antibiotics but receiving only therapeutic antibiotics, 26% among isolates from swine never having received antibiotics and in 100% of the isolates from swine continuously exposed to subtherapeutic levels of antibiotics. Langlois et al.[11] found less of a decrease in antibiotic-resistant coliform organisms in pigs given therapeutic levels of antibiotics compared to pigs fed continuous subtherapeutic levels of antibiotics once the drugs were withdrawn. Therefore, Langlois et al.[11] has suggested therapeutic doses of antibiotics may have a more marked effect than subtherapeutic doses of antibiotics. They found bacterial isolates from pigs that received therapeutic doses of one of the tetracyclines for only 14 days (total of 22.0 μg/g) had more antibiotic resistance than those that received subtherapeutic doses (total of 27.5 μg/g) for 85 days.

Variability in the frequency of antibiotic resistance of salmonellae isolated from healthy poultry has been noted. Salmons[19a] has summarized several industry-sponsored studies. In the survey of chickens, "by far the predominance of Salmonella isolates were sensitive to the broad spectrum of antibiotics used in human or animal therapy. However, isolates from turkeys were 22-23% resistant to streptomycins, tetracycline, panamycin and neomycin."

Other reports have found similar variations between the prevalence of resistance among species, by years and by geographical locations.[2,3,13,14,16,19] They were unable to correlate level of antimicrobial drug use and percentage of resistance in most of these studies; percentage of resistance was reported for tetracycline as 50% to 85% and for ampicillin as 16% to 80% which agrees with the results submitted to the committee. Two labs also reported a decreased percentage of resistant organisms.[14,16]

In conclusion, there have been several reports and surveys of resistance of various enterobacteriaceae to various antibiotics in cattle, swine, and poultry within the United States. These reports generally agree that feeding subtherapeutic antibiotics to animals or therapeutically treating animals with various antibiotics causes an increased in the frequency of isolation of Salmonella spp. and E. coli that are resistant to those antibiotics. However, there appears to be a regional and temporal difference in the percentage of resistance and some variation of resistance expressed between animal species. These results probably reflect the difference of usage both subtherapeutically and therapeutically of the various antibiotics between poultry, cattle, and swine specimens submitted to these labs. Varying production methods, stress, and management practices could also explain some of the differences and reported decreases in resistance.

Also, these data indicate that the percentage of resistance to antimicrobial agents in isolates of salmonellae

from animals in the U.S. is 3 to 5 times greater than that in isolates from humans. Greater differences are seen in the data for isolates of E. coli in humans or animals, if we assume that hospitalized patients or range cattle represent a small portion of the total human or animal populations, respectively. Since farm animals outnumber humans in the U.S. (see Chapter IV), they harbor in their intestinal flora a reservoir of resistance genes that may be an order of magnitude larger than that of the flora in the total human population.

EFFECT OF BANNING THE USE OF SUBTHERAPEUTIC DOSES OF ANTIBIOTICS

To assess the impact of subtherapeutic use of antibiotics on the selection of antimicrobial-resistant bacteria isolated from animals that may also cause human disease, it is critical to review the experience in England and the other countries where growth promoting use of antibiotics has been prohibited. In 1969, Swann et al.[24] were appointed to the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine in England to obtain information about an increase in resistant strains; it produced a report that attempted to explain in simple and straightforward terms how the use of antibiotics in animals may affect both humans and animals.

Other concerns that influenced the Swann committee included the presence of trace amounts of antibiotics in meat or poultry products consumed by humans and their potential for causing allergic or toxic reactions and allowing the selection of resistant bacteria from among the nosocomial flora, and the possibility that S. typhi would develop resistance to chloramphenicol, the drug of choice at that time.

Recommendations adopted included the division of antibiotics for agricultural use into two classes: therapeutic antibiotics, for use in treating bacterial infections in animals and available only by prescription from a veterinarian; and "Feed" antibiotics used in subtherapeutic doses for growth promotion that is available to the farmer without a prescription through feed merchants or farm stores. It was recommended that the latter class be restricted to those drugs that have no use in human medicine. Thus, Zinc Bacitracin, Virginiamycin, Avopracin have been used and apparently do not select strains that would be resistant to the tetracyclines, penicillin, and other antibiotics. Penicillin and the tetracyclines have not been used for feed additives or growth promotion. Although those two antibiotics could be added to animal feed if the purpose was for treatment or prevention of a bacterial infection, neither

could be included at low concentrations for promoting growth. Such use of an antibiotic in feed is prescribed by a veterinarian for a particular disease episode, generally for no longer than 4 weeks.

The subtherapeutic dose (200 g/ton) of antimicrobials in the United States is considered a therapeutic dose in the United Kingdom. Therefore, it is difficult to compare the impact due to the use of this concentration of antibiotics in each of these two countries on the selection of resistance because the applications for this concentration have been different. This difference in application of dosage is critical to our understanding in the United States about the position of the British scientists who feel that 200 g/ton is a concentration that is important in selecting resistance in infectious bacterial strains.

Many changes have occurred in animal husbandry since 1969, and there was no systematic collection of data before that date, so the effect of the Swann Committee recommendations cannot be accurately assessed.[18,27,28] Interested investigators and government groups have gathered data on the number and types of some resistant organisms in animals and humans in the British Isles, but no comprehensive prospective study has been initiated to evaluate the effect of the recommendations. Antibiotic use apparently had increased in both humans and animals since the Swann report (see Table 1 in Braude[4]). Also, human use of antibiotics in the United Kingdom increased rapidly; it was 17 times the veterinary use in 1980, but before the Swann report, the human use was only 1.4 times the veterinary use.[30]

Walton and other researchers have become convinced that the therapeutic use of antibiotics in humans, as well as in animals, causes the selection of resistance in the bacterial strains in humans.[18,25,27,28] They contend that concentrations of antibiotics achieved in animals receiving subtherapeutic concentrations of antibiotics (presumably less than 200 g/ton of penicillin or the tetracyclines) did not reach the critical points necessary for the selection of resistant strains.[28] Although there is concern that some bacterial strains found in animals have multiple antibiotic resistance that could be a hazard to human health, the situation has not worsened despite increasing antibiotic use in animals. Furthermore, it is the general feeling of some scientists in the United Kingdom that the Swann Committee recommendations have had no impact in reducing this hazard.

