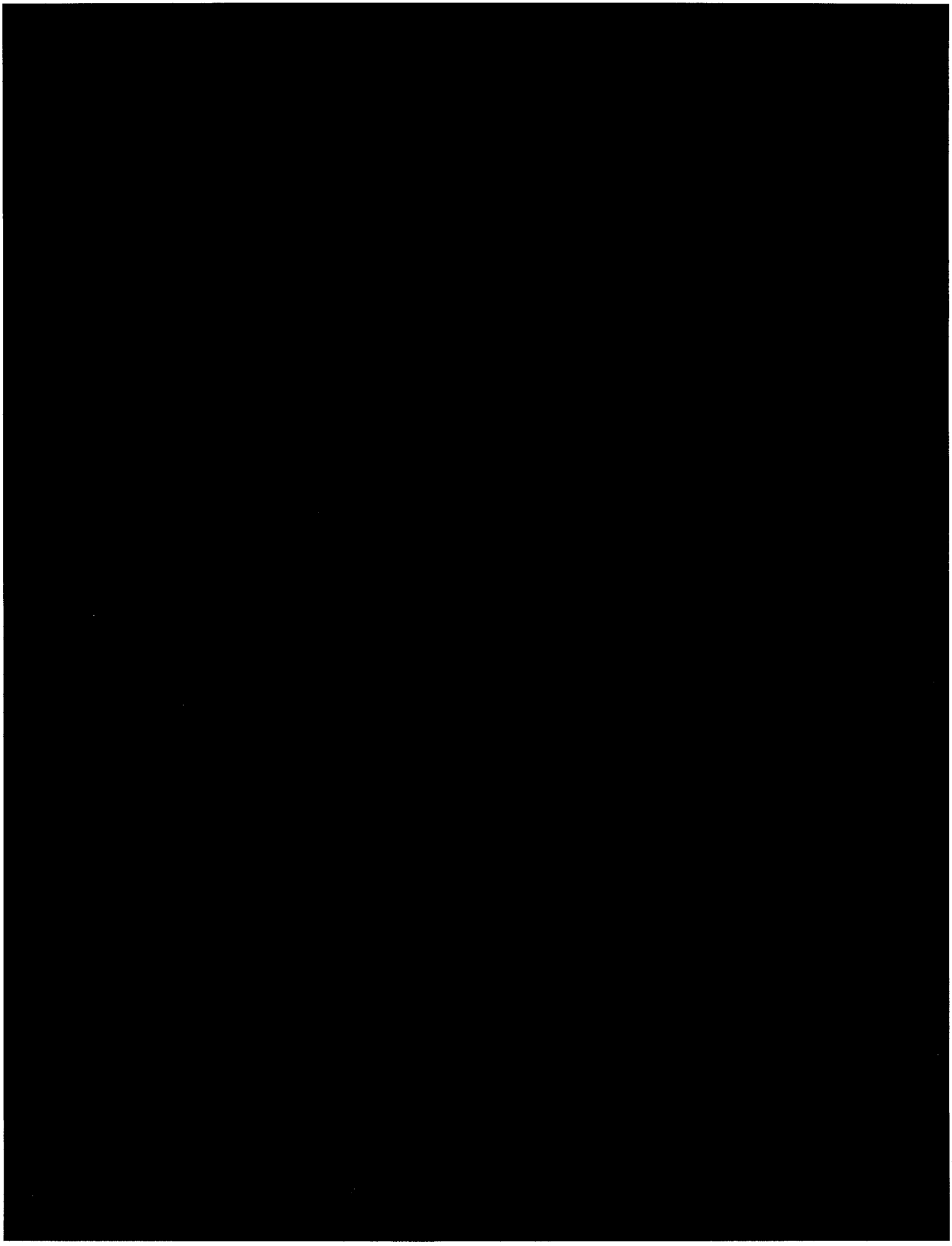
If we do not object to your notification prior to February 12, 2013, the notification will become effective on that date. If your notification becomes effective, it will be added to the list of effective notifications available on the agency's internet site. This can be accessed from the Internet in the

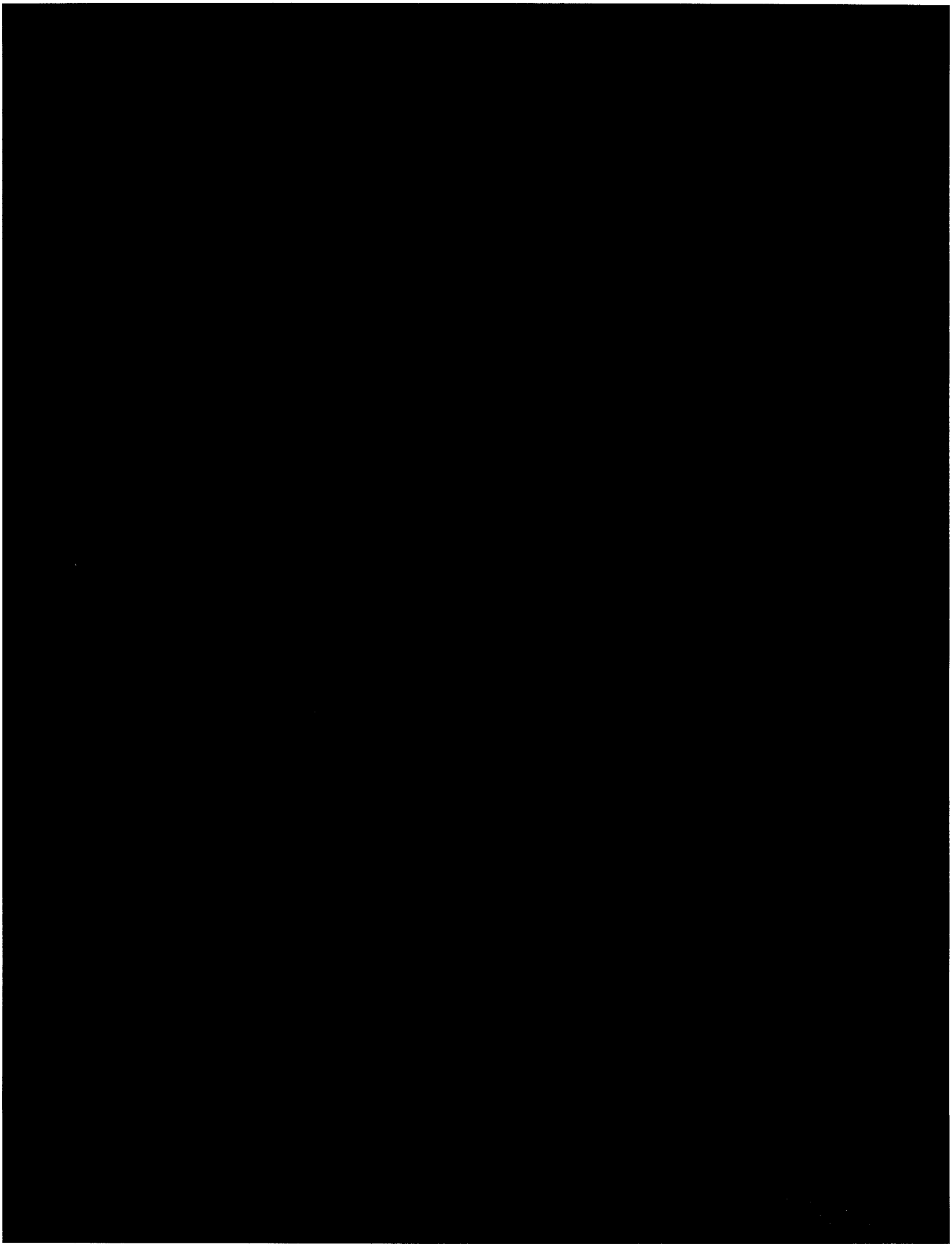
Food Ingredients and Packaging section under the Food Topic of www.fda.gov. The above description will be used by FDA to describe your notification should it become effective. Accordingly, please review the description for technical accuracy, review the environmental assessment for confidential information and provide us with any comments within 30 days from the date of this letter. If your comments result in changes to the identity or intended use of the substance, FDA will evaluate whether the changes affect the adequacy of information in your original FCN. If that adequacy is affected, the agency will request additional information to support the changes in identity or intended use. A new 120-day statutory time period will begin the date we receive the requested information.

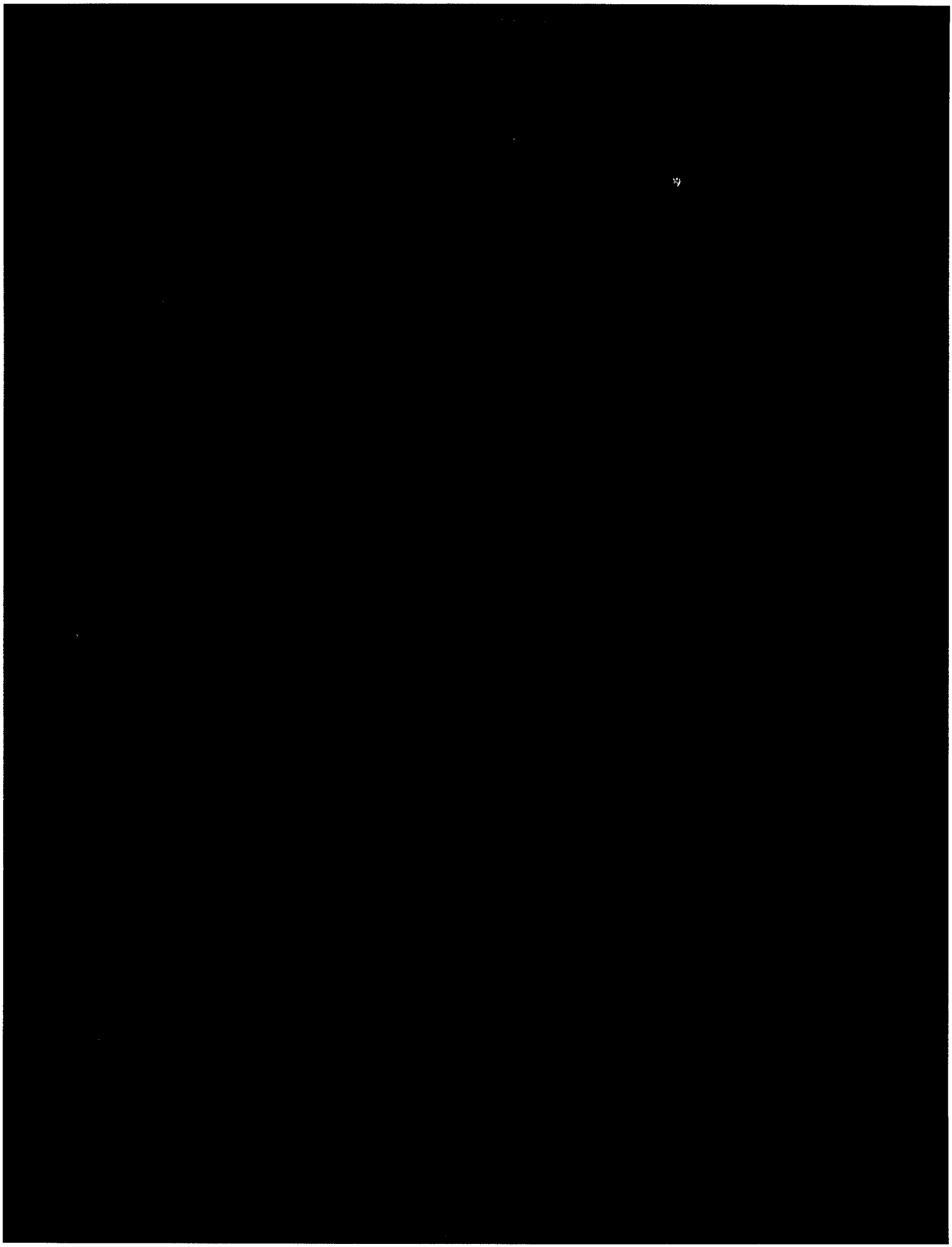
If you have any further questions concerning this matter, please do not hesitate to contact us.

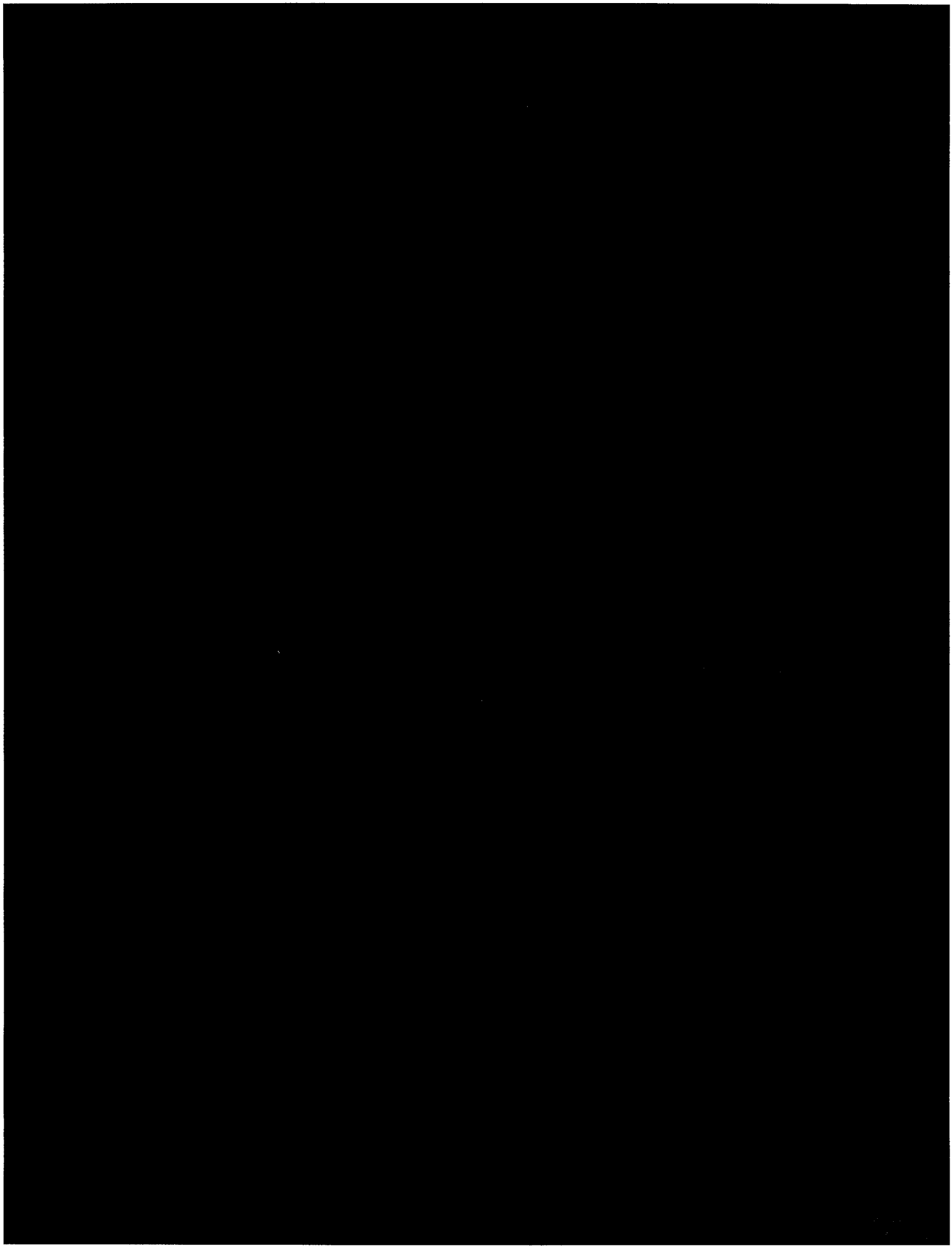
Sincerely,

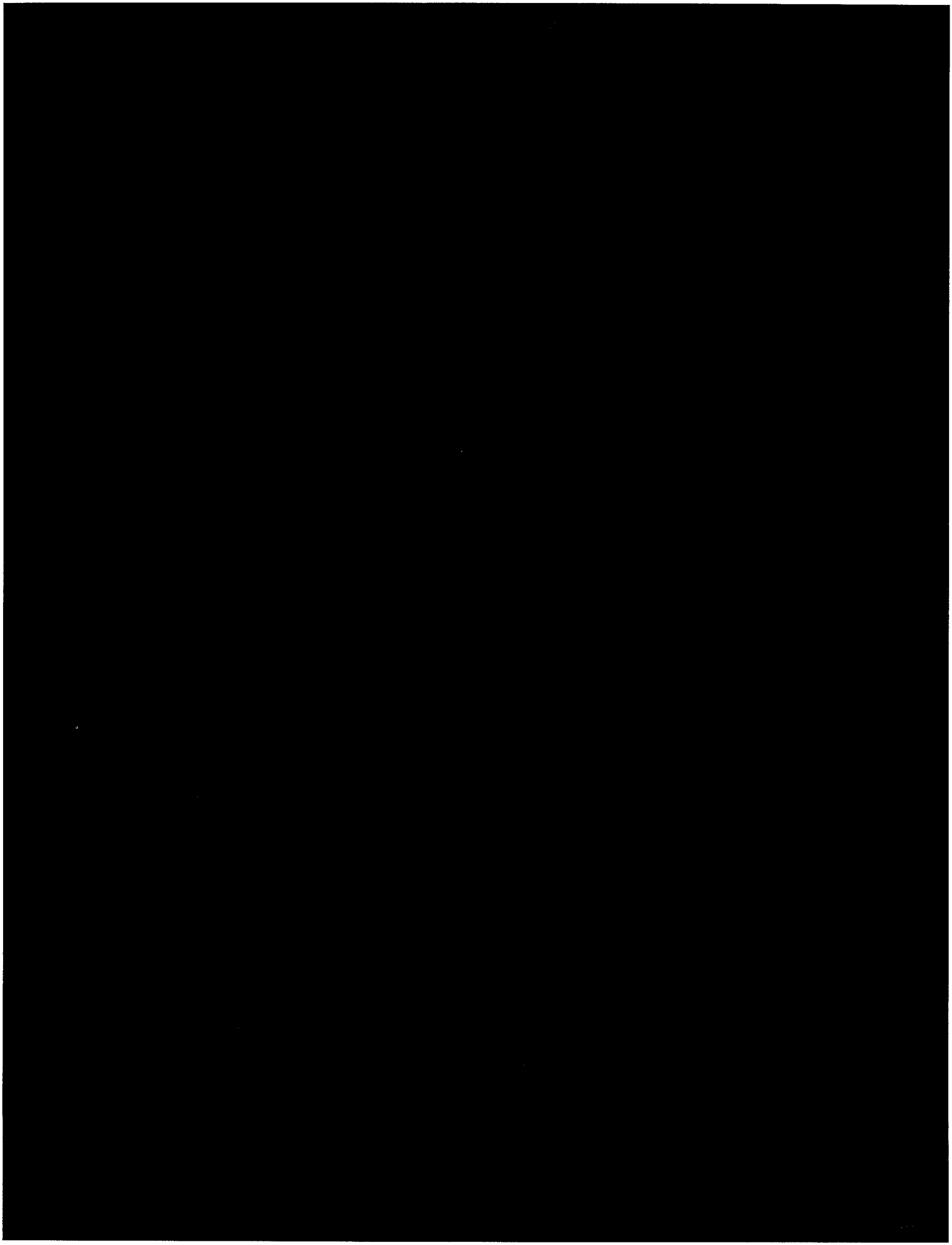
Kelly M. Randolph, D.V.M., M.P.H.
Division of Food Contact Notifications, HFS-275
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