The available data are sufficient for an assessment of the changes in resistance patterns as well as assessment of the numbers of isolations of various species of salmonellae in England since 1970. Sojka et al.[23] have periodically reported similar results over the period from 1972-1986. Those surveys clearly indicate that the recommendations in the Swann report did not stop the development of antibiotic

resistance, especially resistant to penicillins and tetracyclines. Chloramphenicol resistance has steadily increased in some isolates, despite the prohibition of the use of this antibiotic in feed. The authors of those surveys conclude that the therapeutic use of antibiotics in animals, combined with poor animal handling and management practices, especially regarding calves, does continue to promote the development of resistant strains.[18,25,27,28]

Resistance of salmonellae to penicillins and tetracyclines in animals varies with the animal; those of bovine origin are less likely to be sensitive to either kind of drug than those from poultry. In all isolates of resistant S. typhimurium, predominately phage type 204C, and related types 49 and 204--accounted for most of the resistant strains. Those phage types appeared in calves in 1979, spread widely in the next 2 years, and they remain the predominant types in cattle (59% in 1985). Phage type 204C has also caused enteritis in humans as observed in 4% of the patients in 1985. The disease has usually consisted of mild to moderate diarrhea, but several of the 677 patients with S. typhimurium infection in 1977-1984 had to be hospitalized for severe diarrhea. The cases in two outbreaks might have been due to consumption of raw milk, while most of the other cases were thought to be farm workers, but that has not been confirmed. Most people with infections had no farming connections. The bacterial strains isolated from these cases were resistant to ampicillin, chloramphenicol, gentamicin, tetracycline, and trimethoprim. Strain of 204C phage type accounts for 77% of all the Salmonella strains isolated from calves in 1985.[9] Apparently, infected calves stop shedding S. typhimurium before they reach slaughter weight and, therefore do not serve as a source of infection to humans because they do not enter the food chain in great numbers. The spread of phage type 204C probably occurred because of the practice of selling colostrum-deprived calves from broker to broker several times during the first 56 days of life. Calves apparently are susceptible to Salmonella infection during this early period and poor management practices contribute to the problem during frequent trips to market, whereby they acquire salmonellae from other animals. It is speculated that the resistant salmonellae became resistant because of futile attempts to treat calf scours with numerous antibiotics.[25] These salmonellae also appear to have a predilection for acquiring plasmids. Each year since isolation the resistance pattern of phage type 204C has broadened; gentamicin is the most recent antibiotic to which the strain has developed resistance.[23] The plasmid that codes for resistance to gentamicin also confers resistance to netilmicin, tobramycin, and apramycin. The last named is an aminoglycoside that is used to treat salmonella infections in calves, its use is probably the reason that resistance to

apramycin and gentamicin appeared. Phage type 204C has also appeared in the Netherlands[26] and Denmark,[8] and was imported into the European countries via veal calves.

The appearance among bacterial isolates of S. typhimurium phage type 204C with multiple antibiotic resistance has been an isolated event in England. Other Salmonella species have not shown the same rapid increase in acquiring resistance. There have been dramatic shifts in the number of isolations of various other resistant Salmonella species. For example, S. agona appeared in the early 1970s in England and the United States, having been imported from Peru with contaminated fish meal (to be used as poultry feed).[9] By 1975, there were 1,821 human isolates, the peak number. The number fell to around 450 for the years 1979-1983; reasons for the decline are unknown. S. hadar appeared in 1971 and peaked in 1979 at 2,480 isolates. This strain was isolated from turkey breeding stock, and the meat from contaminated, undercooked large birds caused outbreaks.[9] In 1984, only 496 isolations were reported; again, the reasons for the decline are unknown. Those two examples demonstrate that the presence of antibiotics in animals did not cause the strains to proliferate or to develop resistance to antibiotics, such as occurred with S. typhimurium phage type 204C and related types.

Walton noted several lessons that were learned from the United Kingdom's experience with the Swann Committee recommendations.[27,28] Antibiotics, such as the tetracyclines and penicillin used in the production of meat products, did not become ineffective, despite the development of resistance by bacteria. Those two classes of drugs continue to be used and to be effective prophylactically and therapeutically. Food animals have short life spans--broiler chickens, 35-56 days; pigs, 3.5 to 5 months; and cattle 2.5 years--and the rapid turnover results in the destruction of large numbers of bacteria.[27,28] When large batches of animals leave their quarters, cleaning is carried out with high-pressure water, which not only removes most gross amounts of offal but also dilutes and kills bacteria. Better control of antibiotics has been instituted because of the requirement that veterinarians write prescriptions for the use of antibiotics in feed.

One item that has not been clear from the discussions presented above is the human health hazard associated with the increasing number of salmonellae isolated in the United Kingdom. Figure V-1 shows the incidence of salmonellosis in England and Wales in 1941-1984. This shows a significant increase in the numbers of incidents during these years. Interpretation of the trends indicates an epidemic of S. typhimurium infection in the United Kingdom. Total figures for mortality caused by salmonellae is not available to the committee. However, some figures have been obtained;