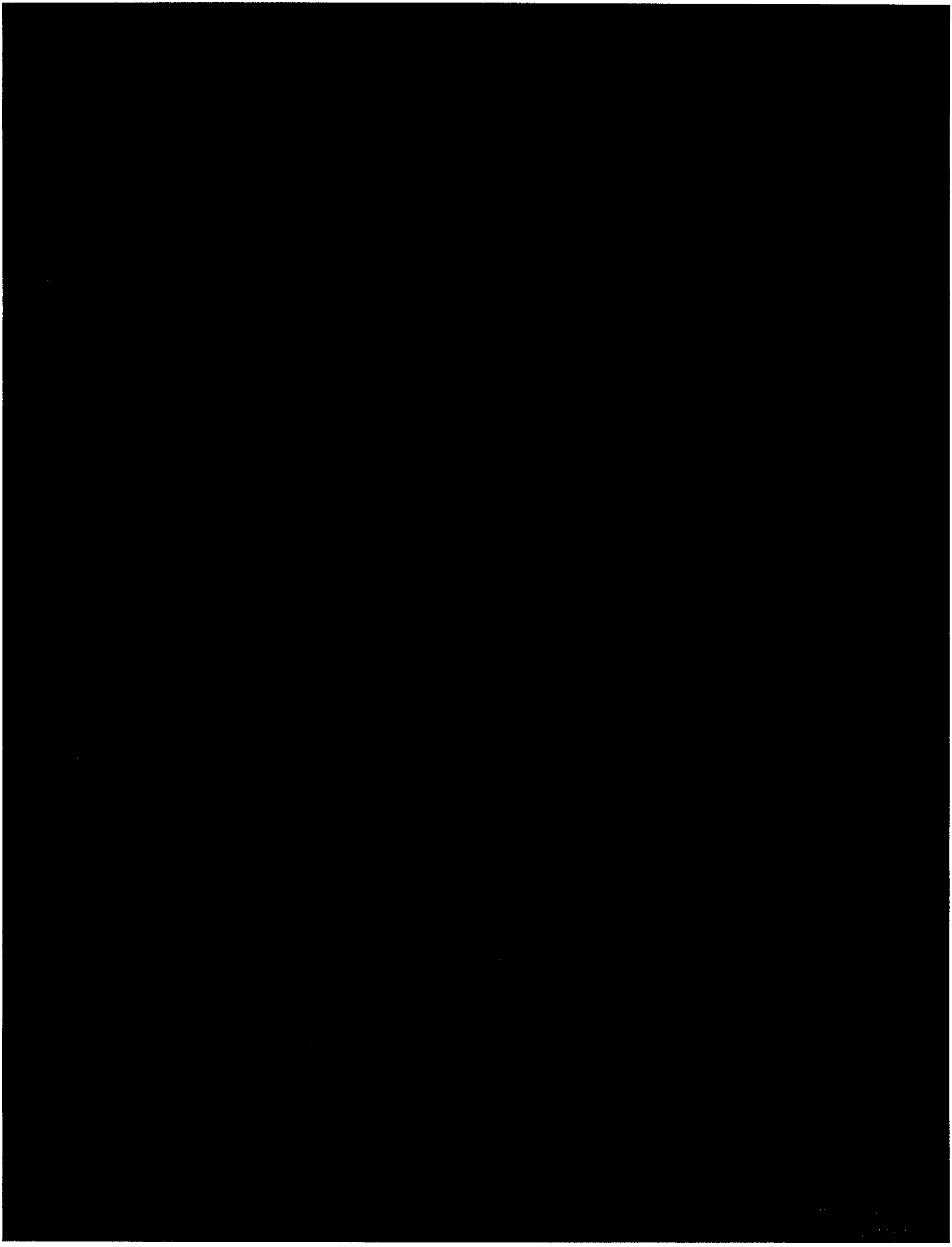


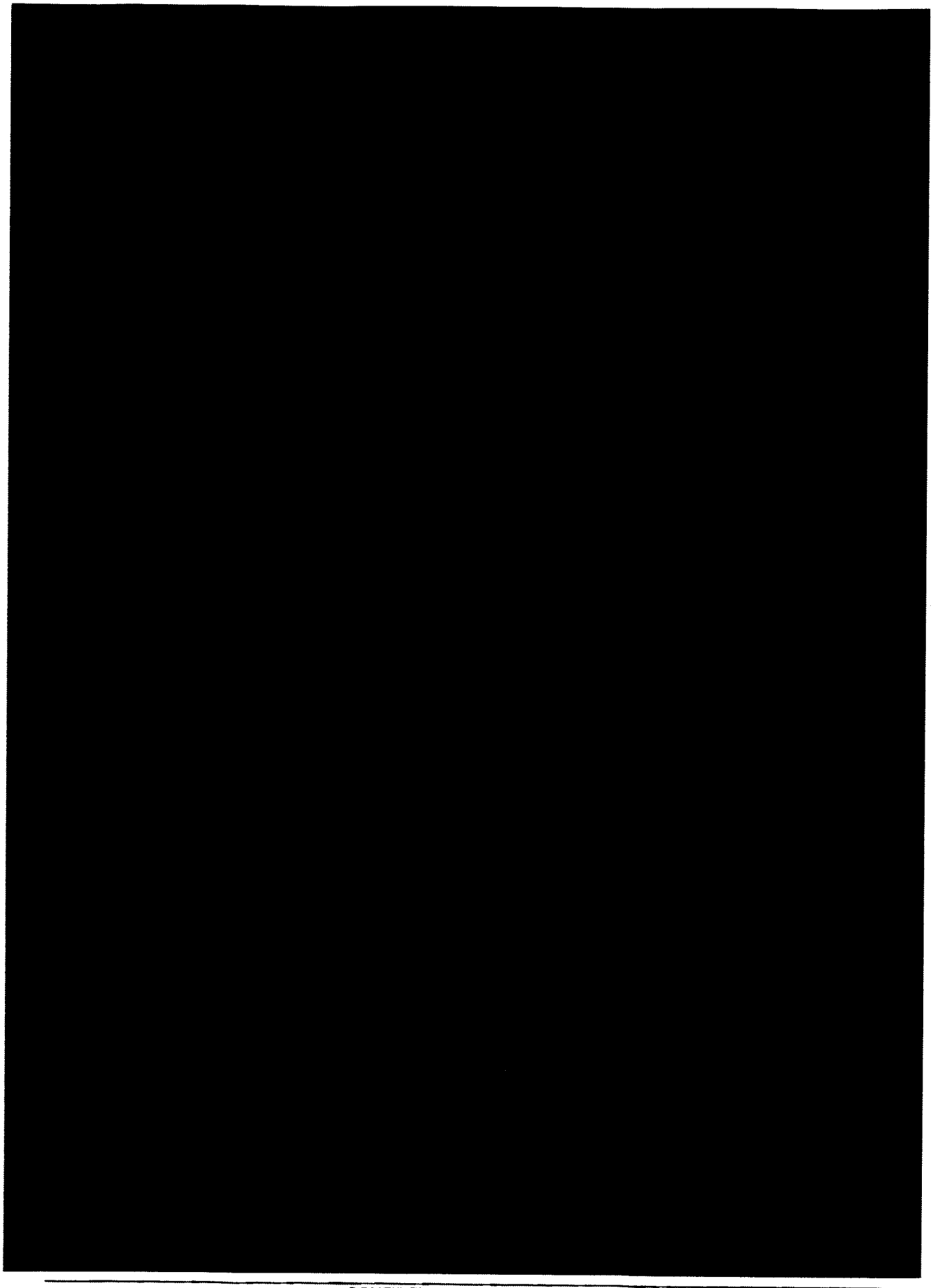


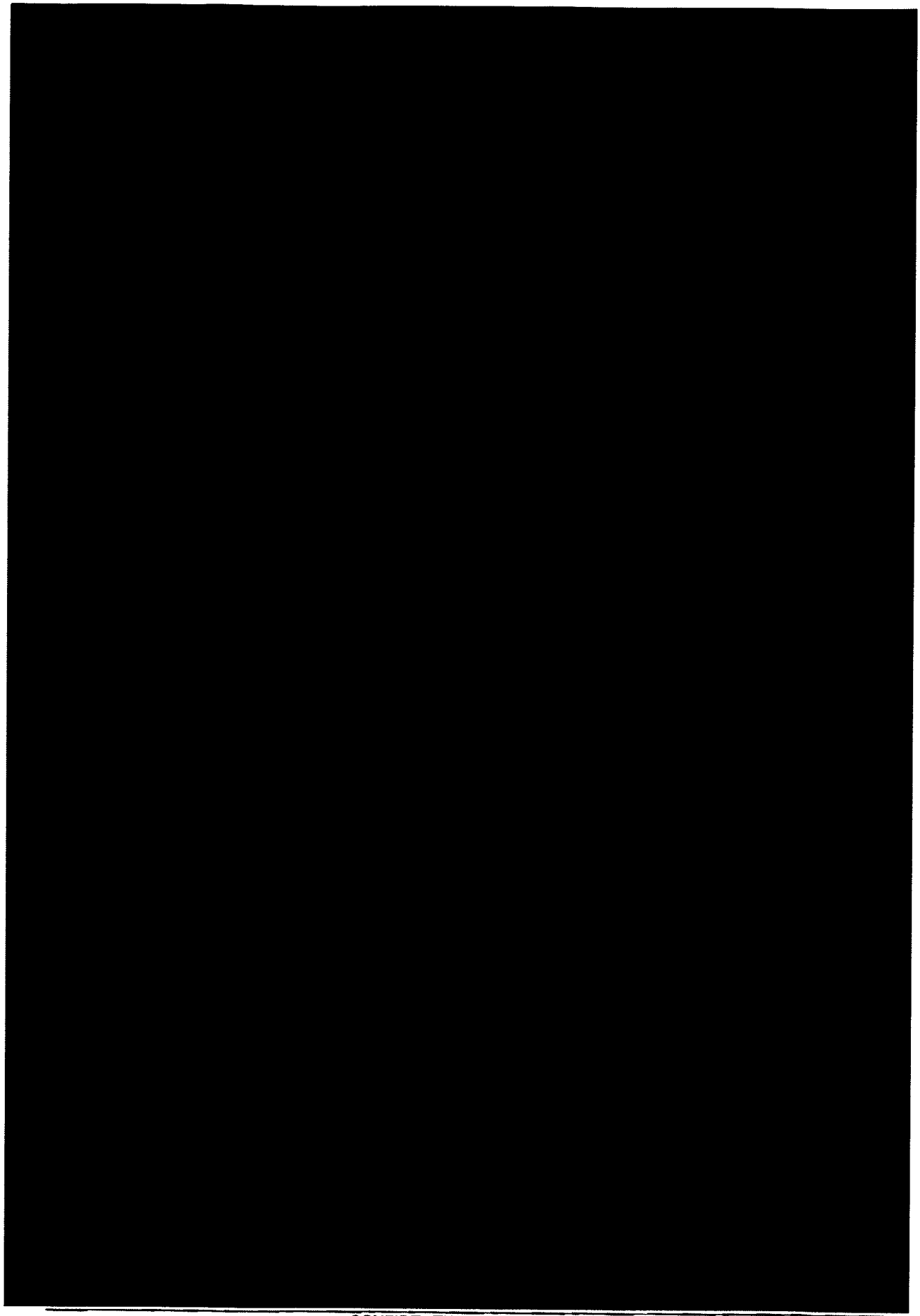


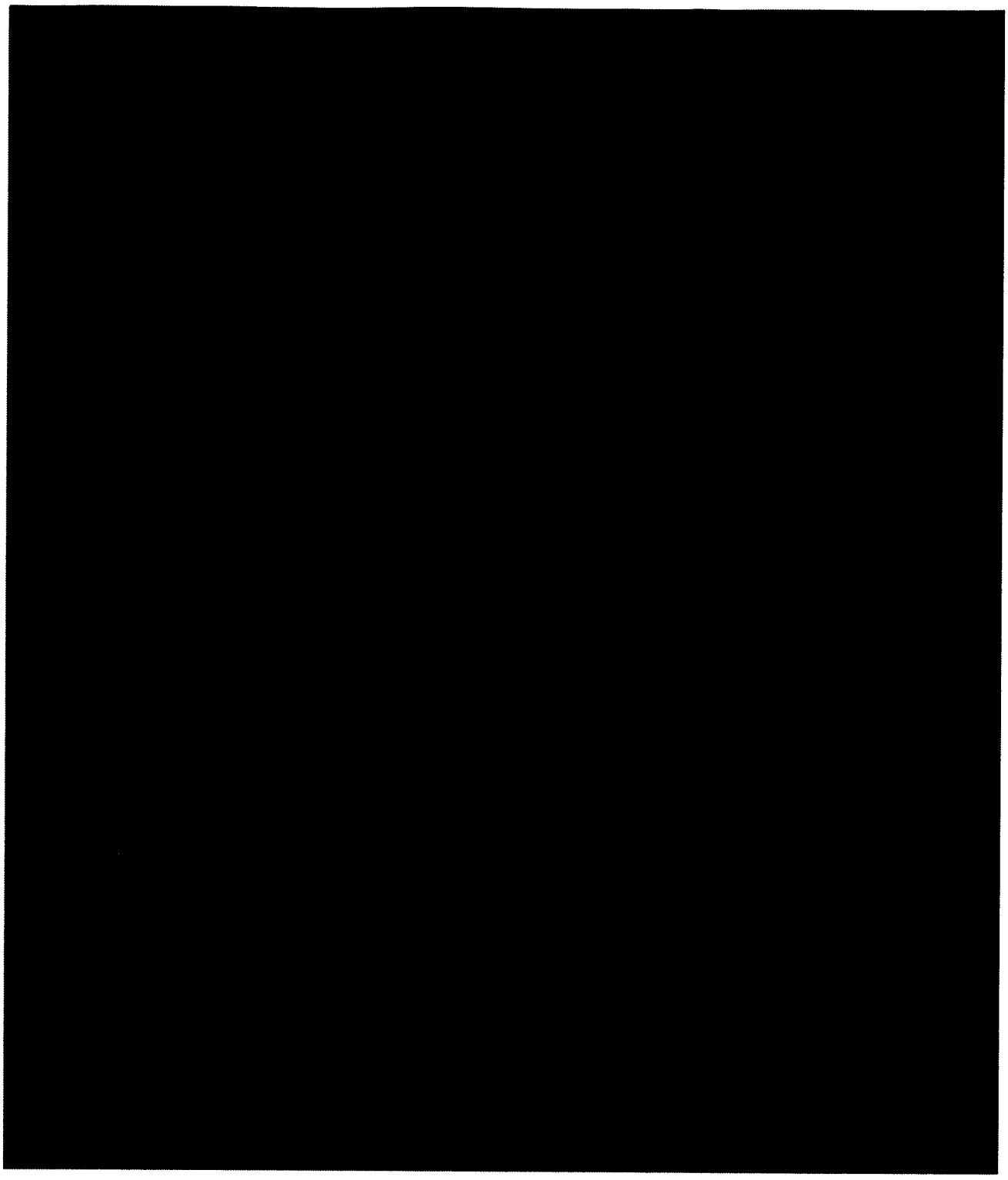


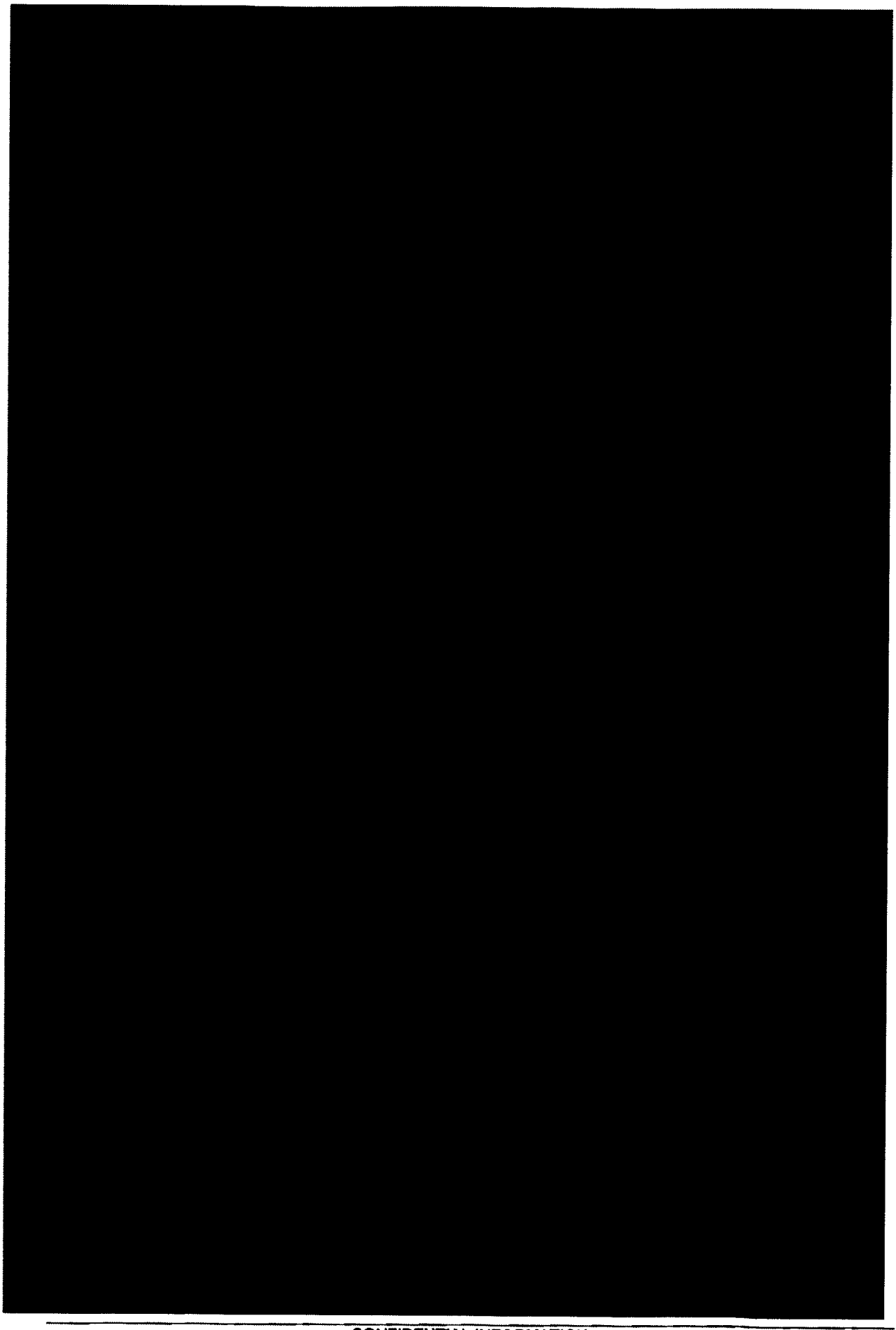


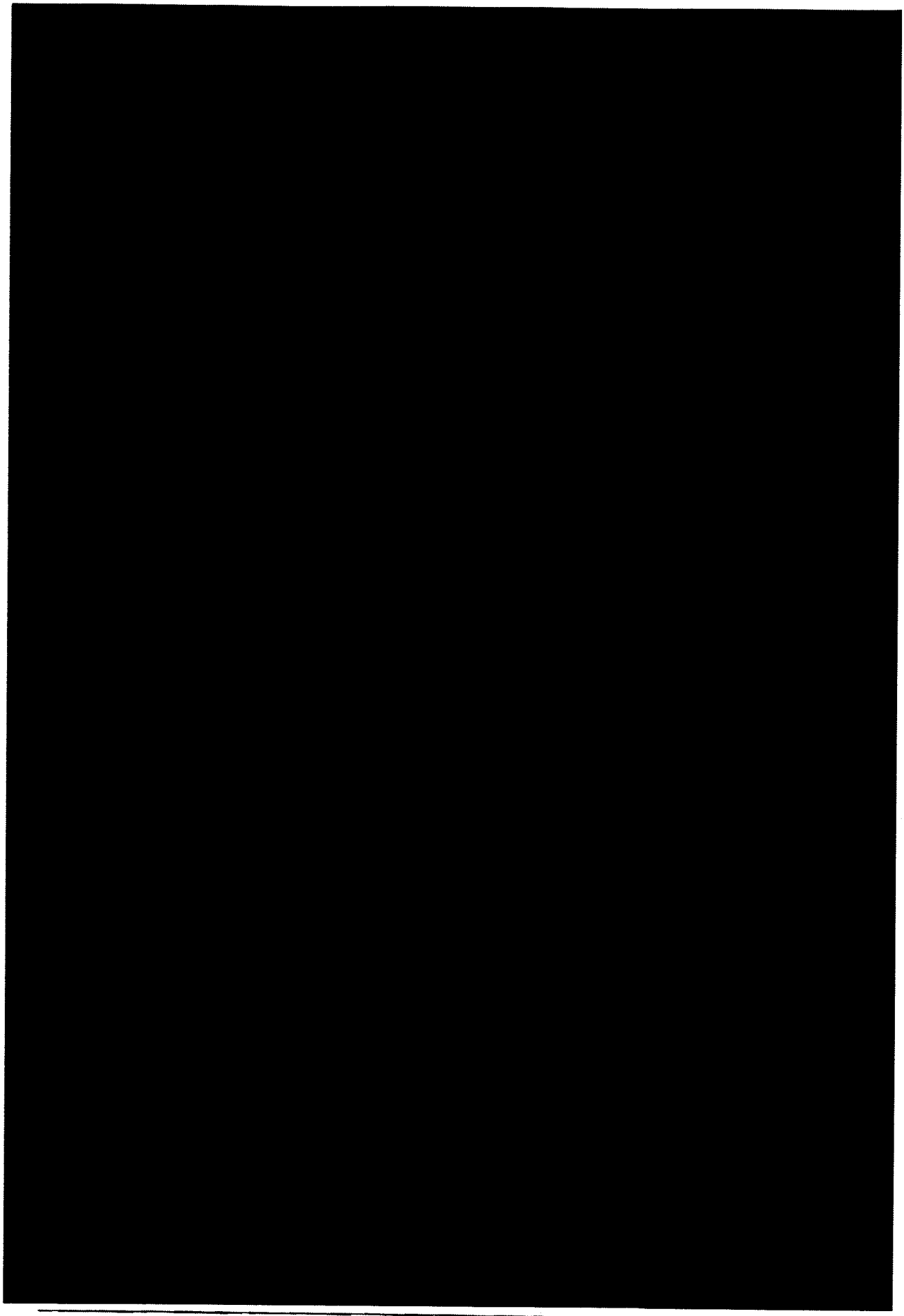


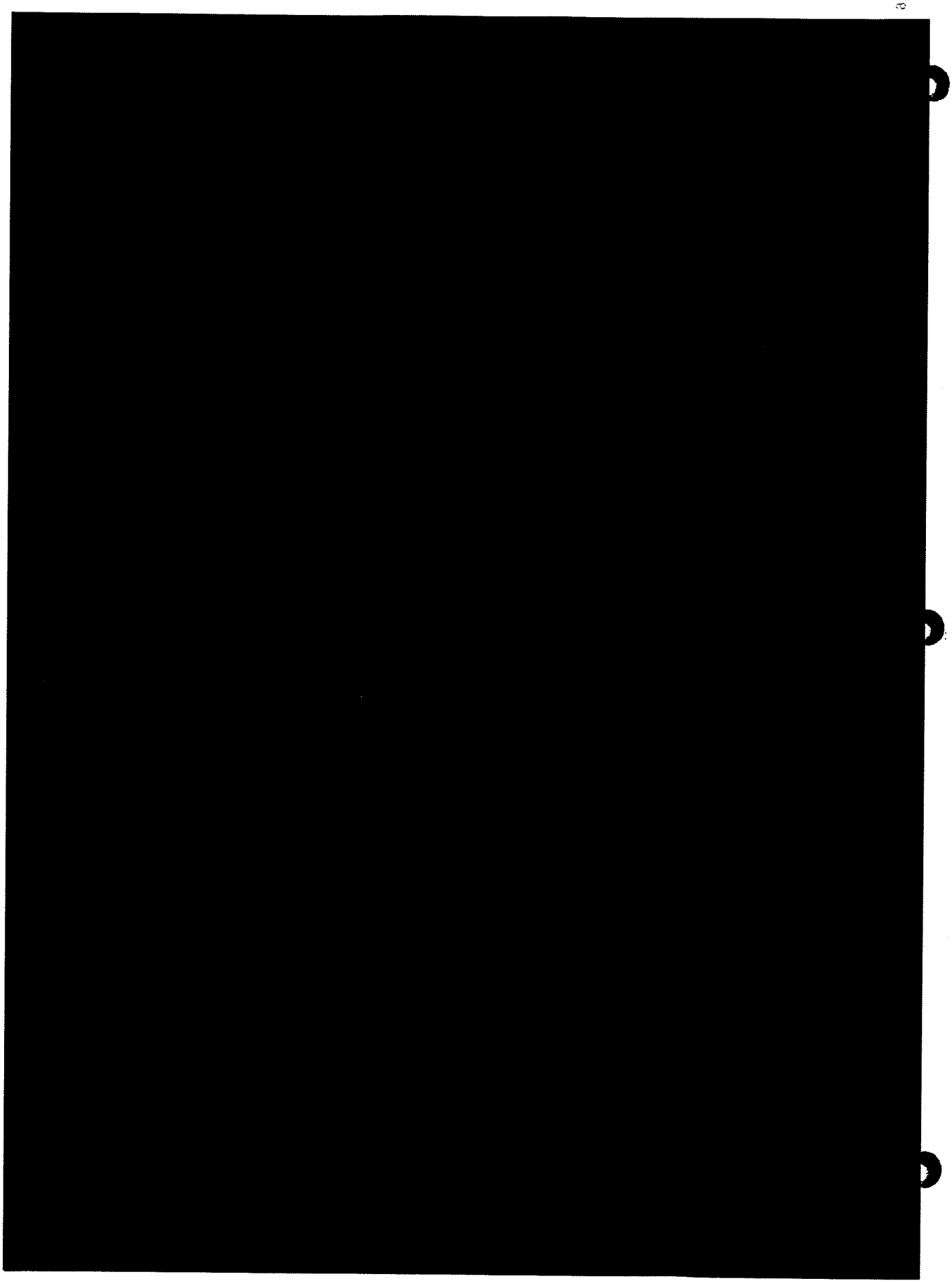


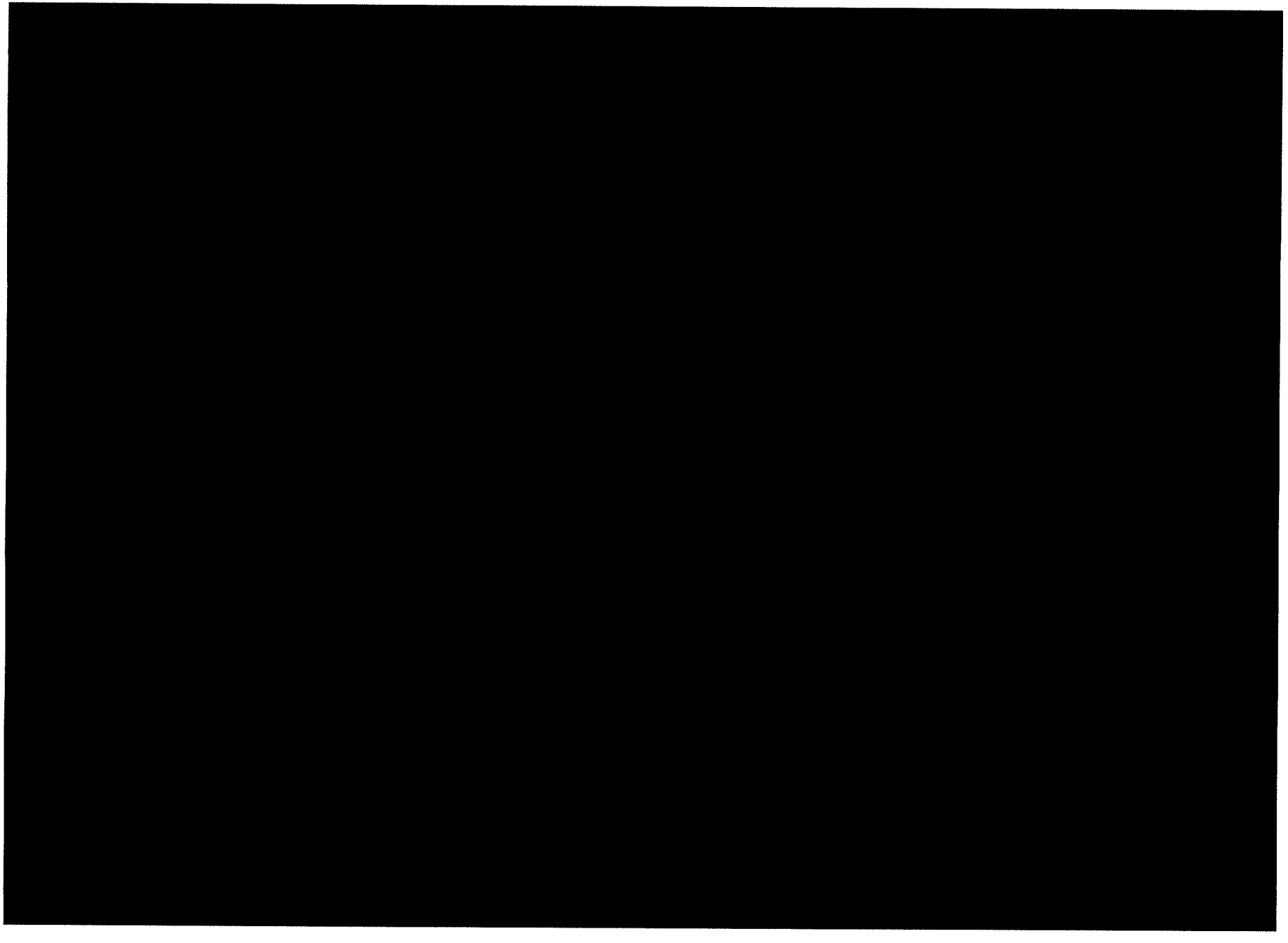


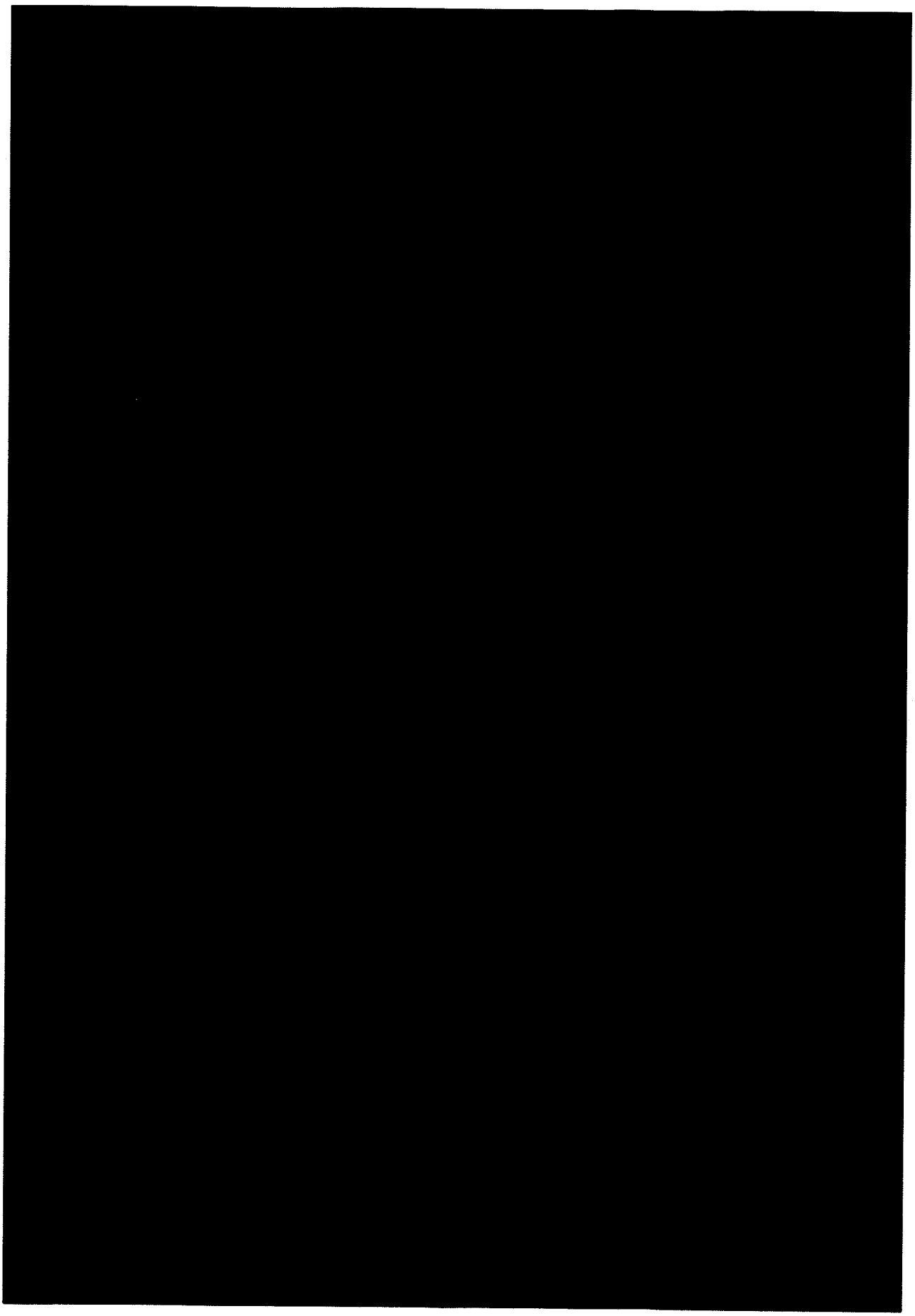


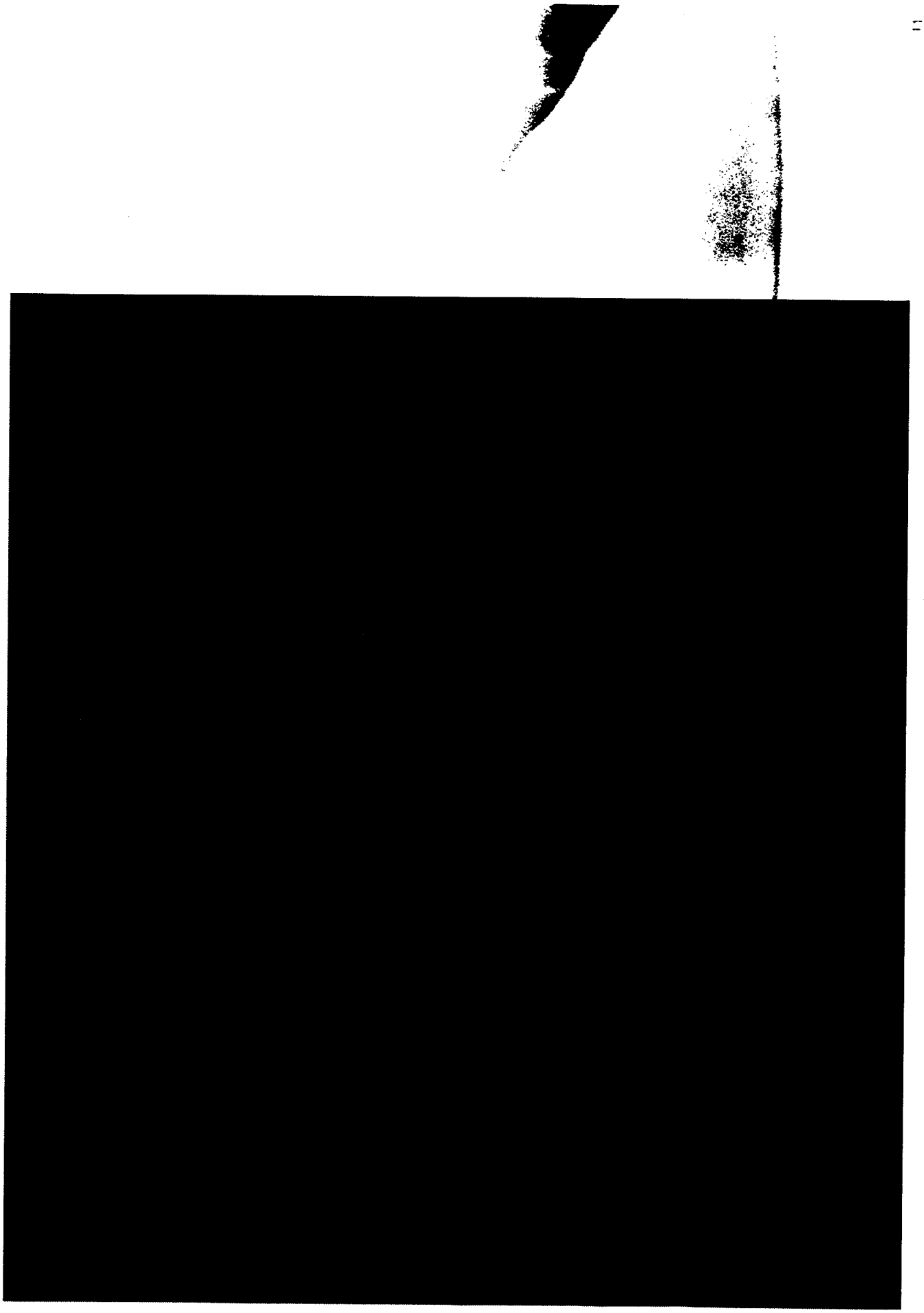




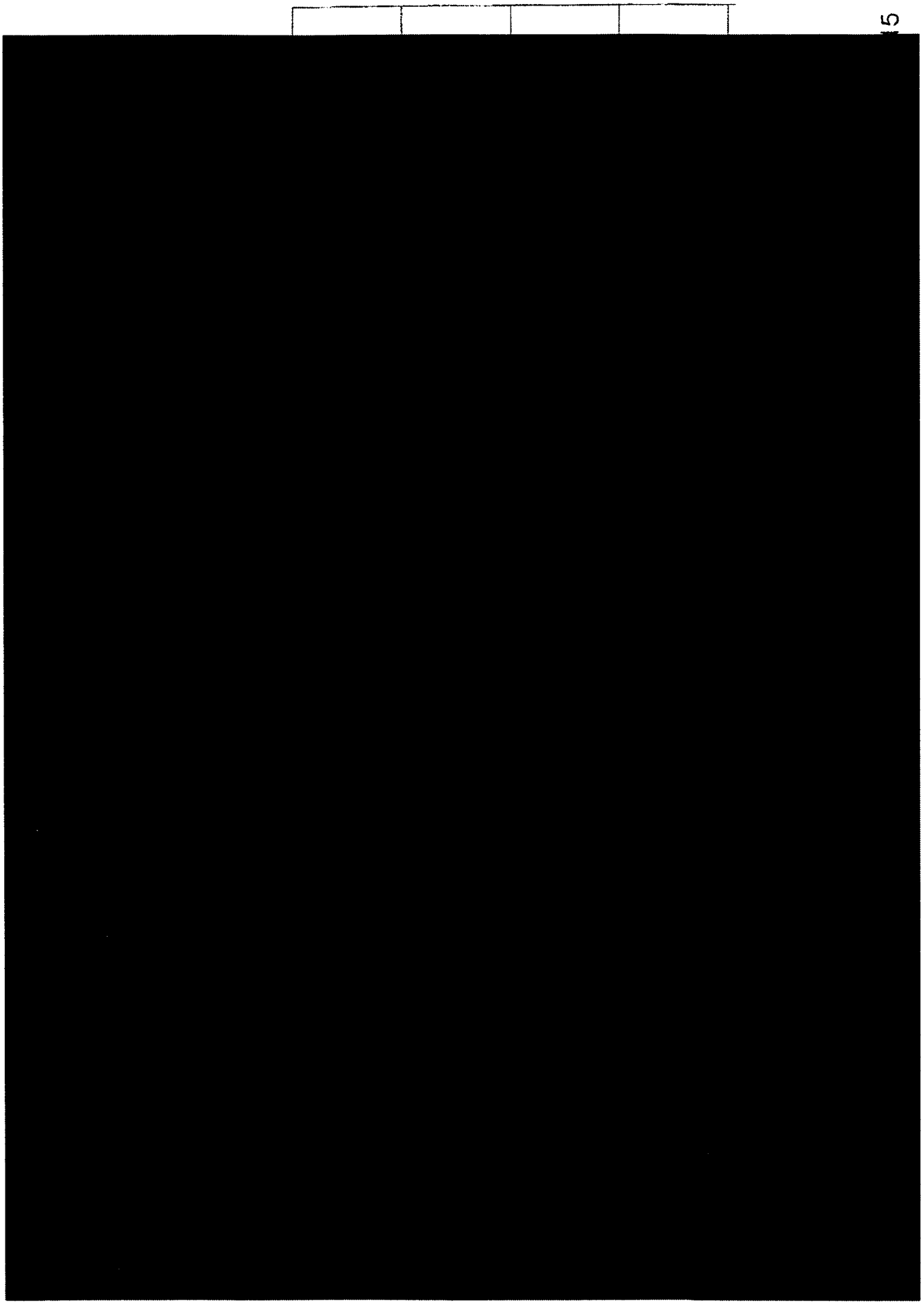


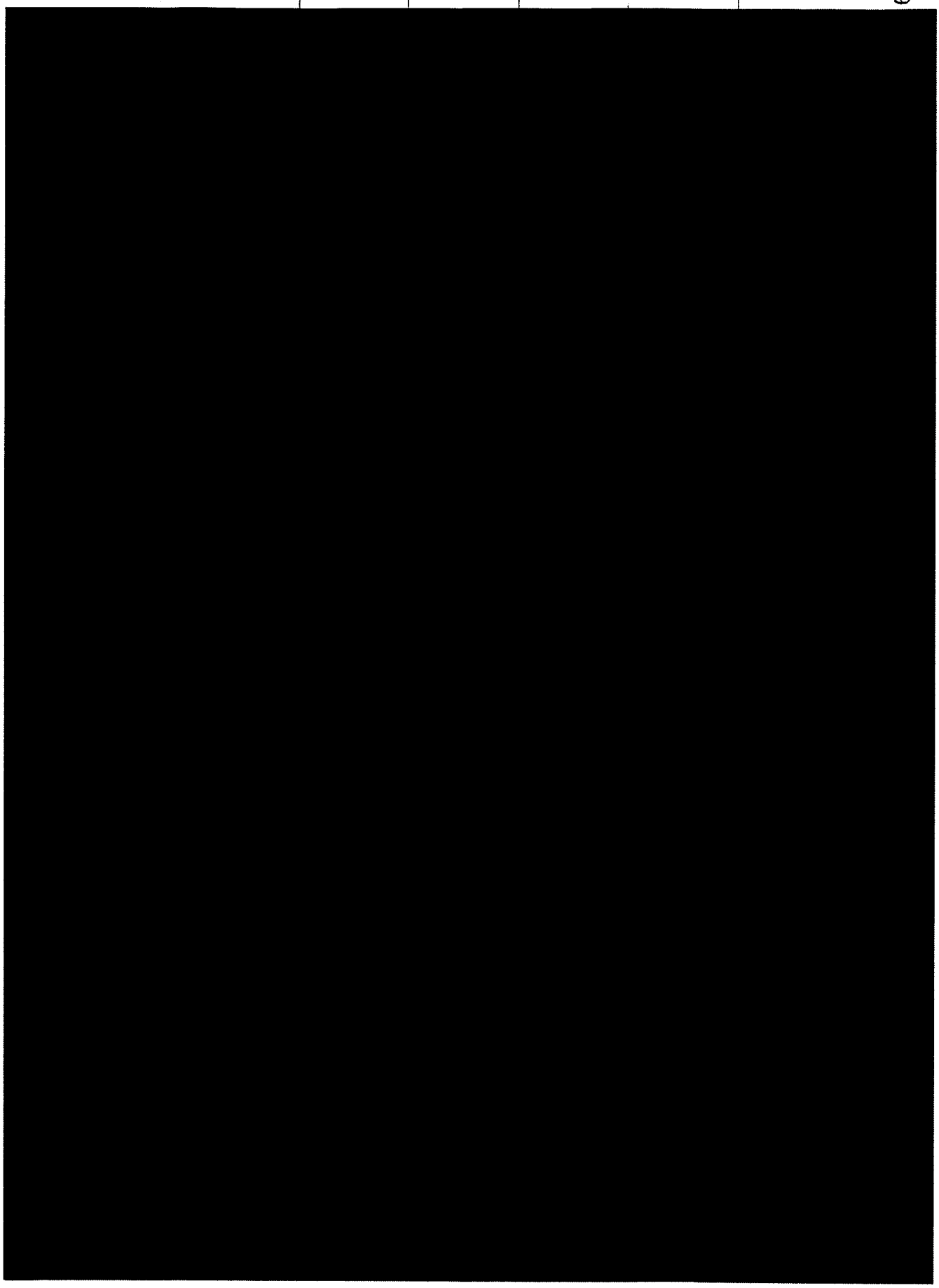






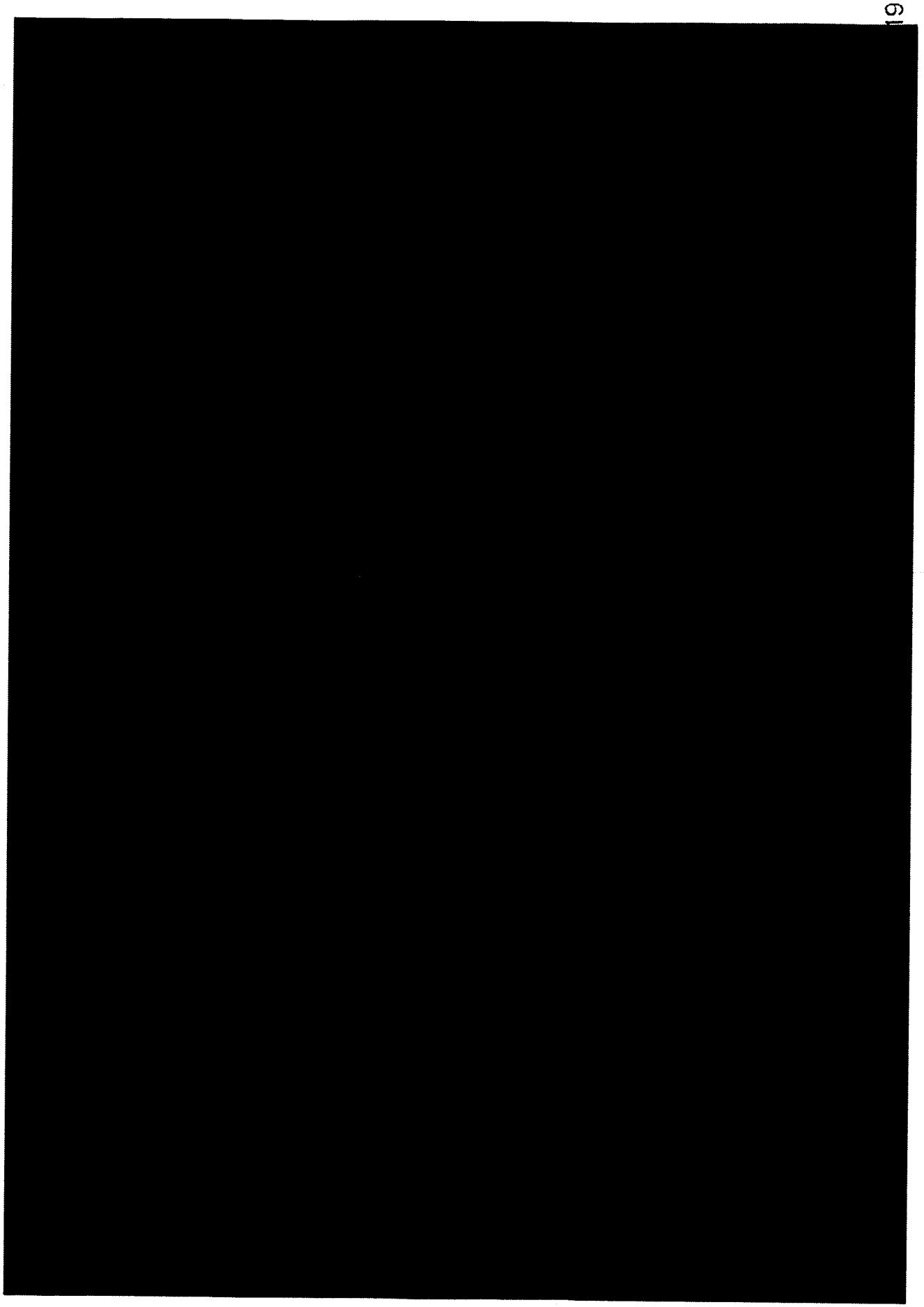
[redacted]





[redacted]

[redacted]



[redacted]

Foodborne Disease Significance of *Escherichia coli* O157:H7 and Other Enterohemorrhagic *E. coli*

A PUBLICATION OF
THE INSTITUTE OF FOOD TECHNOLOGISTS'
EXPERT PANEL ON FOOD SAFETY AND NUTRITION

This Scientific Status Summary addresses the virulence and disease characteristics of EHEC, their reservoirs and sources, survival and growth, and disease prevention strategies

ROBERT L. BUCHANAN AND MICHAEL P. DOYLE

Author Buchanan, a Professional Member of IFT, is Research Microbiologist, Food Safety Research Unit, U.S. Dept. of Agriculture, Agricultural Research Service, 600 E. Mermaid Ln., Wyndmoor, PA 19038. Author Doyle, a Professional Member of IFT, is Director of the Center for Food Safety and Quality Enhancement, University of Georgia, Georgia Station, Griffin, GA 30223-1797 and Head of the Dept. of Food Science and Technology, University of Georgia, Athens, GA 30602.

The unusually virulent enterohemorrhagic strains of *Escherichia coli*, including the O157:H7 serotype, have prompted food microbiologists to rewrite the rule book on food safety. These pathogens are more significant than other well-recognized foodborne pathogens for reasons including the severe consequences of infection that affect all age groups, their low infectious dose, their unusual acid tolerance, and their apparent special but inexplicable association with ruminants that are used for food.