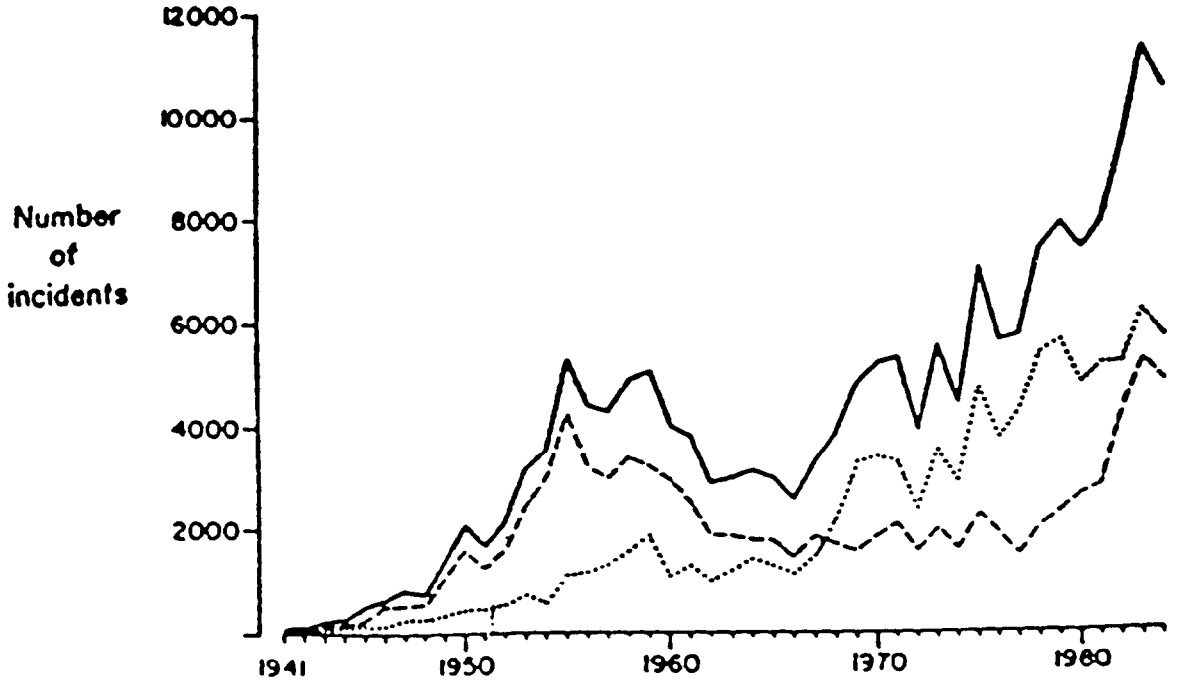

Figure V-1. Salmonellosis, England and Wales, 1941-1984. Reprinted from Palmer and Rowe.[17]

——— Total Salmonellae
- - - Salmonella typhimurium
····· Other salmonella serotypes

a major hospital outbreak in 1984 involved about 350 patients and 50 staff members; 19 patients died. S. typhimurium was the causative organism. Other less striking examples of the deaths during outbreaks listed were as follows: 2 of 654 patients infected by S. typhumurium from raw milk in 1981; none of 500 patients infected by S. montevideo from chicken in 1981; none of 245 patients infected by S. napoli from chocolate in 1982; 2 of 766 patients infected by S. enteritidis from a spicy glaze in 1984; 4 of 274 patients infected by S. virchow from cooked meats in 1985; and 1 of 60 patients infected by S. ealing from infant dried milk in 1985. Those are selected outbreaks and do not represent a thorough survey. The resistance of these bacteria to various antimicrobial drugs was not reported. No great increase in mortality occurred inasmuch as the authors who reported on the incidence of the disease and the apparent increase in numbers of bacterial isolates did not indicate any increase in mortality.[18,25,27,28]

Walton[27,28] also suggested in 1985 that the 15 years of antibiotic controls in the United Kingdom as recommended by the Swann Committee and similar controls in Europe had provided guidance for other countries that wanted to develop antibiotic control policies. Other authors such as Rowe and Threlfall[18] appeared to concur, with the following suggestion: Total control of antibiotic use is neither possible nor even necessary. Rather a redefinition of the current policy is needed, plus updated practical measures to assess the most effective use of the drugs.

In summary, the United Kingdom's experience with restricting the use of antibiotics in feeds has shown that resistance in bacteria probably develop in spite of the controls on "feed" (subtherapeutic concentrations) antibiotics not used in humans. Thus, prohibition of subtherapeutic doses of antibiotics in animals has not prevented or even affected the prevalence of resistant bacteria in the United Kingdom.

In conclusion, it is impossible to ascertain the effectiveness of the Swann Committee recommendations, because agricultural practices have changed substantially and because the therapeutic use of antibiotics--is a more important stress in the selection of resistant organisms than subtherapeutic. Resistant strains of salmonellae and other bacteria have persisted; some have increased in incidence, and others have decreased. The reasons for the changes are unknown, but do not appear to be related solely to the presence of antibiotics in the gastrointestinal tract. Human health hazards persist, perhaps they have increased. Human cases of salmonellosis have increased, but whether mortality from this disease has changed cannot be ascertained.

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VI

EVIDENCE OF TRANSMISSION OF PATHOGENS OF FARM ORIGIN TO HUMANS

Evidence of transmission of bacteria from farm-animal-origin to humans has been found in two genera of bacteria: Escherichia and Salmonella.

ESCHERICHIA COLI

Escherichia. coli and other enteric bacteria resistant to multiple drugs have been found to spread from farm animals into farm workers, their families, and the nearby community has been investigated. Such studies have, in general, indicated that multiple-drug-resistant E. coli organisms do indeed colonize farm workers, and to a lesser extent their families, and at times even spreads to nearby non-farm populations. No evidence has suggested, however, that multiple-drug-resistant E. coli of farm origin is associated with a higher risk of serious infection than E. coli of non-farm origin.

Perhaps the first systemic study of the change of coliform organisms from susceptible to multiple-drug-resistant in farm animals and in eleven members of the family on this farm. This was a prospective study carried out by Levy and Associates.[11] The systemic fecal sampling showed an increase in resistant E. coli within a week after start of the feeding of tetracycline-supplemented feed to a flock of chickens. The numbers of tetracycline-resistant intestinal coliforms also increased in the eleven members of this farm family, but not in their neighbors. Within 3-5 months after medicating the chickens, 31% of the fecal samples taken each week from each member of the farm family yielded bacterial populations of which 80% of the coliform bacteria colonies were tetracycline-resistant, compared with 6.8% of the samples from neighbors. About 6 months after the tetracyclines had been removed from the animal feed, the percentage of resistance organisms in farm dwellers' fecal samples that yielded coliform organisms over 80% of which were tetracycline-resistant had decreased to approximately the magnitude found before use of the tetracyclines was started. The rapidity with which commercially processed poultry is marketed precludes a study over a long time period of the change in the percentage of tetracycline-resistant

coliform bacteria isolated from chickens after discontinuation of tetracycline-supplemented feed.