New safety recommendations for destroying enterohemorrhagic *E. coli* (EHEC) include cooking hamburgers thoroughly, incorporating a procedure that kills EHEC in the manufacture of raw fermented sausage, such as salami, and pasteurizing or using an equivalent processing method for apple cider. Public health problems with EHEC are being recognized throughout the world. The need for consumer education on the safe handling of foods has never been more acute.

Historical Perspective

E. coli O157:H7 (designated by its somatic, O, and flagellar, H, antigens) was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982 (Riley et al., 1983). The first outbreak, with 26 cases of which 19 were hospitalized, occurred in Oregon, and the second, with 21 cases and 14 hospitalizations, followed three months later in Michigan. Undercooked hamburgers from the same fast food restaurant chain were identified as the vehicle, and *E. coli* O157:H7 was isolated from patients and a frozen ground beef patty.

Shortly after *E. coli* O157:H7 was determined to be a human pathogen, Karmali et al. (1983) observed that stool samples from children with hemolytic uremic syndrome (HUS) contained a substance that was toxic to Vero (African green monkey kidney) tissue culture cells. This verocytotoxin was produced by *E. coli* isolates, with O157:H7 the prominent serotype causing infection.

Enterohemorrhagic *E. coli* and Foodborne Illness

E. coli has been used since 1890 as a nonpathogenic indicator of enteric pathogens, such as *Salmonella*. However, as knowledge of enteric diseases increased, investigators began isolating strains of *E. coli* that had acquired virulence characteristics causing pathogenicity to humans or animals. Six classes of diarrheagenic *E. coli* are recognized: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC), and diffusely adherent (DAEC).

• **Definition of EHEC.** EHEC are loosely defined by a combination of the symptoms they produce and the virulence factors they possess (Neill et al., 1994). The disease-defining symptom of EHEC is hemorrhagic colitis (HC), i.e., bloody diarrhea. Not all EHEC infections, however, produce overt blood in the stools. While *E. coli* O157:H7 infections have a high rate of bloody stools, this may not be the case for other EHEC strains.