The potential for spread of antimicrobial-resistant E. coli between farm animals and from farm animals to farm workers and the environment was further demonstrated in very recent experiments by Levy and Marshall.[11a] In this study a calf was fed a marked strain (containing both chromosomally mediated nalidixic acid resistance and a large plasmid encoding multiple antimicrobial resistance including that of tetracycline) capable of colonizing the human as well as bovine intestinal tracts. In the absence of any antimicrobial administration the marked strain was detected in the feces of the calf, and of another bovine kept in an adjacent stall, for at least 3 months. The same market strain was also present in the excreta of mice kept caged in the stall with the calf and in flies trapped in the farm. In addition, two farm workers caring for the bovines began excreting the marked strain in the stools 4-7 days after the experimental strain had been fed to the calf. Colonization of the intestinal tract of these two farm workers, who were not receiving antimicrobials continue for 30-45 days.

That antibiotic-resistant coliform organisms of farm origin sometimes can cause disease in humans was suggested by Hummel and colleagues.[10] They studied a pig-farming in a defined territory in which the streptothricin antibiotic nourseothricin was added to pig feed to promote growth. After 2 years of nourseothricin use in pig feed, they reported that coliform organisms containing plasmids encoded for nourseothricin resistance were found in 33% of the isolates from fecal cultures from pigs with diarrheal disease, in 18% in those from employees of the pig farms, 17% among isolates from families of employees, and 16% in those of outpatients living in nearby communities. Although no nourseothricin had been used in the human population in the territory, 1% of the isolates from urinary tract infections of outpatients were nourseothricin-resistant E. coli. Examination of cultures from pigs, farm employees, and outpatients in neighboring territories that did not use nourseothricin in pig feed revealed no nourseothricin-resistant E. coli.

Much of the important information needed to evaluate the results of the study of Hummel et al. is lacking. The dates of the study were not specified, nor were demographic data on the territories studied well defined. There was no information on the density of the pig population or the human population, and it has been difficult to assess the degree of contact with pigs on different farms in the various populations studied. Similarly, the timing of cultures and their study thereof for nourseothricin resistance in the various populations was not specified. However, the available data do suggest that nourseothricin-resistant

E. coli was transmitted from pigs to humans and from humans to other humans. Once the human gastrointestinal tract had been found to be colonized, it was not surprising that the organisms were occasionally found in urinary tract infections; however, it was not determined whether the organisms were more or less virulent than E. coli not resistant to nourseothricin.

Parsonnet and Kass[14] compared the antibiotic-resistance patterns of E. coli isolated from the urine of bacteriuric female slaughterhouse workers with those of E. coli from poultry in the processing line. E. coli was found in 95% of the cultures from poultry; 96% of them were resistant to antibiotics, and 87% were resistant to more than one antibiotic. The microorganisms isolated from the bacteriuric women's urine, however, only infrequently showed similar resistance patterns or identical patterns with those of the microorganisms from the poultry to which they were heavily exposed. Unfortunately, the bacteria in the women's feces were not studied, so direct spread from the processing line to their gastrointestinal tracts could not be determined.

Such a direct analysis of antimicrobial resistance patterns in fecal E. coli strains (rather than strains causing urinary tract infections) would seem necessary to document spread from animal foodstuff to humans, since E. coli strains causing human urinary tract infections represent only a small nonrandom group of clones, not found with equal probability among those which colonize the intestinal tracts of humans and animals. E. coli strains causing urinary tract infections in individuals without underlying microbiologic abnormalities belong to a limited number of O.K. serogroups and possess specific virulence factors.[10a,24a]

The antibiotic-resistance plasmids of the poultry and slaughterhouse workers from the latter study were examined for matching restriction endonuclease digestion-fragment patterns (T. F. O'Brien, 1988, personal communication). If a plasmid had been endemic among the poultry isolates, as found earlier among cattle isolates of Salmonella typhimurium var. copenhagen, its presence or absence in the human isolates would support or argue against the spread of drug resistance from the poultry to the workers.[10,11] In fact, the same plasmid could be found in two isolates in only a few instances, so the result had little power to exclude the possibility of spread. Extensive spread, however, might have been expected to yield human isolates with higher rates of resistance or more antibiotypes closely matching those of the poultry isolates.

The human health hazards attributable to infection with multiple-drug-resistant E. coli of animal origin were studied more directly by Habte-Gabr and colleagues in Iowa.[8] In 1972-1973, they studied 148 Iowa families: 51 families exposed to livestock given antibiotic-supplemented feed, 43

rural families with no exposure to livestock, and 54 urban families. Multiple-drug-resistant E. coli was found in 15% of the stool cultures from members of animal-exposed families, in 6% of those from members of rural families not exposed, and in 7% of those from members of urban families. A follow-up health survey was conducted 12 years later with 126 of the original 148 families. The incidence of serious infections was 6% in members of rural families exposed to livestock, 13% in members of rural families not exposed to livestock, and 12% in members of urban families. Thus, colonization by multiple-drug-resistant E. coli of farm origin did not appear to be a factor in infection in members of those populations. More extensive bacteriologic studies were not carried out and the populations studied were not large, so the study could "detect" only a high level of transmission of multiple-drug-resistant E. coli of farm origin that caused serious infections. If such spread of infection occurs at all, it is likely to be infrequent.

SALMONELLAE

Most evidence linking human disease to multi-resistant bacteria of farm origin has been found in salmonellae. Data detailing the incidence and associated morbidity and mortality of salmonella infections in farmers, slaughterhouse workers, and their families are not available. Comparison of case reports on farmers who used subtherapeutic antibiotics as livestock feed additives with those on farmers who did not might be particularly informative. The only information available is in the form of case reports or descriptions of small numbers of outbreaks in farmers and their families, but not in slaughterhouse workers.

The paucity of reports might suggest that the occurrence of salmonella infection in the rural or urban population is rare, indeed. In a 10-month study of 279 second-grade farm children in a rural county of Virginia, 149 episodes of diarrheal illness occurred in 97 children; salmonellae were isolated from only one of over 400 stool samples in the 149 cases of diarrhea.[5] The children were in two groups: 92 lived on commercial poultry farms, and 187 did not. The occurrence of diarrheal episodes was almost identical in the two groups. Despite the high prevalence (27%) of salmonella infection among the poultry flocks, only one culture-proven case of salmonella gastroenteritis (antibiotic susceptibilities not known) occurred, and it was in a child who did not live on a poultry farm.

Williams[25] described two veterinarians with pustular forearm lesions due to salmonellae (S. dublin and S. typhimurium) that occurred several days after they delivered an infected stillborn calf or cleaned a cow that had recently

aborted. But they did not describe the antimicrobial susceptibilities of the isolates.

Through 1980, five outbreaks of human salmonellosis directly linked to contact with farm animals have been reported. In the mid-1960s a multiple-antibiotic-resistant strain of S. typhimurium (phage type 29) caused a large outbreak of bovine infection in Great Britain.[1] Infection occurred in farmers, their families, and veterinarians who treated infected calves; spread from animals to humans was implicated. Spread of infection to dairy cows led to 59 human cases of milk-borne salmonella gastroenteritis. Prophylactic use, in healthy animals, of antibiotics to which the epidemic Salmonella strain was resistant might have favored infection with the pathogen by reducing the numbers of competing nonpathogenic antibiotic-susceptible intestinal bacteria.

Salmonella gastroenteritis occurred in a 12-year-old Canadian farm boy who cared for an infected dairy cow and its new calf.[7] The strains of S. typhimurium isolated from the cow and the boy were of the same phage type and antibiotic-resistance pattern (resistant to tetracycline and chloramphenicol). Spread of infection from the cattle to the child was considered most likely. Administration of antibiotics to the sick cow by the farmer 5-6 days before his son became ill might have led to selection of salmonellae with the aforementioned resistance pattern. However, possible use of subtherapeutic doses of antibiotics in feed was not mentioned.

An outbreak of salmonellosis involving several newly arrived calves on a Connecticut farm occurred in 1976.[12] S. heidelberg that was resistant to chloramphenicol, sulfamethoxazole, and tetracycline was responsible. The farmer and his pregnant daughter cared for the sick animals and became infected themselves. The daughter gave birth to a son 9 days after the calves arrived on the farm; 3 days after delivery, her newborn infant developed salmonella gastroenteritis and bacteremia. Infection spread in the nursery to two other babies, most likely by contact with nursery staff. The strain of S. heidelberg isolated from three calves and the farmer had identical antimicrobial susceptibilities, and those isolated from the farmer's daughter and the three infants were very similar (resistant to chloramphenicol, sulfamethoxazole, and tetracycline), but lacked resistance to neomycin, streptomycin, and kanamycin. Information on subtherapeutic or therapeutic use of antibiotics in the calves is not available.

In the late 1970s, numerous outbreaks of salmonellosis due to multiple-antibiotic-resistant S. typhimurium of phage types 204 and 193 occurred among calves on over 300 farms throughout Great Britain.[17,24] The two strains of S. typhimurium made up 28% of all S. typhimurium isolates from

cattle that were sent to the Central Public Health Laboratory for evaluation in 1978. The same two strains were later isolated from 211 human infections, including one that ended fatally, in the British Isles. In most human cases, no apparent connection with cattle could be found, but the same strains were later isolated from minced meat and sausage, suggesting entrance at some point into the human food supply. However, 30 of the human infections occurred in persons on farms where outbreaks of bovine infection with the multiple-drug-resistant strains of S. typhimurium were occurring or had previously occurred.[17]

Before 1977, the predominant-antibiotic resistance pattern in S. typhimurium of phage type 204 responsible for several outbreaks of salmonellosis in cattle and humans in Great Britain consisted of resistance to sulfonamide (nontransferable) and tetracycline (not directly transferable, but mobilizable by F-like plasmids). In 1977, a strain of phage type 204 that had gained an additional transmissible R plasmid (H2 compatibility group) bearing resistance to chloramphenicol (C), streptomycin (Sm), sulfonamide (Su), and tetracycline (T) was responsible for a small outbreak of salmonellosis on a farm in Leicestershire. The farm was involved extensively in calf-trading, which resulted in wide distribution of calves infected with the multiple-drug-resistant strain. It was thought that acquisition of the new H2 plasmid probably resulted from selective pressure introduced by the use of chloramphenicol in treatment of a calf infected with a type 204 strain that had the original R plasmid (SuT), which was predominant before 1977. Alternatively, the multiple-drug-resistant (C Sm Su T) plasmid might have been brought in with a newly purchased, already infected animal that could have been introduced into the herd shortly before the outbreak.[24]

An outbreak of multiple-drug-resistant salmonella infections involving three of four members of a family who worked on a dairy farm in Kentucky occurred in 1977.[4] Infection appeared to have been transmitted through ingestion of unpasteurized milk.

These data are not sufficient to support any conclusions concerning the relative incidence of infections with salmonellae (either antibiotic-susceptible or -resistant) in farm workers or their families, compared with other population groups. Data are not available to this committee on the frequency or severity of infections with salmonellae (either antibiotic-susceptible or -resistant) in slaughterhouse workers. In the five salmonella outbreaks described above, there are no data on the role of subtherapeutic use of antibiotics in feed, although the use of therapeutic dosages of antibiotics in the first four was considered to have important effects.

Since 1980, several additional outbreaks of multiple-

drug-resistant salmonellosis provide some evidence, and in one case compelling evidence, that the resistant salmonellae originated in farm animals fed antimicrobial drugs. Holmberg et al.[9] reported on an outbreak of S. newport resistant to ampicillin, carbenicillin, and tetracycline that occurred in several midwestern states. Food histories and plasmid profiles of the organisms isolated from both humans and animals led the authors to conclude that the resistant organisms infecting the patients were of animal origin and that the probable source was contaminated hamburger, the meat of which was derived from a single herd. The subtherapeutic use of chlortetracycline in this herd was admitted by the farmer, but this has not been analyzed or proven. Although the editorial that accompanied the report of Holmberg et al.[9] suggested that the study provided the "important missing link" between human disease and resistance in the infecting bacteria due to the feeding of subtherapeutic antibiotics to animals, the evidence is incomplete. (Note: Dr. Holmberg's comments, in personal communication, about this article are inserted parenthetically below.)

First, as pointed out by DuPont and Steele,[5] the pathogenic bacterial strain was not recovered from the slaughterhouse or from the hamburger (all the hamburger meat had already been consumed and none of the slaughterhouse animals were available for study), and no cases of S. newport disease occurred in the cattle or in the people associated with the farm that reared the animals or the processing plants (living cows remaining on the farm were excreting S. newport). Second, another processing plant in another state received half the carcasses from this herd of cattle and had no apparent problem (actually there were only 12 animals out of the 105 animal herd sent to another state; that cases traceable to these were not uncovered may only mean that some or all of these 12 animals were not infected or that ill persons were not ascertained or reported). Third, the only S. newport isolated from an animal and of a strain identical with the outbreak strain was isolated from a calf that died in an adjacent dairy herd. That calf might have been the source of the infection (this calf was not the only animal from which S. newport was isolated, as stated above, some of the cows on the farm were excreting the bacterium).