All EHEC strains produce Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2), also referred to as verotoxin 1 (VT1) and verotoxin 2 (VT2). The ability to produce Shiga toxin was acquired from a bacteriophage, presumably directly or indirectly from *Shigella*.

The toxin is a 70,000 dalton protein composed of a single A subunit (32 kDal) and five B subunits (7.7 kDal). The B subunits provide tissue specificity by binding to globotriaosylceramide (Gb_3) receptors on the surface of eucaryotic cells. The A subunit has an N-glycosidase that inactivates the 28S ribosome, thus blocking protein synthesis. Endothelial cells high in Gb_3 receptors are the pri-

mettwurst (CDC, 1995). EHEC isolates of serotypes O111:H- and O157:H- were isolated from both patients and product (Paton et al., 1996). *E. coli* isolates capable of producing one or more Shiga toxins can be isolated readily from meat, poultry, and seafoods (Samadpour et al., 1994); however, most do not possess the other virulence determinants associated with fully pathogenic EHEC.

Other foods have been associated with EHEC outbreaks worldwide (Table 1). Unpasteurized apple juice and cider have received considerable attention due to local and multistate outbreaks (Besser et al., 1993). A 1980 outbreak of HUS involving fresh apple juice is now suspected of being caused by EHEC (Steele et al., 1982). Sources are not identified in a substantial portion of EHEC cases, but a nonspecific association has often been made with the consumption of food in restaurants (Waters et al., 1994). This may be attributed in part to secondary person-to-person (Griffin and Tauxe, 1991) or animal-to-person (Wilson et al., 1996) spread of EHEC. For example, *E. coli* O157:H7 is similar to *Shigella* in its association with day-care centers, which are often foci for infections (Belongia et al., 1993). The largest reported *E. coli* O157:H7 outbreak, which caused thousands of illnesses, occurred in Japan in 1996. This outbreak and a second one a year later were associated with radish sprouts. Alfalfa sprouts were also implicated in a recent outbreak in the U.S.