More recently, an outbreak of multiple-drug-resistant S. newport in California in 1985 convincingly demonstrated the entire chain of transmission.[22] The outbreak strain was resistant to chloramphenicol, tetracycline, kanamycin, ampicillin, and sulfisoxazole and was characterized by a single large plasmid. Epidemiologic studies identified ground beef as the suspect food vehicle, and many of the patients had consumed the ground beef at fast-food restaurants. Microbiologic and epidemiologic studies traced

the epidemic strain through the hamburger, back to meat processing plants, and ultimately back to the farms from which the animals were sent for slaughter. The isolates were from ill calves and cows at a number of dairies in important dairy-farming areas. Isolation of chloramphenicol-resistant salmonellae was associated with chloramphenicol use at those dairies. Such use of chloramphenicol as a feed additive is not approved by the Food and Drug Administration.

Several recent milk-borne outbreaks of multiple-drug-resistant salmonellae provide additional information, but do not directly link the organism to a farm source, or to subtherapeutic use of antibiotics. Tackett et al.[23] reported an outbreak of multiple-drug-resistant S. typhimurium that occurred in Arizona caused by the ingestion of raw milk. This bacterial strain was isolated from the raw milk samples. Further investigations into the source of contamination was not done because the implicated dairy withdrew the product from the market and would not permit any examination of the facility, its employees, or its animals.

The largest outbreak of salmonellosis ever recorded in the United States occurred in Illinois and Wisconsin in 1985 and involved over 16,000 bacterial-culture-confirmed cases.[18] In these studies, the estimates of cases derived from telephone surveys estimated the actual number of people infected was about 175,000. The epidemic strain was resistant to ampicillin, tetracycline, carbenicillin, streptomycin, sulfisoxazole, erythromycin, and penicillin. The outbreak ultimately was traced to two brands of pasteurized milk produced by a single dairy plant. The source of the infecting bacterial organism was presumed to be the dairy cattle, although this could not be demonstrated conclusively because no isolates either from the dairy-animals or the farm had exactly the same plasmid profile as the strain isolated from the milk.

One might speculate on whether those outbreaks due to multiple-drug-resistant salmonellae might still have occurred had the salmonellae been fully susceptible. The cause of each of the outbreaks appeared to be defects in food processing or inappropriate food preparation, rather than being due to the fact that the salmonellae were multiple-drug-resistant. Such defects would allow the persistence of any salmonellae, whether antibiotic susceptible or resistant. Hence, it would be difficult to argue that the outbreaks would not have occurred at all had the salmonellae been fully susceptible.

A recently reported outbreak of egg-associated fully-drug-susceptible S. enteritidis infections underscores the ease with which salmonellae can enter the food chain, in spite of the usual food processing and food preparation safeguards. Epidemiologic data suggested that, rather than the usual mechanism of contamination of the shells,

especially cracked shells, by salmonella-containing chicken feces, the mode of transmission was transovarial, with infection of the yolk before shell deposition. That mechanism would thwart the usual method to decontaminate eggs; and any use of such eggs that involved little or no cooking--e.g., use with hollandaise sauce, eggnog, or Caesar salad dressing--would likely result in cases of human salmonellosis.

The evidence of farm-to-human spread of salmonellae derived from the study of outbreaks should be put into the broader context of the overall epidemiology of human salmonellosis. In a study of farm children regularly exposed to poultry, Marx[13] could find no evidence of a greater occurrence of salmonellosis and diarrheal illness than in a control group. In a multivariate analysis of clinical and epidemiologic features of multi-drug-resistant salmonellae causing salmonellosis in humans, Riley and colleagues[16] found no evidence that exposure to animals or pets was a significant risk factor. Risk factors identified in their investigation included the recent use of antimicrobial agents by patients, a Hispanic ethnic background, regular antacid use, and age over 60 years. Thus, although transmission of salmonellae from farm animals to humans has been documented in several instances, it is not frequently recognized. It would appear that the best protection against multiple-drug-resistant salmonellae is the same as that against fully susceptible salmonellae; that is, accepted sanitation and sterilization (cooking) techniques of food processing and food preparation.

OTHER ENTERIC PATHOGENS

The available information on three enteric pathogens--enterohemorrhagic *E. coli*, *Yersinia enterocolitica*, and *Campylobacter* spp.--is sparse, but they are responsible for important clinical infections and should be mentioned. The committee did not search the data files of clinical or diagnostic laboratories in the United States for information on those organisms, but has relied on published summaries. The committee acknowledges that the hazard associated with transmission to humans of these bacteria that might have originated on the farm cannot be evaluated.

Enterohemorrhagic *E. coli* was recognized in 1982 as a major etiologic agent of the syndrome of hemorrhagic colitis, a diarrheal syndrome characterized by rapid progression from watery to bloody diarrhea and marked by severe morbidity.[3,15,19] Signs of enteroinvasive infection, such as fever or the presence of fecal polymorphonuclear leukocytes, are usually lacking or are not prominent. A particular serotype of *E. coli*, O 157:H7, is especially associated with

the syndrome and has been found to produce cytotoxins similar to the shiga toxin of Shigella dysenteriae type 1.

Outbreaks of hemorrhagic colitis due to enterohemorrhagic E. coli have occurred in persons of all ages, but have been prominent in elderly residents of nursing homes. Deaths were frequent in nursing home outbreaks. Several outbreaks have been traced to the consumption of beef and dairy products, and the organisms have been isolated from cattle. Thus, cattle are suspected of being a major reservoir. There is no comprehensive information on the epidemiology of the syndrome, and national surveillance data do not yet exist.

Antibiotic resistance of enterohemorrhagic E. coli has not been an issue. Indeed, the role of antibiotics is paradoxical; their use appears to be a risk factor for development of hemorrhagic colitis if they are given during exposure; but they appear to have little therapeutic value in the disease, in that the pathogenesis is toxin-mediated, rather than enteroinvasive infection.[15]

Infection caused by Yersinia enterocolitica, although moderately common in some European countries, is rarely recognized in the United States. There might be serious underdiagnosis of infection caused by this species, but far more extensive data would be needed to establish yersiniosis as an important clinical problem in this country. Most isolates are susceptible to tetracycline, although ampicillin resistance is common. The committee knows of no nationwide database that permits estimation of the incidence of infection with Y. enterocolitica.

Results of recent surveys in several areas of the United States suggest that Campylobacter spp. might cause at least as much illness and death as salmonellae. However, nationwide data on infections caused by campylobacters are not available yet, and antimicrobial resistance is not a recognized issue in the treatment of infections with them.[2,6,20,21]

In summary, studies have indicated spread into farm workers and their families of E. coli originating in farm animals and poultry. If a drug-resistant enteric flora is selected in farm animals or poultry by the use of antibiotic supplemented feeds, the drug-resistant enteric flora might spread into the farm workers, their families, and ultimately, to some extent into the community at large. There is no evidence, however, to suggest that drug-resistant E. coli of farm origin are more infective or more virulent than drug-susceptible E. coli of non-farm origin. Farm workers and their families have not been found in limited studies to have an increase in serious infections with diarrheal diseases, as compared to the population at large.

There is evidence, derived from the study of food-borne outbreaks of salmonellosis, that the causative salmonellae

were of farm origin, and entered the human food chain. In a number of outbreaks of multiple drug-resistant salmonellosis, an animal or poultry source was implicated, and the multiple drug-resistance was believed to be due to the use of antibiotics in animal feeds. In only one such outbreak was the evidence compelling, with full documentation of the entire chain of transmission from infected cattle to infected humans.

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VII

THE RISK MODEL: OVERVIEW OF THE PROBLEM AND NEED FOR A MODEL

The committee took as its principal charge the quantitative assessment of hazards to human health from the subtherapeutic administration of penicillin/ampicillin and the tetracyclines to farm animals. The committee deliberately chose to consider the tetracyclines and penicillin G together, rather than separately, for several reasons:

- Antimicrobial resistance in salmonellae and E. coli to each of these drugs is predominantly plasmid-mediated.

- Simultaneous resistance to both ampicillin and the tetracyclines is commonly found in the same individual animal isolates of salmonellae. Of 717 isolates of S. typhimuriun (see Table V-2), 52% had tetracyclines resistance, and 37% had both tetracycline and ampicillin resistance.

- Exposure to either penicillin G or a tetracycline of E. coli or salmonella strains bearing R plasmids that encode both tetracycline and ampicillin resistance markers selects for such R-plasmid-containing strains in a mixed population. Thus, exposure to either of the two antibiotics would enrich the population of microorganisms resistant to the other, as well as resistant to it itself.

- The tetracyclines far exceed penicillin G in use in livestock and poultry feeds. For example, in 1985, tetracycline accounted for 49% of annual sales of antimicrobials for animal feeds, and penicillin accounted for only 5% (Table IV-6). Because penicillin use was only 10% of that of the tetracyclines, it did not seem to the committee that performing a separate risk analysis for penicillin G would provide useful information.

- In performing the risk assessment, the committee could not find evidence sufficient to justify the use of different death rates for strains resistant to ampicillin (penicillin), as opposed to the tetracyclines.

This task requires the study of broad questions regarding the effects of drug resistance on the epidemiology of various pathogens and diseases and the effects of feeding subtherapeutic doses of antimicrobial agents on (a) the

prevalence of carriage of various pathogens by farm animals; (b) the antimicrobial susceptibility patterns of these pathogens; (c) the prevalence of infections caused by these pathogens in humans.

Data do not exist to answer directly the principal question posed to the committee even for a well-recognized pathogen such as Salmonella spp. Indeed, the data are sparse and conflicting even with regard to the subordinate questions cited above. To illustrate some of the problems confronting the committee, Figures VII-1 and VII-1A provide a summary of current information about the impact of drug resistance of salmonellae on the epidemiology of salmonellosis in human or animal populations exposed or not exposed to antimicrobial agents. In constructing Figures VII-1 and VII-1A, it was assumed that there is a gradual shift of strains from drug susceptibility to drug resistance. The committee believes that drug resistance is a manifestation primarily of exposure of bacteria to antimicrobial agents for long periods with ultimate selection of resistant strains; thus, it is anticipated that with increasing time of exposure, the prevalence of resistant strains in the animal and human populations will increase. For certain elements in both Figures VII-1 and VII-1A, no data are available, as indicated in parentheses after the item. The studies cited in Figures VII-1 and VII-1A are limited in applicability by the fact that they were not done as part of a cohesive attempt to address the overall issues posed to this committee, but rather were done to address more limited aspects of the problem.

As illustrated in the first horizontal line of Figure VII-1, the "majority" of the reports show that salmonellae in the fecal flora of farm animals are resistant (i.e., resistant to at least one antimicrobial) and a minority are susceptible (meaning susceptible to ampicillin or the tetracyclines). By contrast, the majority of the reports show that human isolates are still susceptible to commonly tested antimicrobials (Figure VII-1A). The prevalence of resistance appears to be rising both for E. coli and Salmonella spp.

No data prove directly that administering antimicrobial agents in subtherapeutic doses to farm animals increases the prevalence of carriage of susceptible salmonella in farm animals; the argument (a highly unlikely one) would be indirect by analogy with the effect of antimicrobial agents on infections by enterohemorrhagic E. coli (EHEC) in humans.[7] By contrast, the 1980 NRC report[21] cited various studies showing that the feeding of antimicrobial drugs to farm animals enhanced the rate of elimination of susceptible strains of Salmonella spp.; this effect would result in a decrease in the prevalence of these susceptible isolates.

	Susceptible Strains	Resistant Strains
1. Current prevalence	Minority of strains	Majority of strains
2 Effect of subtherapeutic administration on prevalence	+ (no data except by analogy with EHEC** strains in humans, see text)	++ (numerous studies of E. coli in animals [see text] but for Salmonella, and extra polation from "etiologic fraction" concept in humans)
	-- (1980 NAS [21])	- (no data)
		o (Fagerberg [13])

FIGURE VII-1. Potential Effects of Antimicrobial Use on Prevalence of Antimicrobial-Susceptible and -Resistant Salmonella Strains in Farm Animals. Figure prepared by the committee.