The infectious dose (2–2,000 cells) associated with foodborne *E. coli* O157:H7 outbreaks has been consistently low—a characteristic associated with the organism's acid tolerance. It has been suggested that outbreak-associated strains of *E. coli* O157:H7 may have enhanced acid tolerance (Buchanan and Edelson, 1996). The inability of *E. coli* O157:H7 to ferment sorbitol, however, is not associated with its virulence (Fratamico et al., 1993).

Reservoirs and Sources of *E. coli* O157:H7

Several reservoirs and sources of *E. coli* O157:H7 have been identified:

Cattle. The association of *E. coli* O157:H7 with undercooked ground beef and raw milk led to investigations of the role of cattle as a reservoir of the pathogen. Several surveys of fecal shedding of *E. coli* O157:H7 produced the following general observations:

- Young animals tend to carry *E. coli* O157:H7 more frequently than adults (Zhao et al., 1995).
- Prevalence of fecal excretion varies substantially among positive herds (Hancock et al., 1994; Zhao et al., 1995).
- Reported incidence among cattle varies widely, in part because of differences in sensitivity of procedures used for detecting *E. coli* O157:H7.
- Results of two major U.S. surveys indicated that 31 (3.2%) of 965 dairy calves (Zhao et al., 1995) and 191 (1.6%) of 11,881 feedlot cattle were positive for *E. coli* O157:H7. An additional 0.4% of feedlot cattle were positive for *E. coli* O157:H- (USDA/APHIS, 1995).
- *E. coli* O157:H7 levels in calf feces range from $<10^2$ CFU/g to 10^5 CFU/g (Zhao et al., 1995).
- Fecal shedding of *E. coli* O157:H7 frequently is intermittent and of short duration, i.e., several weeks to months (Brown et al., 1997; Cray and Moon, 1995).
- Strains of *E. coli* O157:H7 with indistinguishable pulsed field gel electrophoresis (PFGE) genomic DNA profiles can be isolated from calves in different states or farms (Faith et al., 1996; Meng et al., 1995).
- More than one strain of *E. coli* O157:H7 can be isolated from feces of the same animal or different animals within the same herd (Faith et al., 1996; Meng et al., 1995).

Calves have been experimentally infected with *E. coli* O157:H7 (Brown et al., 1997; Cray and Moon, 1995); results revealed that:

- *E. coli* O157:H7 is not pathogenic to calves; inoculation with 10^{10} CFU did not induce significant clinical disease.
- The numbers of *E. coli* O157:H7 shed in feces decreased dramatically during the first 14 days postinoculation (e.g., from 10^4 to 10^6 CFU/g after 48 hr to $5–10^2$ CFU/g at 14 days).
- *E. coli* O157:H7 is confined to the gastrointestinal tract, with the forestomachs (rumen, omasum, and reticulum) and distal sites (distal ileum, proximal cecum, spiral colon, and descending colon) being the principal sites of localization.
- Fasting increases the levels of *E. coli* O157:H7 shed in the feces of some animals, but not in most.
- *E. coli* O157:H7 did not form attaching and effacing lesions and did not colonize mucosal surfaces.

Oral inoculation of calves and steers with 10^{10} *E. coli* O157:H7 induced prompt and sustained increases in serum antibodies to the O157 antigenic lipopolysaccharide and to a lesser extent to Stx1 (Johnson et al., 1996). The serological responses, however, do not correlate with elimination of carriage by cattle or protection of calves against reinfection by the same strain. The ability of *E. coli* O157:H7 to persist in and reinfect cattle that have a strong immune response is likely to contribute to the introduction and persistence of infection in herds.

Table 1 Foods or food handling practices implicated or suspected of being associated with *Escherichia coli* O157:H7 outbreaks

Undercooked ground beef
Raw milk
Unpasteurized apple juice/cider
Dry cured salami
Lettuce
Produce from manure-fertilized garden
Handling potatoes
Radish sprouts, alfalfa sprouts
Yogurt
Sandwiches
Water

Deer. Recent *E. coli* O157:H7 investigations have established that deer are a source of the pathogen and that transmission of the pathogen may occur between deer and cattle (Keene et al., 1997; Rice et al., 1995). For example, in a recent outbreak involving contaminated venison jerky, *E. coli* O157:H7 with the same distinctive PFGE profile were isolated from the human cases, leftover jerky, uncooked meat from the same deer, a saw used to cut up the carcass, and fragments of the deer hide. Deer and cattle fecal samples obtained from a ranch in Texas had the same Shiga toxin-producing *E. coli* O157:H7 isolate (Rice et al., 1995).

Sheep. Sheep have also been identified as a reservoir of *E. coli* O157:H7 (Kudva et al., 1996). A six-month study of healthy ewes revealed that fecal shedding of the pathogen was transient and seasonal, with 31% of sheep positive in June, 5.7% positive in August, and none in November. The sheep showed no signs

occur at limiting a_w values, differences among humectants were minimal at a_w 0.98 (Buchanan and Bagi, 1997). Growing *E. coli* at elevated levels of NaCl induces *rpoS* expression with associated increases in thermotolerance and H_2O_2 resistance (Hengge-Aronis et al., 1993). *E. coli* O157:H7 can survive for many weeks when desiccated, particularly at refrigeration temperature (Bagi and Buchanan, 1993).

• Antimicrobials. *E. coli* O157:H7 does not appear to have any increased resistance to antimicrobial food additives.

Disease Prevention

E. coli O157:H7 represents unique challenges to preventing foodborne disease. Its low infectious dose in combination with the disease severity means that successful prevention strategies must focus on reducing or eliminating the presence of the microorganism, rather than on preventing pathogen growth, as is done in more traditional approaches. This focus is particularly important for raw products that may not be thoroughly cooked before consumption (e.g., ground beef) or ready-to-eat products that do not receive a definitive treatment that assures elimination of *E. coli* O157:H7 (e.g., fermented sausages, apple cider).

• HACCP. The Hazard Analysis and Critical Control Point (HACCP) system continues to be the most effective means for systematically developing food safety protocols that can reduce the risk of EHEC infections. EHEC, however, pose some unique problems when developing and implementing HACCP plans. For example, the low incidence of *E. coli* O157:H7 in foods makes direct microbiological testing for the pathogen as a means of verifying the effectiveness of a HACCP program of limited benefit. In such instances, verification based on microbiological analysis would have to depend on the use of an appropriate indicator organism that could provide a measure of how well a process controls factors associated with risk of *E. coli* O157:H7 contamination.

Most desirable is a process that includes a step lethal to the pathogen. This reduces the critical control points to assuring the effectiveness of that step and preventing subsequent cross contamination. For products that depend on nonthermal interventions to assure product safety (e.g., fermented meats), validation that the integrated process can achieve the desired level of inactivation may be a necessary part of the hazard analysis phase of HACCP implementation.

HACCP plans that do not include a step that kills pathogens are more complex, since the focus is on risk reduction instead of risk elimination. Typically, there is one or more critical control points associated with steps that either reduce the likelihood that the pathogen has gained access to the product or actively reduce (but not eliminate) the levels that may be present.

Since such processes cannot assure complete absence of the pathogen, there will also be critical control points associated with preventing pathogen growth. For example, the generic HACCP plan for beef slaughter and fabrication developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1993) included *E. coli* O157:H7 as a hazard. The HACCP plan listed skinning, post-skinning rinsing/bactericidal spray, evisceration, final bactericidal rinse, chilling, and maintenance of refrigeration as likely critical control points. In addition to these specific activities associated with slaughter, the committee identified factors associated with animal production practices and with the distribution, marketing, and consumption of the final products that would have to be considered in a farm-to-table HACCP plan.

• Farms. An important component of HACCP application in animal production is reducing the carriage of *E. coli* O157:H7 by animals. Two approaches that have potential are competitive exclusion and vaccination.

Competitive exclusion involves the use of microbial cultures that out-compete pathogens from colonizing specific niches. This approach uses defined bacterial cultures that can greatly reduce colonization of *Campylobacter jejuni* in poultry (Schoeni and Doyle, 1992).

Vaccination involves exposing an animal to an attenuated pathogen or an antigen of a virulent microorganism to produce immunity. However, traditional vaccination approaches are not likely to be successful with *E. coli* O157:H7. Recent observations showed that *E. coli* O157:H7 does not form attaching and effacing lesions or colonize mucosal surfaces of the gastrointestinal tract (Brown et al., 1997; Cray and Moon, 1995), and cattle exposed to *E. coli* O157:H7 are not protected from reinfection (Johnson et al., 1996). Hence, innovative approaches will be needed for vaccines to be effective.

• Slaughterhouse. Like other *E. coli*, it is assumed that the ultimate source of *E. coli* O157:H7 on carcasses is fecal contamination during animal production and slaughter operations. Fecal contamination is associated primarily with contamination of the carcass during hide removal and spreading of contamination to other carcasses by equipment and workers' hands (Dickson and Anderson, 1992).

Traditional trimming procedures can reduce *E. coli* O157:H7 levels on areas of the carcass with visible fecal contamination (Hardin et al., 1995). Various alternatives to trimming have been investigated for the removal of enteric pathogens. Recent studies with *E. coli* O157:H7 suggest that rinsing of carcass surfaces with solutions of organic acids may have limited effectiveness. Spray chilling with 1–2% acetic acid only produced a 1-log cycle (tenfold) reduction of *E. coli* O157:H7 on lean tissue; a slightly greater effect was observed on fat tissue (Dickson, 1991). Holding the meat for 24 hr indicated only a small residual effect on lean, but a substantial effect on fat tissue. Several investigators observed differences in the effectiveness of acid treatments between lean and fat tissue and among different portions of the carcasses (Cutter and Siragusa, 1994; Fratamico et al., 1996; Hardin et al., 1995).

Investigators found that acid rinses had little effect on eliminating *E. coli* O157:H7 from the surface of beef tissues (Brackett et al., 1994; Fratamico et al., 1996), possibly due to difficulty in removing *E. coli* O157:H7 from beef surfaces previously chilled (Hardin et al., 1995).

Preevisceration washing decreased the subsequent attachment of *E. coli* O157:H7 to beef carcasses (Dickson, 1995). Trisodium phosphate has been evaluated as a sanitizing agent for carcass surfaces and equipment. Its overall effectiveness, due to its high pH, was similar to that achieved with organic acids (Fratamico et al., 1996). Trisodium phosphate can increase the removal of *E. coli* O157:H7 from equipment surfaces (Somers et al., 1994).

The actual fate of *E. coli* O157:H7 cells that have been removed from carcass surfaces by rinses with sanitizing agents is still unclear. Model system studies on the microorganism's ability to survive acids and other agents at non-

developing means for controlling them in foods. It is also evident, however, that there are major scientific questions that must be answered before we will be able to fully assess and manage public health concerns associated with their foodborne transmission. Addressing these questions will require the continued effort and support of basic and applied scientists from a variety of disciplines.

On a broader front, a key lesson dramatically reinforced by the emergence of *E. coli* O157:H7 is that both the macroscopic and microscopic worlds change continually. We cannot take for granted that foods and food practices that have been traditionally safe will remain that way in the future. Continued vigilance and the ability to rapidly mobilize research capabilities must be an integral part of food safety programs if we are going to minimize the impact of new foodborne microbial threats to human health.

References

Abdul-Raouf, U.M., Beuchat, L.R., and Ammar, M.S. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999–2006.

Ahmed, N.M., Conner, D.E., and Huffman, D.L. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. J. Food Sci. 60: 606–610.

Arocha, M.M., McVey, M., Loder, S.D., Rupnow, J.H., and Bullerman, L. 1992. Behavior of hemorrhagic *Escherichia coli* O157:H7 during the manufacture of cottage cheese. J. Food Protect. 55: 379–381.

Bagi, L.K. and Buchanan, R.L. 1993. Preservation of *Listeria monocytogenes* and other pathogenic foodborne bacteria on silica gel. Lett. Appl. Microbiol. 17: 37–39.

Belongia, E.A., Osterholm, M.T., Soler, J.T., Ammend, D.A., Braun, J.E., and MacDonald, K.L. 1993. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. J. Am. Med. Assn. 269: 883–889.

Benjamin, M.M. and Datta, A.R. 1995. Acid tolerance of enterohemorrhagic *Escherichia coli*. Appl. Environ. Microbiol. 61: 1669–1672.

Besser, R.E., Lett, S.M., Weber, J.T., Doyle, M.P., Barrett, T.J., Wells, J.G., and Griffin, P.M. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. J. Am. Med. Assn. 269: 2217–2220.

Beutin, L., Aleksic, S., Zimmermann, S., and Gleier, K. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol. 183: 13–21.

Bitzan, M., Lidwig, K., Klemt, M., Konig, H., Buren, J., and Muller-Wiefel, D.E. 1993. The role of *Escherichia coli* infections in the classical (enteropathogenic) hemolytic uraemic syndrome: Results of a central European, multicenter study. Epidemiol. Infect. 110: 183–196.

Boyce, T.G., Swerdlow, D.L., and Griffin, P.M. 1995. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome. N. Engl. J. Med. 333: 364–368.

Brackett, R.E., Hao, Y.-Y., and Doyle, M.P. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. J. Food Protect. 57: 198–203.

Brown, C.A., Harmon, B.G., Zhao, T., and Doyle, M.P. 1997. Experimental *Escherichia coli* O157:H7 carriage in calves. Appl. Environ. Microbiol. 63: 27–32.

Buchanan, R.L. and Bagi, L.K. 1994. Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. Intl. J. Food Microbiol. 23: 317–322.

Buchanan, R.L. and Bagi, L.K. 1997. Effect of water activity and humectant identity on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiol. (In press).

Buchanan, R.L. and Edelson, S.G. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. Appl. Environ. Microbiol. 62: 4009–4013.

Buchanan, R.L. and Klawitter, L.A. 1992. The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiol. 9: 185–196.

CDC. 1993. Update: Multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1992–1993. Centers for Disease Control and Prevention, Atlanta, Ga., Morb. Mortal. Weekly Rept. 42: 258–263.

CDC. 1995. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia. Centers for Disease Control and Prevention, Atlanta, Ga., Morb. Mortal. Weekly Rept. 44: 550–551, 557–558.

Cheville, A.M., Arnold, K.W., Buchrieser, C., Cheng, C.-M., and Kaspar, C.W. 1996. *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 62: 1822–1824.

Clavero, M.R.S. and Beuchat, L.R. 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. Appl. Environ. Microbiol. 62: 2735–2740.

Clavero, M.R.S., Monk, J.D., Beuchat, L.R., Doyle, M.P., and Brackett, R.E. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. Appl. Environ. Microbiol. 60: 2069–2075.

Cray, W.C. and Moon, H.W. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 61: 1586–1590.

Cutter, C.N. and Siragusa, G.R. 1994. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. J. Food Protect. 57: 97–103.

Dickson, J.S. 1991. Control of *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 on beef in a model spray chilling system. J. Food Sci. 56: 191–193.

Dickson, J.S. 1995. Susceptibility of preevisceration washed beef carcasses to contamination by *Escherichia coli* O157:H7 and salmonellae. J. Food Protect. 58: 1065–1068.

Dickson, J.S. and Anderson, M.E. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Protect. 55: 133–140.

Dorsa, W.J., Cutter, C.N., and Siragusa, G.R. 1996. Effectiveness of a steam–vacuum sanitizer for reducing *Escherichia coli* O157:H7 inoculated to beef carcass surface tissue. Lett. Appl. Microbiol. 23: 61–63.

Doyle, M.P. and Schoeni, J.L. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48: 855–856.

Doyle, M.P., Zhao, T., Meng, J., and Zhao, S. 1997. *Escherichia coli* O157:H7. In "Food Microbiology: Fundamentals and Frontiers," ed. M.P. Doyle, L.R. Beuchat, and T.J. Montville, pp. 171–191. ASM Press, Washington, D.C.

Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.-S., Luchansky, J.B., and Kaspar, C.W. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl. Environ. Microbiol. 62: 1519–1525.

Fratamico, P.M., Buchanan, R.L., and Cooke, P.H. 1993. Virulence of an *Escherichia coli* O157:H7 sorbitol-positive mutant. Appl. Environ. Microbiol. 59: 4245–4252.

Fratamico, P.M., Schultz, F.J., Benedict, R.C., Buchanan, R.L., and Cooke, P.H. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. J. Food Protect. 59: 453–459.

Gill, C.O. and Phillips, D.M. 1985. The effect of media composition on the relationship between temperature and growth rate of *Escherichia coli*. Food Microbiol. 2: 285–290.

Goldwater, P.N. and Bettelheim, K.A. 1995. The role of enterohemorrhagic *Escherichia coli* serotypes other than O157:H7 as causes of disease in Australia. Commun. Dis. Intell. 19: 2–4.

Griffin, P.M. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In "Infections of the Gastrointestinal Tract," ed. M.J. Blaser, P.D. Smith, J.I. Ravdin, H.B. Greenberg, and R.L. Guerrant, pp. 739–761. Raven Press Ltd., New York.

Griffin, P.M. and Tauxe, R.V. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13: 60–98.

Hancock, D.D., Besser, T.E., Kinsel, M.L., Tarr, P.I., Rice, D.H., and Paros, M.G. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington state. Epidemiol. Infect. Dis. 113: 199–207.

Hao, Y.Y. and Brackett, R.E. 1993. Growth of *Escherichia coli* O157:H7 in modified atmosphere. J. Food Protect. 56: 330–332.

Hardin, M.D., Acuff, G.R., Lucia, L.M., Oman, J.S., and Savell, J.W. 1995. Comparison of methods for decontamination from beef carcass surfaces. J. Food Protect. 58: 368–374.

Hengge-Aronis, R., Lange, R., Henneberg, N., and Fischer, D. 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. 175: 259–265.

Jackson, T.C., Hardin, M.D., and Acuff, G.R. 1995. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. J. Food Protect. 59: 230–237.

Johnson, R.P., Cray, Jr., W.C., and Johnson, S.T. 1996. Serum antibody responses of cattle following experimental infection with *Escherichia coli* O157:H7. Infect. Immun. 64: 1879–1883.

Karmali, M.A., Steele, B.T., Petric, M., and Lim, C. 1983. Sporadic cases of hemolytic uremic syndrome associated with fecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet i: 619–620.

Keene, W.E., McAnulty, J.M., Hoesley, F.C., Williams, L.P., Hedberg, K., Oxman, G.L., Barrett, T.J., Pfaller, M.A., and Fleming, D.W. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N. Engl. J. Med. 331:579–584.

Keene, W.E., Sazie, E., Kok, J., Rice, D.H., Hancock, D.D., Balan, V.K., Zhao, T., and Doyle, M.P. 1997. Outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. J. Am. Med. Assn. 277: 1229–1231.

Kudva, I.T., Hatfield, P.G., and Hovde, C.J. 1995. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. Appl. Environ. Microbiol. 61: 1363–1370.

Kudva, I.T., Hatfield, P.G., and Hovde, C.J. 1996. *Escheri-*

Small Plant Intervention Treatments to Reduce Bacteria on Beef Carcasses at Slaughter

Dennis Buege
Animal Sciences Department

Steve Ingham
Food Science Department

University of Wisconsin-Madison - - June 2003

The slaughter process for cattle and other meat-producing animals involves the removal of the bacteria-free meat from between two contaminated surfaces - the hide and the GI tract. In this process, no matter how carefully it is carried out, there will invariably be transfer of bacteria to the carcass. The food safety goal of the slaughter process is minimize bacterial contamination of the carcass, and effectively remove contamination which has occurred.

The primary weapon in reducing bacterial contamination of beef carcasses is employing effective sanitary dressing procedures during slaughter. There is no substitute for trying to keep bacteria off the carcass in the first place. Workers should know, understand and use the recommended sanitary dressing techniques in whatever slaughter method is used. A list of current "best practices" as developed by the beef slaughter industry, is included at the end of this report.

However, no matter how carefully a plant dresses beef carcasses, it is inevitable that bacteria will contaminate the carcass, some of which could potentially be fecal pathogens such as *E. coli* O157:H7 or *Salmonella*. Therefore, applying "interventions" to carcasses during and after the dressing procedure to effectively remove or inactivate bacterial contamination and improve meat safety is important. Such "interventions" include trimming, steam vacuuming, carcass washing; hot water rinses, organic acid rinses and steam pasteurization. In addition, it has been demonstrated that the process of dry chilling and refrigerated storage of beef carcasses likewise causes a decline in bacteria numbers.

In the fall of 2002, the USDA issued a directive calling for beef slaughter plants (and also beef grinding and fabrication operations) to reassess their HACCP plans. If at slaughter *E. coli* O157:H7 is a hazard "reasonably likely to occur" (and from industry experience and research data it is difficult to argue that it isn't), then a validated intervention must be present in the slaughter process and operated as a critical control point. "Validated" means that there must be scientific evidence that the intervention can reduce the likelihood of *E. coli* O157:H7 being present on the carcass. Besides a CCP associated with a validated intervention, a CCP is required to assure zero fecal contamination on the carcass at the end of slaughter.

The USDA has not mandated the size of the bacteria/*E. coli* O157:H7 reduction required by an intervention process. Reduction in bacteria numbers is usually expressed in terms of "logs" of reduction. A one log reduction means that the number of bacteria has been reduced by 90% (100 to 10). A two log reduction would be from 100 to 1 (99% reduction) and so on. No intervention can be guaranteed to completely eliminate all pathogens all of the time, but significant reductions are a move in the right direction, and a lowering of the risk of food-borne illness.

Currently we are hearing that small slaughter plants are testing or using a wide variety of interventions. The purpose of this summary report is make our recommendations about interventions that are possible and make sense for a smaller-scale beef slaughter plant.

- apply at solution temperature of ambient to 130°F. The warmer the temperature the more effective the kill (do not go over 130°F - acetic acid will evaporate out of solution).
- we recommend two thorough passes over the entire carcass surface with a garden type sprayer.
- suggested critical limits: (1) documenting the proper concentration of solution at make-up, and (2) documenting application to each carcass.
- (Note: acetic acid will be cheaper than lactic acid. One source preferred lactic acid because it was easier on floors, and not as irritating to people).

Fresh Bloom

- available from Excalibur Seasonings - contains citric acid, ascorbic acid and eythorbic acid.
- in one UW in-plant test, Fresh Bloom was only slightly less effective than lactic acid in reducing total bacteria counts (effects on *E. coli* O157:H7 not evaluated)
- use a thorough warm-water carcass wash before applying Fresh Bloom solution.
- use 8 ounces of Fresh Bloom per gallon of water.
- apply at solution temperature of ambient to 130°F. The warmer the temperature the more effective the expected kill.
- we recommend two thorough passes over entire carcass surface with a garden type sprayer.
- suggested critical limits: (1) documenting the proper concentration of solution at make-up, and (2) documenting application to each carcass.

Hot Water Rinse

- use 150 to 180°F water (the higher the temperature the greater the effect)
- must be careful in using - hazardous to people. May cause condensation problems in plant.
- we suggest two thorough passes over entire carcass surface.
- suggested critical limits: (1) periodic check of water temperature, and (2) documentation of application to carcass.

Dry Aging

- a UW in-plant test found a 1.2 log reduction in total bacteria due to the final carcass wash (tap water), a 0.6 log additional reduction from wash through 2 days of aging, and 0.4 log additional reduction from day 2 through 6 days of aging (total reduction of aerobic plate count was 2.2 logs, from before carcass wash through 6 days of aging).
- follow-up laboratory tests simulating slaughter cooler conditions found generic *E. coli* and *E. coli* O157:H7 to die off more than total bacteria (so above tests may have showed even more effective kill for O157:H7).
- suggest cooler be at less than 90% RH and less than 41°F.
- suggest 2 critical limits: (1) cooler temperature less than 41°F, and (2) document that carcasses are chilled/aged for at least 6 days.
- considering dry chilling/aging as an intervention is a new concept (most large plants spray chill and fabricate carcasses after 2 days). However our UW tests support that generic *E. coli* and *E. coli* O157:H7 die off under dry chilling/aging conditions.

Treatment	Microbial Contaminant	Reduction (log CFU/cm^2)	Reference
3% lactic acid (75°F)	*E. coli* O157:H7	1.7	6
5% lactic acid (75°F)	*E. coli* O157:H7	2.6	6
2% lactic acid (100 - 138°F)	Aerobic Plate Count	0.7	13
2% lactic acid (tap water)	*E. coli* O157:H7 in feces	2.4	10
2% lactic acid (tap water)	*E. coli* O157:H7 in feces	2.2	10
2% lactic acid (tap water)	*E. coli* O157:H7 in feces	2.7	10
2% lactic acid (tap water)	*E. coli* O157:H7 in feces	1.3	10
Water (165°F) + 2% acetic acid (61°F)	*E. coli* (resistant) in feces	3.0	4
Water (95°F) + 2% acetic acid (131°F)	*E. coli* O157:H7 in feces	2.4 - 3.7	1
1% acetic acid (75°F)	*E. coli* O157:H7	1.6	6
3% acetic acid (75°F)	*E. coli* O157:H7	1.9	6
5% acetic acid (vinegar) (75°F)	*E. coli* O157:H7	2.0	6
1% citric acid (75°F)	*E. coli* O157:H7	1.2	6
3% citric acid (75°F)	*E. coli* O157:H7	1.7	6
5% citric acid (75°F)	*E. coli* O157:H7	1.8	6
5.7% Fresh Bloom (ambient temperature)	Aerobic Plate Count	0.5	13
Wash + Hot Water (203°F)	*E. coli* O157:H7 in feces	4.0	7
Hot Water Wash (165°F)	*E. coli* O157:H7 in feces	2.6	8
Hot Water (146-162°F)	Aerobic Plate Count	0.3	13
Hot Water (146-162°F) + 2% lactic acid (100-138°F)	Aerobic Plate Count	1.3	13
Dry Chilling/Aging (1 day)	*E. coli* (manure)	1.3	11
Dry Chilling/Aging (7 days)	*E. coli* (manure)	2.1	11
Dry Chilling/Aging (1 day)	*E. coli* O157:H7 in feces	1.7	10
Dry Chilling/Aging (7 days)	*E. coli* O157:H7 in feces	3.3	10
Dry Chilling/Aging (1 day)	*E. coli* O157:H7	0.9	10
Dry Chilling/Aging (3 days)	*E. coli* O157:H7	2.0	10
Dry Chilling/Aging (1 day)	*E. coli* O157:H7	1.3	10
Dry Chilling/Aging (3 days)	*E. coli* O157:H7	2.1	10

5. Davey, K.R., and M.G. Smith. 1989. A laboratory evaluation of a novel hot water cabinet for the decontamination of side of beef. Int. J. Food Sci. Technol. 24:305-316.
6. Cutter, C.N., and G.R. Siragusa. 1994. Efficacy of organic acid against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. J. Food Prot. 57:97-103.
7. Castillo, A., L.M. Lucia, K.J. Goodson, J.W. Savell, and G.R. Acuff. 1998. Comparison of water wash, trimming, and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. J. Food Prot. 61:823-828.
8. Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Prot. 60:114-119.
9. Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Prot. 60:619-624.
10. Calicioglu, M., C.W. Kaspar, D.R. Buege, and J.B. Luchansky. 2002. Effectiveness of spraying with Tween 20 and lactic acid in decontaminating *Escherichia coli* O157:H7 and indigenous *Escherichia coli* Biotype I on beef. J. Food Prot. 65:26-32.
11. Calicioglu, M., D.R. Buege, S.C. Ingham, and J.B. Luchansky. 1999. Recovery of *Escherichia coli* Biotype I and Enteroccus spp. during refrigerated storage on beef carcasses inoculated with fecal slurry. J. Food Prot. 62:944-947.
12. Ingham, S.C., J. Losinski, and D.R. Buege. 2003. Change in aerobic plate count on beef carcasses during dry-aging. University of Wisconsin Research Report.
13. Ingham, S.C., and D.R. Buege. 2001. Evaluation of small-scale intervention treatments for improvement of beef carcass hygiene. University of Wisconsin Research Report.
14. Ingham, S.C., and D.R. Buege. 2003. Validation of dry-aging as an effective intervention step against E. coli O157:H7 on beef carcasses. University of Wisconsin Research Report.
15. Chang, V.P., E.W. Mills, and C.N. Cutter. 2003. Reduction of bacteria on pork carcasses associated with chilling method. J. of Food Prot. 66:1019-1024.

Verification

Under HACCP, "verification" is designed to check that the controls at the CCP are effective. For beef slaughter, the USDA directive wants plants to do some level of testing of carcasses to verify the elimination of *E. coli* O157:H7. Below are some suggestions related to this verification testing.

- we suggest bi-monthly or quarterly testing of one carcass for the pathogen (*E. coli* O157:H7), using the 3 carcass-site sponge technique.
- be sure to hold the tested carcass until the test results are known.
- if verification test results are consistently negative for 2 years or longer you might consider reducing the frequency of carcass testing.
- if verification test results find a positive *E. coli* O157:H7 result, evaluate your slaughter process for potential problem areas, and consider increasing your frequency of carcass testing for the pathogen. Re-apply intervention to positive carcass and retest.
- in Wisconsin state-inspected plants, the carcass verification testing for *E. coli* O157:H7 may be done by the state inspection program.

3

Animal Product Manual

Reference

Foreign Meat and Meat Products, Equine

Contents

Introduction and Subsidiary Locator

The *Foreign Meat and Meat Products, Equine* section covers horse meat and horse meat products.

No specific regulations govern the importation of horse meat. Horses **do not** get BSE and FMD. However, unless horse meat can be differentiated from that of ruminants, then horse meat **cannot** enter U.S. commerce if the meat is from a country affected with BSE or FMD. When a VS permit does **not** authorize entry, continue to **Table 3-10-1** which directs you to the final regulatory action to take. Inspect the importation to determine if there is bone-in meat with hoof attached (the hoof is attached by natural attachments to each portion of the carcass).

TABLE 3-10-1 Regulatory Action on Meat and Meat Products of Horse

If there is:	And the country or region of export is:	And:	Then:
Hoof attached	→	→	REFER to FSIS[1]
No hoof attached	Affected with BSE or FMD	Argentina or Paraguay	SEE **Table 3-10-3**
		Other than Argentina or Paraguay	REFUSE ENTRY PROVIDE the importer with the appropriate options including the option to have an identification test done (*see* **Table 2-2-11**)
	Minimal risk for BSE[2] and **free** from FMD	→	SEE **Table 3-10-4**
	Free from BSE and FMD	→	

1 Importer/broker **must** coordinate with FSIS prior to shipment as FSIS may refuse entry if hoof is attached.

2 Currently Canada is the **only** country designated at minimal risk for BSE.

OFFICIAL HORSE MEAT CERTIFICATE FOR HORSE MEAT FOOD PRODUCTS

Place: _____ _____ Date: _____
(City) (Country)

The undersigned Official Medical Veterinary, CERTIFIES: That the horse meat and/or horse food products herein described were derived from horses which received ante mortem and post mortem veterinary inspection at the time of slaughter, and that such horse meat products are sound, healthful, wholesome, and otherwise fit for human consumption and have not been treated with, and do not contain any preservative, coloring matter, or other substance not permitted by the regulations governing the horse meat inspection of the U.S. Department of Agriculture, filed with me, and that said horse meat and horse meat food products have been handled only in a sanitary manner in this country.

Kind of Product	Species of livestock derived from	Number of pieces or containers	Weight
_____	_____	_____	_____
_____	_____	_____	_____

Identification marks on products and containers: _____

_____

Consignor: _____
(Address)

_____
(Address)

Establishment number: _____

Consignee: _____

Destination: _____

Shipping marks: _____

(Name of official authorized by the national foreign government to issue inspection certificates for meat food products exported to the United States.)

Official title: _____

FIGURE 3-10-1 Example of a USDA Approved Horse Meat Certificate (Blank)

Horse Meat from a Country Known to Be FREE from FMD

TABLE 3-10-4 Regulatory Action to Take on Horse Meat from a Country Known to Be FREE from FMD

If the importation is:	And is:	And is:	Then:
Horse meat from a country known to be free from FMD[1]	Accompanied by an official certificate[2] verifying the species as horse, burro, or mule	→	REFER to FSIS
	Not accompanied by an official certificate verifying species	Consigned to an approved establishment for rendering or processing into pet foods	AUTHORIZE shipment under seal with VS Form 16-78 (*see* **Appendix K** to complete form)
		Not consigned to an approved establishment	**DO NOT** RELEASE the HOLD PROVIDE the importer with the appropriate options including the option to have an identification test done (*see* **Table 2-2-11**) SEE Identification Tests on page 3-10-5 if the importer requests an identification test

1 Currently Canada and New Zealand are the only FMD-free countries eligible to export horse meat for human consumption to the United States.

2 *See APM* Figure **3-10-1** on page **3-10-3** for an example.

Identification Tests

Procedures for these identification tests will **not** be listed in this manual because they are specific to those designated ports who communicate directly with laboratories.

TABLE 3-10-5 Identification Tests of Horse Meat

If your port of arrival:	And after conducting a test the results are:	Then:
Conducts identification tests	Negative for containing ruminant tissue	RELEASE
	Positive for containing ruminant tissue	REFUSE ENTRY PROVIDE the importer with the appropriate options (*see* **Table 2-2-11**)
Does not conduct identification tests	→	1. DO NOT RELEASE the HOLD 2. PROVIDE the importer with the appropriate options (*see* **Table 2-2-11**)

[redacted]

Equine Drug Testing Services offered by [redacted]

For Responsible Transportation, Sigourney, Iowa

We appreciate the opportunity to offer testing to Responsible Transportation.

[redacted]

[redacted]

Veterinary Residues Committee

Position Paper – Residues of Phenylbutazone in horses Published July 2012

Issue

Phenylbutazone (also known as 'bute') is a non-steroidal anti-inflammatory (NSAID) medicine that is authorised for medicinal use in horses that are not intended for human consumption, and in dogs.

It is used in these species to treat musculoskeletal disorders, such as rheumatoid and arthritic diseases. Phenylbutazone must not be administered to any animals destined for the food-chain; however phenylbutazone residues continue to be found in horses that have been slaughtered in the UK for human consumption.

Relevance to Consumers

Throughout the EU horses are normally regarded as being food-producing animals, although in the UK horse meat is not commonly eaten. Around 8000 horses are slaughtered for human consumption every year in the UK, mostly for export to other EU countries; this figure has been increasing in recent years.

The Veterinary Residues Committee (an independent scientific advisory committee that advises the Government) has repeatedly expressed concern over residues of phenylbutazone entering the food chain. This is because this substance has the potential for serious adverse effects in consumers, such as blood discrasia (a rare but very serious, life-threatening, condition).

Background

Horse meat is included in the Veterinary Medicines Directorate's UK surveillance programme for residues of veterinary medicines. The surveillance programme is a requirement of EU legislation and its purpose is to check that home-produced food derived from animals does not contain residues of veterinary medicines at levels that would be harmful to consumers. Samples are taken at abattoirs, from animals that were sent for slaughter for human consumption; the number of samples is directly related to the level of throughput of horses in the preceding year (in 2011 the number of samples taken represented 1.86% of all horses sent for slaughter for human consumption).

Since 2005 all horses have been required by law to have a passport for identification. This document must accompany the horse whenever it is sold or transported, and contains a declaration as to whether or not the horse is intended for human consumption. If it is, the medicines that may be administered to that horse are limited. The Veterinary Medicines Directorate (VMD) advises vets that if an owner or keeper of a horse does not have the passport to hand, and the vet has not previously seen the passport, the horse should be regarded as intended for the food-chain when medicines are being selected.

However, Defra's follow-up investigations in recent years have found that some vets are still prescribing phenylbutazone without checking the passport or ensuring that the horse is subsequently signed out of the food-chain. Phenylbutazone residues have also been found in horses that have changed owners prior to going to slaughter, and whose passports do not indicate that they have been signed out of the food-chain. Other residues have occurred because feed containing phenylbutazone intended for one horse has allegedly been eaten by another horse. Therefore care must also be taken to avoid other food-producing animals gaining access to treated feed.

What happens when a non-compliant sample is found?

It is an offence to present an animal for slaughter that contains a substance not allowed in food–producing animals. The source of the sample will be investigated by a Defra Animal Health Officer, who will try to ascertain who is responsible for the horse entering the food-chain. It is normal for written advice to be given to the person responsible; in serious or persistent cases further action could be taken.

It is possible to recall the consignment, even though this will be after it has been exported. This process is carried out by the Food Standards Agency (FSA) once the European Commission has been informed; in accordance with the EU Rapid Alert System for Food and Feed (RASFF) an alert is issued to other Member States to instigate withdrawal of the product from the market if necessary.

The VRC members receive regular reports at our meetings of any follow-up actions that are taken. Our meeting papers are published on our website – www.vmd.defra.gov.uk/vrc

The number of horse samples which have tested positive for residues of phenylbutazone has varied between 2-5% over the last five years. Annex A provides more information about the number of non-compliant samples found since 2007.

The Committee is hopeful that the availability of improved guidance is now having an impact and we will continue to monitor the number of non-compliant samples that occur each year.

What guidance is available?

New combined VMD and Defra guidance for veterinary surgeons and horse owners was produced in 2010 and further updated during 2011 - Veterinary Medicines Guidance Note 16 on the VMD Website – www.vmd.defra.gov.uk. The Guidance Note contains information on how to complete the medicines section of a horse passport and guidance for vets prescribing medicines for horses.

Conclusion

The Committee understands that phenylbutazone is an important medicine to maintain the welfare of older horses and is widely used to treat horses that are kept as companion animals. However, keepers of horses and veterinary surgeons must comply with their obligations under the legislation in relation to administering medicines to horses that may be destined for the food chain, to avoid consumers being exposed to potentially harmful residues.

The simplest way to do this is to ensure that the passport of any horse that has been treated with phenylbutazone has been appropriately signed to declare that it cannot enter the food chain.

The VRC would like this message to be communicated widely, with the support of representative groups such as the British Horse Society (BHS), British Equine Veterinary Association (BEVA) and the Responsible Use of Medicines in Agriculture Alliance (RUMA).

Veterinary Residues Committee (VRC)

For more information about the work of the VRC please visit our website:
www.vmd.defra.gov.uk/vrc

#374804

Veterinary Residues Committee – Position Paper on Residues of Phenylbutazone in horses

Table 1: Number of non-compliant samples found since 2007

Year	Number of Samples Taken	Type of Sample	Positive Samples
2007	30	Plasma	1
2008	56	Plasma - 30	1
		Kidney - 26	2
2009	40	kidney	0
2010	60	kidney	5
2011	68	kidney	1

There is a useful webpage on Horse Medicines on the VMD website – and the VRC urges all horse owners and vets to visit it:

http://www.vmd.defra.gov.uk/vet/horses.aspx

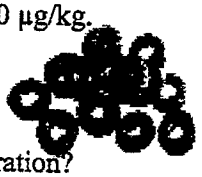
Table 2: Follow-up Information recently received

Examples of Follow up Information provided by Defra Animal Heath officers 2010/11:
• The horse was administered phenylbutazone by the keeper, however, the prescribing vet failed to sign the horse out of the food-chain. The keeper was also aware of the requirement to remove treated horse from the food-chain and realises he made a mistake. The trainer and the vet have been advised by the inspector about food-chain requirements.
• Another horse at this livery yard was administered Danilon, however, both horses were kept in the same area. The livery owner was not aware that this horse had access to the feed. There was a possibility that a member of the public gave the feed to this horse and the livery owner was reminded of the importance of security with any medicated feed.
• No investigation was carried out as the horse originated from an address in the Republic of Ireland. The Chief Veterinary Officer for Great Britain wrote to his counterpart in Ireland to advise him of the residue.
• The owner of this racing stable also buys horses in poor condition to be given some care and rehabilitate, and bought this horse from an elderly couple in May 2011. It came with a passport which was unsigned regarding human consumption and as the horse had a problem in one of its legs, the owner decided to have it slaughtered. The medicine cabinet only contained herbal remedies minerals and vitamins. The vet had not been to the premises for over a year. Unfortunately, the previous owner could not be traced to comment on whether phenylbutazone had been administered prior to sale, which is the most likely cause of this residue.

The Concentration Shell Game; or How to Promote Hysteria Over Minutia

The European Food Safety Authority tested 672 samples of horse meat. One of 672 had a phenylbutazone (PBZ) residue at a concentration of less than 20 µg/kg.

One gram of this meat would be the size of a single Cheerio
It would contain .02 µg of phenylbutazone.



How small is that concentration?

Phenylbutazone is still used in the EU to treat humans. In order to ingest the lowest therapeutic PBZ dose (200mg) idiosyncratically implicated in the very rare development of aplastic anemia:

Eat 22,000 pounds (11 tons, half a semi truckload) of this meat in a single sitting.
Chance of dying from overeating – 100%
Chance of getting sick from the PBZ residue in the meat - much less than 1:30,000.

Here's another example:

To create a solution of the same concentration as the PBZ residue found in the single horse meat sample (20 µg/kg):

Take an Olympic sized swimming pool

Add 3½ Tablespoons of pure phenylbutazone

Mix well

To ingest the smallest average dose of the phenylbutazone still used in the EU to treat humans (200mg), and that may be idiosyncratically associated with the < 1:30,000 chance of disease:

a) Drink 10,000 liters (2,641 gallons) of water from the pool.
b) Die of water poisoning (hyponatremia).
Actually, you will die after drinking less than 0.11% of this volume.

How much water is that, exactly?

16,949 20 oz bottles of Sam's Choice water

About one small tank truck full of water

When will death-by-water occur?
The body's response to water is highly individualized, with some people being more sensitive to excessive water intake than others.
In other words, water poisoning is an ***idiosyncratic*** condition.

Safe maximum levels of water intake can be recommended and the incidence of water intoxication is extremely rare at these levels of ingestion, so long as the human is otherwise healthy. There are some conditions that would make even otherwise normal levels of water intake potentially risky to some unhealthy humans, however.

Sounds like a familiar argument.

Canadian Food Inspection Agency

5.3 Bacteria

5.3.5 *Campylobacter coli* and *Campylobacter jejuni*

5.3.5.1 Description

Campylobacter coli and *C. jejuni* are slender, non-spore forming, spirally curved, rod-shaped organisms, that are gram-negative, have a minimum growth temperature of 28°C and are resistant to freezing.

5.3.5.2 Occurrence

Campylobacter jejuni, recognized in 1980 as a foodborne pathogen, and more recently *C. coli*, are emerging as important public health concerns. The organisms are relatively ubiquitous in the environment, commonly found in untreated water and in the intestines of poultry, cattle, swine, rodents, wild birds. Poultry products, beef and liver are most commonly implicated in disease outbreaks, primarily due to consumption of raw meats or inadequate cooking. Meat products should reach an internal temperature of at least to 69°C to eliminate the risk of infection.

5.3.5.3 Concern

The minimum infective dose appears to be quite low and toxicological manifestations include headache, fever and muscle pain, followed by self-limiting enterocolitis with severe abdominal pain, anorexia, malaise, and vomiting primarily in young adults. Occasionally, other complications such as septicemia, short-term arthritis, Guillain-Barré syndrome or meningitis have been reported. Symptoms of campylobacteriosis occur within 2 to 10 days after ingesting contaminated food and recovery may take from a few days to a few weeks.

5.3.5.4 Program

Sampling programs are implemented on a rotating basis in the form of surveys or targeted monitoring.

5.3.5.5 Sampling

Sampling is normally limited to ready-to-eat products and testing is conducted to discern the absence or presence of the organism. Specific instructions accompany the call for sampling.

5.3.7 Generic *E. coli*

5.3.7.1 Introduction

While of little significance in raw commodities, the presence of these non-hazardous organisms in processed products serves as a useful indicator that contamination may have occurred. As an index for sanitation, they permit monitoring of plant hygiene for a wide range of processed foods and are therefore indispensable to HACCP approaches. This is also the case for the broader categories of coliforms and fecal coliforms.

5.3.7.2 Testing

E. coli counts are routinely performed on multiple analysis submissions (MASS) of ready-to-eat meat products including fermented commodities.

5.3.7.3 Follow-up

Test results are interpreted based on the specific commodity, as follows:

Product	Standard/guideline				Assessment	
	n	c	m*	M*	Investigative	Unsatisfactory
Non-fermented RTE products	5	1	10^2	10^3	>60/g on composite	$>10^3$/g or $>10^2$/g in more than 2 units
Heat treated fermented RTE sausage	5	1	10	10^3	10^3 if any detected on composite	$>10^3$/g or >10/g in more than 1 unit
Raw fermented RTE sausage	5	0	10^2	10^3	>40/g on composite	$>10^3$/g or $>10^2$/g in more than 1 unit

*measured in cfu/g

5.3.8 Verotoxigenic *E. coli*

5.3.8.1 Description

Escherichia coli O157:H7, as well as several other related strains, are gram-negative facultatively anaerobic rod-shaped microorganisms with unusually severe pathogenic characteristics not normally observed for the genus of *Escherichia*.

5.3.8.2 Occurrence

These bacteria live in the intestines of animals such as cattle, pigs, sheep and poultry. During slaughter, they may spread to the outer surfaces of the meat. *E. coli* O157:H7 infection can also be spread by hand-to-hand contact with an infected person or by contact with a contaminated surface. Aside from the O157:H7, there are other dangerous strains of *E. coli*.

Although Hemolytic Uremic Syndrome (HUS) is commonly called "hamburger disease", other kinds of undercooked meat and poultry, fermented meat products, unpasteurized milk, non-chlorinated water, and raw apple juice contaminated with *E. coli* O157:H7 have made people ill. Ground beef may be easily contaminated, due in part to the grinding process which spreads the bacteria, generally found on the surface, throughout the meat.

5.3.8.3 Concern

Enteropathogenic *Escherichia coli* were not recognized as significant foodborne pathogens until the early 1970s, while the O157:H7 strain was first identified as causing human illness in 1982 in U.S. and Canadian outbreaks. While the former type is known to cause gastroenteritis with self-limiting non-bloody diarrhea due to toxin production, the latter strain is characterized by bloody diarrhea (hemorrhagic colitis) and, in 10% of all infected humans (notably children), by being causative of the HUS, which interferes with normal renal functions and the blood coagulation mechanism and may require blood transfusions and kidney dialysis. Chronic kidney failure in the aged and susceptible (diabetics) and child mortality due to HUS have been stated as reaching 30% of all affected cases. Seizures or strokes are not uncommon among the elderly.

Symptoms may develop as stomach cramps, vomiting and a mild fever within 2 to 10 days after ingesting contaminated food. Unless accompanied by severe complications, most people recover within 7 to 10 days.

5.3.8.4 Program

Raw or semi-cooked meat products, and more recently fermented products as well, are primary objects for monitoring, while ready-to-eat products remain prime suspects due to recontamination.

5.3.8.5 Sampling

Meat products are sampled and submitted for laboratory analysis to determine specific strains of verotoxic *E. coli*. In addition, rapid testing is employed to ascertain the presence or absence of these organisms.

Samples are interpreted as follows:

Analysis	Standard/guideline				Assessment	
	n	c	m*	M*	Investigative	Unsatisfactory
E. coli O157:H7	5	0	0	-	n/a	present in 65 g

*measured in cfu/g

5.3.9 *Salmonella*

5.3.9.1 Description

Salmonella organisms are known to exist in well over 2,000 serotypes. They are readily inactivated by pasteurization temperatures in foods with a water activity greater than 0.95. Heat resistance increases with lowering of the water activity. In dried foods, *Salmonella*survive longer at water activity values below 0.20 than at higher values. Dependent on acid type, they are generally killed by a pH below 4.5 and are injured by cooling to below 7°C or freezing.

5.3.9.2 Occurrence

They are widely distributed in the environment through the discharge of natural animal and human waste to land and water. Raw poultry is often contaminated with at least one strain of *Salmonella*. Primary sources of human salmonellosis are foodstuffs of animal origin, particularly raw or undercooked meat and poultry and, in

some instances, unbroken eggs and unpasteurized egg and dairy products. Red meat and poultry become contaminated during slaughter and processing from the gut content of healthy excreting animals. In a similar way, every food that is produced in a contaminated environment may become exposed to *Salmonella* and may in turn be responsible for foodborne disease outbreaks as a result of faults in transport, storage or preparation.

5.3.9.3 Concern

Salmonella organisms are in many countries the most prevalent causative agent in foodborne disease outbreaks. *Salmonella* act directly as a viable organism without producing an enterotoxin and the likelihood of illness is therefore proportional to the number of organisms ingested. The exact number or organisms necessary to produce human salmonellosis depends on the serotype; in some cases as little as a few viable cells per 100 g of minced meat have caused an outbreak of serious consequences. Symptoms include diarrhea, abdominal cramps, vomiting and fever. In more serious cases, salmonellosis may cause dehydration, or it may infect the entire body. These symptoms are usually not felt for 6 to 48 hours and last from one to three days.

5.3.9.4 Program

In spite of controls at the farm level (*Salmonella*-free livestock, breeding stock, feed and sanitary environment) and at the slaughterhouse (sanitation of holding pens, hygiene during slaughter, avoidance of cross-contamination), *Salmonella*-contaminated food commodities remain on the market and every possible opportunity must be taken to inform the food service industry and the general public about the basic principles of food hygiene.

5.3.9.5 Sampling

Salmonella evaluations are routinely performed on multiple analysis submissions of domestic ready-to-eat meat products including fermented commodities (sampling schedule M-200) and imported ready-to-eat products (sampling schedule M-203). For each sample of domestic product, five (5) subsamples of 150 g each or five units will be sent to the designated laboratory. A similar sample consisting of five (5) subsamples of 150 g each will be collected from every re-inspected shipment of imported ready-to-eat meat products and submitted to the designated laboratory.

Sample results are interpreted as follows:

Analysis	Standard/guideline				Assessment	
	n	c	m*	M*	Investigative	Unsatisfactory
Salmonella spp.	5	0	0	-	n/a	present in 125 g

*measured in cfu/g

Concentration of Phenylbutazone (PBZ) residue in horse meat sample – how much is it, really?

Concentration of PBZ in one sample (of 672 samples tested for the European Food Safety Authority, or .1% incidence) = 19.2 µg/kg

Let's round to 20 µg/kg. That would be .02 µg/g of "horse steak" (a gram of meat would be about the size of a single Cheerio). A generous steak is 300 g (recommended single serving of meat = 100 g), a super steak is about 500 g. This 300 g horse steak would then contain 6 µg of PBZ and the super steak would have 10 µg PBZ.

Phenylbutazone is recommended for therapeutic use in humans for certain conditions, and is still in use in the EU. The smallest average therapeutic dose of PBZ implicated in the rare (in this case, an incidence of <1:30,000) correlation with a blood dyscrasia (such as aplastic anemia) is 200 mg, or 200,000 µg.

To get a possibly idiosyncratic dose of PBZ from eating this generous horse steak, at a concentration of 20 µg PBZ/kg, one would need to consume 10,000 kg, or 22,000 lbs (11 tons) of steak in a single sitting. You would only need to eat 6,000 kg or 13,200 lbs (6.6 tons) of the super steak. Either way, you will die from overconsumption.

Here's another way to look at the relative dilution factor of PBZ in this horse meat sample:

An Olympic swimming pool holds 2.5 million liters of water.

To get the same relative concentration of PBZ found in the single ESFA meat sample (20µg/kg):
a) Take one Olympic-sized swimming pool,
b) Add 3 ½ tablespoons of pure phenylbutazone to the pool.
c) Mix well.

If you drink a liter of this water, you will ingest the same amount of PBZ as in the contaminated horse meat sample. This level of PBZ has never been associated with any human disease condition. In fact, in the thousands of years that humans have been eating horse meat, it has never been implicated in the development of blood dyscrasias.

To ingest the same level of PBZ as the lowest human therapeutic dose (200mg) rarely linked to blood dyscrasia:
a) Drink 10,000 liters (2,641 gallons) of water from the pool.
b) Die of water poisoning (actually, you will die after drinking less than 0.11% of this volume).

When will death-by-water occur?
The body's response to water is highly individualized, with some people being more sensitive to excessive water intake than others. In other words, water toxicity is an ***idiosyncratic*** condition.

Safe maximum levels of water intake can be recommended and the incidence of water intoxication (hyponatremia, or too little sodium in the body due to its dilution by water) is extremely rare below these levels of ingestion, so long as the human is otherwise healthy. There are some conditions that would make even otherwise normal levels of water intake potentially risky to some unhealthy humans, however.

Sound like a familiar argument? Maybe we should we consider banning dihydromonoxide?
https://www.youtube.com/watch?v=yi3erdgVVTw

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
WASHINGTON, DC

FSIS DIRECTIVE	6130.1	6/28/13

ANTE-MORTEM, POSTMORTEM INSPECTION OF EQUINES AND DOCUMENTATION OF INSPECTION TASKS

I. PURPOSE

This directive provides instructions to inspection program personnel (IPP) on how to perform ante-mortem inspection of equines before slaughter and post mortem inspection of equine carcasses and parts after slaughter. Additionally, this directive instructs Food Safety and Inspection Service (FSIS) Public Health Veterinarians (PHVs) making ante-mortem and post-mortem dispositions of equines how to perform residue testing, verify humane handling, verify marking of inspected equine products, and document results using the Public Health Inspection System (PHIS) for equine when available.

II. BACKGROUND

A. The Federal Meat Inspection Act (FMIA) provides that there is to be an inspection of horses and other equines, among other species, to assess whether the carcasses of these animals are not adulterated, can be passed for human consumption, and are eligible to bear the mark of inspection (21 U.S.C. 604).

B. The FMIA requires that the slaughter or preparation of products of equines be conducted under inspection. FSIS regulations require that horse slaughter and preparation of products of equines be done in establishments that are separate from any establishment in which cattle, sheep, swine, or goats are slaughtered or their products prepared (9 CFR 305.2 (b)).

C. The Humane Methods of Slaughter Act of 1978 and 9 CFR Part 313 require that all livestock, including horses, slaughtered under inspection be handled humanely. Equines must be rendered insensible to pain (i.e. unconscious) before being shackled, hoisted, thrown, cast, or cut.

III. BEFORE START OF OPERATIONS

A. GRANT OF INSPECTION

1. Before issuing a grant of inspection for equine slaughter, a representative of the District Office (DO) is to verify that the establishment has:

    a. Sanitation Standard Operating Procedures (SSOPs);

    b. Performed a hazard analysis with supporting documentation;

    c. Developed a Hazard Analysis and Critical Control Points (HACCP) plan per 9 CFR 304.3,; And

    d. A recall plan per 9 CFR 418.3.

2. The Frontline Supervisor (FLS) at or prior to the start of operations is to inform the establishment management of applicable Food Safety and Inspection Service (FSIS) regulatory requirements per 9 CFR 305.4.

3. Before recommending approval for the grant of inspection or the start of operations and as necessary, the FLS is to determine whether any modifications to establishment facilities or other conditions are necessary to meet regulatory requirements per 9 CFR 307.2. The FLS is to advise the establishment management that the establishment with deficiencies will not be issued a grant of inspection until specified changes necessary to meet regulatory requirements are made.

4. Upon acceptance and approval of the application for a grant of inspection, the DO is to issue a conditional grant, not to exceed 90 days, to allow the establishment time to validate its HACCP plan.

5. The DO through the FLS or the PHV is to ensure that IPP receive all equine-related training provided by the FSIS Center for Learning (CFL).

B. AWARENESS MEETING

1. Before the start of slaughter operations, the PHV-IIC is to review with the establishment the FSIS procedures used to verify humane handling (9 CFR Part 313), identification (9 CFR Part 320), inspection, and other regulatory requirements referenced in this directive. The PHV-IIC is to document the meeting in a Memorandum of Interview (MOI) with distribution to the establishment and government office files in accordance with FSIS PHIS Directive 5000.1, Ch. 1, VIII. *Weekly Meeting.*

2. In addition, before the start of slaughter operations, the PHV-IIC is to review the information from this awareness meeting with the IPP assigned to the establishment.

IV. HUMANE HANDLING AND ANTE-MORTEM INSPECTION OF EQUINES

A. HUMANE HANDLING

1. IPP are to follow instructions in FSIS Directive 6900.2 Rev. 2; *Humane Handling and Slaughter of Livestock*, for verifying establishment compliance with humane handling and slaughter requirements set forth in 9 CFR Part 313.

2. During official hours of operation and when performing official duties, IPP are to verify the humane handling of all equines on the official premises from the time of unloading up to the time of slaughter. IPP are to verify:

   a. Facilities and handling are maintained at a level to prevent equine injuries per 9 CFR 313.1.

   b. The humane handling, segregation, identification, and slaughter of equines identified as U. S. Suspects per 9 CFR Parts 309 and 313.

   c. The humane handling, identification, stunning, and disposal of equine identified as U. S. Condemned per requirements in 9 CFR Parts 309 and 313.

NOTE: IPP are to immediately contact the District Veterinary Medical Specialist (DVMS) or DO via the PHV or FLS regarding any questions regarding the humane handling of equines.

B. HUMANE ACTIVITIES TRACKING SYSTEM (HATS):

1. FSIS IPP are to follow instructions in FSIS Directive 6900.2 Rev. 2, *Humane Handling and Slaughter of Livestock*, to perform and document HATS activities. See Section VIII of this directive regarding instructions on how to document HATS activities.

2. IPP are to seek guidance and updated instructions from the DVMS on how to perform HATS activities at official establishments slaughtering equines.

C. ANTE-MORTEM INSPECTION OF EQUINES

PHVs or IPP under PHV supervision are to conduct ante-mortem inspection of equines. FSIS IPP are to follow the verification instructions for ante-mortem inspection that are found in FSIS Directive 6100.1, *Ante-Mortem Livestock Inspection*. IPP are to conduct such inspection per the direction in this directive.

1. IPP are to observe:

   a. Equines at rest from outside the pen; and

   b. Equines in motion.

2. IPP are to perform ante-mortem inspection and accept only animals capable of producing products acceptable for use as human food. IPP are to pass equines for regular slaughter when ante-mortem inspection does not reveal diseases or abnormalities.

3. IPP while conducting ante-mortem inspection are to direct establishment employees to segregate all equines found to have any abnormalities or disease conditions into designated (suspect) pens for further examination by a PHV. Such additional inspection ensures removal from human food channels of equines that are:

   a. Obviously unfit for human food because of diseases or abnormalities;

   b. Have diseases or conditions that are difficult to detect on routine post-mortem inspection (e.g., central nervous system disorders, lameness, and chemical poisoning). See 9 CFR Part 309;

   c. Febrile or appear to be ill, depressed, or with a fever; or

   d. Showing indications of zoonotic or reportable diseases as listed in *FSIS Directive 6000.1, Rev. 1, Responsibilities Related to Foreign Animal Diseases (FADs) and Reportable Conditions - Revision 1.*

4. PHVs are to pass for slaughter with restriction suspect equines eligible for slaughter as U. S. Suspects per requirements in 9 CFR 309. 2.

5. In accordance with FSIS Directive 6100.1, *Ante-Mortem Livestock Inspection*, PHVs are to identify as "U.S. Condemned" any equines that found on ante mortem inspection to be:

   a. Dead or in a dying condition when offered for slaughter on the premises of the official establishment;

   b. Plainly showing on ante-mortem inspection any disease or condition that, under 9 CFR Part 311, would cause the PHV to condemn the carcass when inspecting post-mortem;

   c. Febrile with a temperature of 105° F or higher (9 CFR 309.3(c));

   d. In a comatose or semi-comatose condition; or

   e. Other condemnable condition per 9 CFR Part 309.

V. EQUINE POST-MORTEM INSPECTION

A. Head Inspection: IPP are to:

1. Observe head surfaces; and

2. Observe and palpate (incise when necessary) mandibular, pharyngeal, and parotid lymph nodes; guttural pouch; and tongue.

B. Viscera Inspection: IPP are to:

1. Observe and palpate lungs and bronchial and mediastinal lymph nodes (incise when abnormal);

2. Incise and observe heart as for cattle;

3. Observe and palpate spleen, liver (both surfaces), and portal lymph nodes;

4. Open the hepatic (bile) duct as for cattle; and

5. Observe remaining viscera including kidney if removed from the carcass and body cavities.

C. Carcass Inspection: IPP are to perform carcass inspection of equines using the same basic methodology used on cattle as described in FSIS Directive 6100.2, *Post-mortem Livestock Inspection*. IPP are to perform carcass inspection after carcass splitting and before washing. Depending upon facilities available and after approval by the FLS, IPP have two (2) approaches to carcass inspection. IPP may inspect equine carcasses by the quarters (i.e. hind quarters or forequarters; or high and low) or by the side (i.e. side by sides).

1. Carcass Inspection by the Quarters: Similar to inspecting beef carcasses on a high-low final rail, IPP inspect the carcass and viscera as follows:

   a. Hindquarter inspection. Used where viscera and carcass inspections are combined. For each hindquarter on each side:

      i. Observe back of skinned carcass after it has been eviscerated.

      ii. Palpate superficial inguinal, or supramammary, and internal iliac lymph nodes.

iii. Observe body cavities.

b. Perform viscera inspection per B. above.

c. Forequarter inspection. It completes carcass inspection started under "hindquarter inspection." For each forequarter on each side:

i. Observe cut surfaces of muscles and bones, peritoneum, and diaphragm's pillars;

ii. Observe and palpate kidneys and diaphragm in the carcass; and

iii. Observe pleura, neck, and carcass exterior.

2. Carcass inspection by the sides. Alternatively to inspection by the quarters, IPP inspect each side of the carcass to complete carcass inspection. This is typical with other livestock (e.g. cattle) carcass inspection on moving chains with separate carcass inspection stations. Carcass inspection is performed after viscera inspection and splitting of the carcass as follows:

a. Palpate superficial inguinal, or supramammary, and internal iliac lymph nodes;

b. Observe lumbar region;

c. Observe and palpate kidneys;

d. Observe diaphragm's pillars and peritoneum;

e. Observe and palpate diaphragm; and

f. Observe pleura, cut surfaces of muscles and bones, neck, and carcass exterior.

3. Additional carcass inspection. IPP perform the following additional inspections on all or particular retained equine carcasses. IPP are to observe (and incise when necessary):

a. The inner abdominal walls for encysted parasites when IPP observe inflammatory lesions as nodules in the equine stomach, ceacum, colon, or fat along the abdominal wall. IPP are to condemn and verify affected organs and parts are condemned and removed by trimming.

b. Observe after the carcass has been skinned, and before splitting the carcass, the "topped" withers. The upper third of the spinous processes of thoracic vertebrae two through nine are removed and presented for inspection. IPP verify there is no evidence of inflammation and infection that may be occasionally be found in the supraspinous bursa in the withers area.

NOTE: Lesions in this area (fistulous withers) are commonly the result of *Brucella abortus* infection; The incidence of brucellosis in these lesions is high and humans can contract brucellosis. The PHV is responsible to verify IPP and establishment employees maintain sanitary conditions, sanitary implements, and sanitary dressing procedures. IPP in contact with such lesions are to thoroughly wash hands and avoid placing their hands about their face. IPP are to always retain the carcass and parts for veterinary disposition when brucellosis is suspected.

c. Observe the axillary, perineal, and subscapular spaces of gray and white equines for melanosis and metastatic or invasive melanomas. To accomplish this observation effectively, the FLS and PHV are to arrange with the establishment procedures to identify carcasses of white and gray horses after the hide has been removed. To ensure detection of melanosis or metastatic melanoma lesions commonly seen in the axillary and subscapular areas of white or gray equines, per requirements in 9 CFR 305.4, 307.2, 310.2, and 310.3 and as requested by the FLS, the PHV may direct company personnel to routinely "drop the shoulders" of any or all white or gray equines. When "dropping the shoulders," the limb remains attached to the carcass. As usual, the PHV may perform other inspections as necessary at his or her discretion.

NOTE: The FLS or PHV may at the request of the establishment allow the dropping to be accomplished on the following day after the carcass has chilled. The carcasses must be under FSIS control (U.S. Retained) until after the inspection is completed.

VI. RESIDUE TESTING OF EQUINE

A. GENERAL

FSIS recognizes that most equines presented for slaughter will likely not have been raised for human consumption. Therefore, FSIS has concerns regarding the potential presence of chemical residues from drugs not previously approved for use in all food animals including equine. Because of these concerns about residues in horses, IPP should follow instructions in FSIS PHIS Directive 5000.1, *Verifying an Establishment's Food Safety System*, for verifying that the establishment that slaughters horses has addressed violative residues in its hazard analysis and that the establishment's HACCP system is effective in preventing horsemeat containing residues that would adulterate the meat under the FMIA from entering the human food supply.

In addition, FSIS expects many of the drugs used in working or pleasure horses are not antimicrobials and therefore would not be detected by FSIS in-plant antibiotic residue screening tests. Therefore, whenever IPP collect equine tissues for residue sampling as instructed below, IPP are to submit those tissues directly to the specified FSIS laboratory where a complete residue analysis can be conducted. IPP are to select carcasses for residue verification testing according to the two selection methods described below.

B. RESIDUE SAMPLING WHEN IPP FINDINGS SUGGEST INCREASED RISK OF DRUG RESIDUES

IPP are to select carcasses for residue testing when ante-mortem or post-mortem findings suggest an increased likelihood of recent drug treatment. IPP are to use the existing residue policies (including retaining of carcasses) in FSIS Directive 10,800.1, *Procedures For Residue Sampling, Testing, and Other Responsibilities for the National Residue Program*, for residue sampling, testing, and verification of the establishment's residue program and test every time the IPP suspect that there is an increased likelihood of a violative residue. Also, IPP are to use the list of pathologies and conditions in FSIS Directive 10,220.3, as a reference for conditions warranting residue testing. IPP are to retain any carcass suspected of containing a drug residue and follow the sample submission instructions described in part D. of this section for selected carcasses. The policy for testing animals from producers that are listed on the Residue Repeat Violator Lists as described in FSIS Notice 44-12 also applies to horse slaughter.

C. RANDOM RESIDUE SAMPLING OF NORMAL-APPEARING ANIMALS

Because equines are not generally raised as food animals, FSIS will conduct random residue testing of

normal-appearing animals to provide additional assurance that carcasses are free from drug residues. FSIS will conduct random testing of normal-appearing horses at least the same rate as for show livestock as described in FSIS Directive 10,800.1, *Procedures for Residue Sampling, Testing, and Other Responsibilities for the National Residue Program.* IPP are to randomly select, on the slaughter floor from normal-appearing equine, from every lot of animals that passes ante-mortem as follows:

1. A minimum of 1 animal if there are 1 to 10 animals in a lot;

2. A minimum of 2 animals if there are 11 to 50 animals in the lot;

3. A minimum of 3 animals if there are 51 to 100 animals in the lot; and

4. A minimum of 4 animals if there are more than 100 animals in the lot.

IPP are to retain the selected carcasses and follow the sample submission instructions in paragraph D. of this section

D. SUBMITTING RESIDUE SAMPLES

1. From each equine carcass selected for residue sampling under the two scenarios (i.e. Paragraphs B and C) above, IPP are to collect two (2) separate one pound muscle samples; and

    a. Submit one sample containing one pound of muscle to the Western Lab (WL) where it will be tested for pesticides; and

    b. Submit the other one pound sample from each carcass to the Eastern Lab (EL) where it will be tested for multiple chemical class residues and contaminants.

IPP are to follow the instruction provided in FSIS PHIS Directive 13,000.2, *Performing Sampling Tasks in Official Establishments using the Public Health Information System,* and FSIS Notice 58-12, *Scheduling and Submitting Lab Samples in PHIS,* on sample collection and submission of inspector-generated residue samples for laboratory testing. IPP are to create and schedule the sampling task in PHIS by selecting the following projects from the drop down menu in the Sample Management window of PHIS:

    a. Select project CG_EQUINE_EL for the one pound of muscle going to the Eastern Laboratory.

    b. Select project CG_EQUINE_WL for the one pound of muscle going to the Western Laboratory

2. Until the equine class is available in PHIS, unless directed by the DO otherwise, IPP are to verify that the establishment profile includes the slaughter class "GOAT" and enter equine data in PHIS using the goat slaughter class. If the establishment profile does not include the goat slaughter class, IPP are to add "GOAT" slaughter class to the plant profile.

NOTE: "GOAT" is being used at this time in order to capture necessary information in PHIS relative to equine. FSIS will manage PHIS results in a manner to discern goat data separately from equine data until such time that PHIS is modified to accommodate equine data entry. FSIS will rely upon the grant of inspection to discern which establishments in PHIS slaughter goat versus equine.

E. ACCESSING TEST RESULTS

1. IPP are to periodically access LEARN to check the status of tissue samples submitted for chemical residue testing. FSIS Directive 10,200.1, *Accessing Laboratory Sample Information via LEARN*, provides complete information on how to access LEARN on the FSIS intranet. Test results are reported in PHIS upon completion of the sample analysis. IPP can access test results in PHIS through the Laboratory Sample data field on the Inspector Home page.

2. IPP are to provide a printed copy of the test results from LEARN to establishment management and inform the establishment that it can receive sample results by email if it provides an email address to the IIC, who will enter it into the establishment profile information in PHIS. IPP are to advise establishments to add to their address book OPHSLearn@fsis.usda.gov to ensure the emails are not blocked. IPP are to provide a printed copy of sample results to the establishment regardless of whether they receive results via email.

3. Sample discard: If the FSIS Laboratory discards a sample submitted for chemical residue testing, IPP are to take appropriate action based on the reason for sample discard. IPP are to review the reason for sample discard, as indicated in LEARN, and make the necessary adjustments in how they collect, seal, and ship the samples to ensure that the laboratory does not discard future samples because of improper handling or packaging

F. IPP ACTIONS UPON REPORTING OF TEST RESULTS THROUGH LEARN

1. IPP are to check LEARN and review the test results. The PHV is to make a final disposition on the carcass and parts and take any necessary regulatory enforcement actions based on the results.

   a. For residue test results reported as "Not Detected," the PHV is to inform the establishment that the test result is "in compliance" and release the carcass and its parts.

   b. For residue test results reported as "Detected – violative," the PHV is to condemn the carcass and all parts and notify the establishment of the results and the final disposition of the carcass and parts.

2. IPP are to notify the establishment of each new violation, any developing trends, and final disposition of any carcass and its parts at the next weekly meeting and document the meeting in a MOI.

3. IPP are to seek guidance through their supervisory chain of command for any questions regarding residue test results or action to take based on test results. IPP may also submit questions through AskFSIS, using the instructions provided in Section X of this directive.

NOTE: Additional information on how FSIS expects establishments to address residues in a HACCP environment is available in Federal Register: November 28, 2000 (Volume 65, Number 229).

VII. MARKING OF EQUINE CARCASSES, PARTS, AND PRODUCTS

A. IPP are to verify the official inspection legend used in the establishment. 9 CFR 312.3 identifies the official inspection legends that are to be used in equine slaughter establishments.

B. IPP are to verify the establishment uses green ink that is approved to mark equine carcasses and product per 9 CFR 316.5(e).

C. IPP are to verify that the establishment marks equine carcasses, parts, and product per 9 CFR 316.12.

VIII. PERFORMING AND DOCUMENTING INSPECTION TASKS

A. Where no comparable PHIS FSIS Directive is published, IPP are to follow the instructions in the standard (non-PHIS) FSIS Directives for inspection activities applicable to all livestock slaughter and processing.

http://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/5000-series

B. When PHIS is not available, IPP are to contact the DO for additional instructions on how to determine what inspection tasks they are to perform, how often they perform the tasks, and how to document results.

C. Where FSIS Directives specifically provide instructions applicable to specific classes of livestock other than equine, and no specific direction is available for equine, IPP are to refer to and extrapolate instructions applicable to cattle when performing inspection procedures on horses after discussion with the PHV. The PHV may modify such instructions as appropriate. For example, IPP seeking guidance regarding sanitary dressing of horses are to refer to FSIS Directive 6410.1, *Verifying Sanitary Dressing and Process Control Procedures in Slaughter Operations of Cattle of Any Age - Revision 1*, until such information for equine is provided in a revised or new issuance.

IX. EXPORTS

IPP are to follow the instructions in FSIS Directive 9000.1, *Export Certification*, to certify exports of equine products for edible purposes. IPP are to refer to the FSIS Export Library opening page first for any general remarks about equine product exports, as well as the specific requirements for the country to which exports are being considered:

X. QUESTIONS

Refer questions regarding this directive to the Policy Development Staff through askFSIS or by telephone at 1-800-233-3935. When submitting a question, use the Submit a Question tab, and enter the following information in the fields provided:

Subject Field:	Enter **Directive 6130.1**
Question Field:	Enter your question with as much detail as possible.
Product Field:	Select **General Inspection Policy** from the drop-down menu.
Category Field:	Select **Slaughter** from the drop-down menu.
Policy Arena:	Select **Domestic (U.S.) Only** from the drop-down menu.

When all fields are complete, press **Continue**.

Charles E. Williams

(for) Assistant Administrator
Office of Policy and Program Development