+ represents mild increase in prevalence of strains, in degree of virulence, or in the characteristic specified; ++ represents a moderate increase; and +++ represents a major increase.

- represents a mild decrease in prevalence of strains, in degree of virulence or in the character specified; -- represents a moderate decrease; and --- represents a major decrease.

o represents no change.

** EHEC = Enterohemorrhagic E. coli.

	Susceptible Strains	Resistant Strains
1. Current prevalence	Majority of strains	Minority of strains
2. Virulence for humans (ability to colonize and cause disease)	Current level	+ compared to susceptible strains (see Chap III)
	+ (by analogy with EHEC)**	+++ ("etiologic fraction"; see text)
3. Virulence for humans taking antibiotics for other reasons	-- (by analogy with effect on clearance in animals per 1980 NAS report [21]	- (no data)
		--- (great difficulty if "wrong" drug chosen, presumably uncommon)
4. Effiency of treatment of infections	Current effectiveness	

**EHEC = Enterohemorrhagic E. coli.

FIGURE VII-1a. Potential Effects of Antimicrobial Characteristics on Antimicrobial-Susceptible and -Resistant Salmonella Strains in Humans. Figure prepared by the committee.

+ represents mild increase in prevalence of strains, in degree of virulence, or in the characteristic specified; ++ represents a moderate increase; and +++ represents a major increase.

- represents a mild decrease in prevalence of strains, in degree of virulence or in the character specified; -- represents a moderate decrease; and --- represents a major decrease.

o represents no change.

There are few data on animals concerning the effect of feeding subtherapeutic doses of antimicrobial agents on the carriage rate of drug-resistant strains. However, Bohnhoff and colleagues showed many years ago that the feeding of a single oral dose of streptomycin to mice markedly increased their susceptibility to infection by a streptomycin-resistant strain of salmonellae administered orally.[5a] Similar findings were later reported by Meynell,[20a] Bohnhoff and Miller,[5b] Meynell and Sabbaiah,[20b] and Miller and Bohnhoff.[20c] Furthermore, by extrapolation from the "etiologic fraction" in humans (the proportion of infections that would not have occurred but for the resistance of the infecting bacterial strain to the antimicrobial administered), one would expect a marked enhancement of infectivity and hence of prevalence. No data support a diminution in the prevalence of drug-resistant strains as a result of feeding subtherapeutic doses of antimicrobial agents. One study by Fagerberg[13] indicates no difference in the clearance rates of tetracycline-resistant strains of salmonellae between animals given tetracycline and those given another antibacterial drug.

In assessing the second, third, and fourth elements of Figure VII-1A, which deal with the impact of salmonella infections in humans, the committee had the opinion (see below) that the majority of strains of salmonellae that find their way into humans are transmitted from food products which originate on the farm. The second element deals with the effect of antimicrobial resistance on the virulence of salmonellae for humans. Various authors have used the term "virulence" in different ways. Some have restricted the term to the ability to cause disease, particularly toxin-mediated disease, whereas others have incorporated the ability to colonize and to cause disease by any mechanism. The committee decided to use the second definition for this assessment and to use the terms "virulence" and "infectivity" interchangeably. If drug-susceptible strains are considered as a baseline, there is evidence (see Chapter III) that drug resistance may be associated with either a decrease or an increase in virulence. On balance, the committee decided that the data were more compelling for either no change in virulence or an increase in virulence than they were for a decrease in virulence, although the data are weak and rather inconclusive.

In terms of the virulence of Salmonella spp. for humans who are taking antibiotics for other reasons (element 3 in Figure VII-1A), there is strong evidence that drug resistance of the salmonellae facilitates infection. In persons in this category, whose disease is included in the "etiologic fraction," drug-resistant strains are able to colonize the gastrointestinal tract and cause disease even in inocula too small to cause infection in other circumstances, presumably

because the antimicrobial drugs inhibit the normal competing flora (see below). By contrast, the committee is aware of no data indicating that drug resistance diminishes the infectivity of Salmonella spp. for humans. The committee also is unaware of any data on the effect of taking antimicrobial agents on the infectivity of drug-susceptible strains. However, by analogy with data in animals noted under element 2 in Figure VII-1, the administration of antimicrobial agents could enhance the rate of elimination and hence reduce the infectivity of drug-susceptible strains for humans. Nevertheless, using infection by enterohemorrhagic E. coli (EHEC) as an analogy, a study in 1987 by Carter[7] of EHEC infection in a nursing home showed a higher rate of secondary infection among patients who were taking antimicrobial agents to which EHEC was presumably susceptible (isolates of EHEC appear to be almost uniformly susceptible to ampicillin, tetracyclines, chloramphenicol, and trimethoprim-sulfamethoxazole,[22a,23,29a] as opposed to a control group that was not taking such antimicrobial agents. This suggests a facilitating effect of antimicrobial agents even upon infection by susceptible strains.

Finally, the committee considered the effect of drug resistance on the treatability of salmonella infections in humans. In principle, resistance should lead to increased difficulty in treatment. However, considering the epidemiology and the population at greatest risk of death i.e., neonates and the very elderly--it seemed likely to the committee that many patients who die of salmonellosis never receive specific antimicrobial treatment and that failure of treatment because the wrong drug was chosen may be uncommon. However, some patients may be treated inadvertently because their physician does not recognize the infection as salmonellosis. Treatment that prevents bacteremia, a rare event anyway, might lead to an unrecognized benefit. Taking these considerations into account, the committee considered that drug resistance is an uncommon cause of treatment failure.

Overall, the committee concluded that the major consequences of feeding antimicrobial agents to animals or humans are likely to be: 1) a tendency to increase the prevalence of drug-resistant strains; 2) an effect on both the pathogen and the fecal flora that might alter their usual interaction; and, thus, the relative infectivity of the pathogen.

The number of reported cases of salmonellosis in the U.S. has risen progressively over the past three decades, a period during which the practice of subtherapeutic administration of antimicrobial agents to farm animals has been steadily increasing (Figure VII-2). However, this observation does not prove that the increase in salmonellosis is related to antibiotic use because other potentially

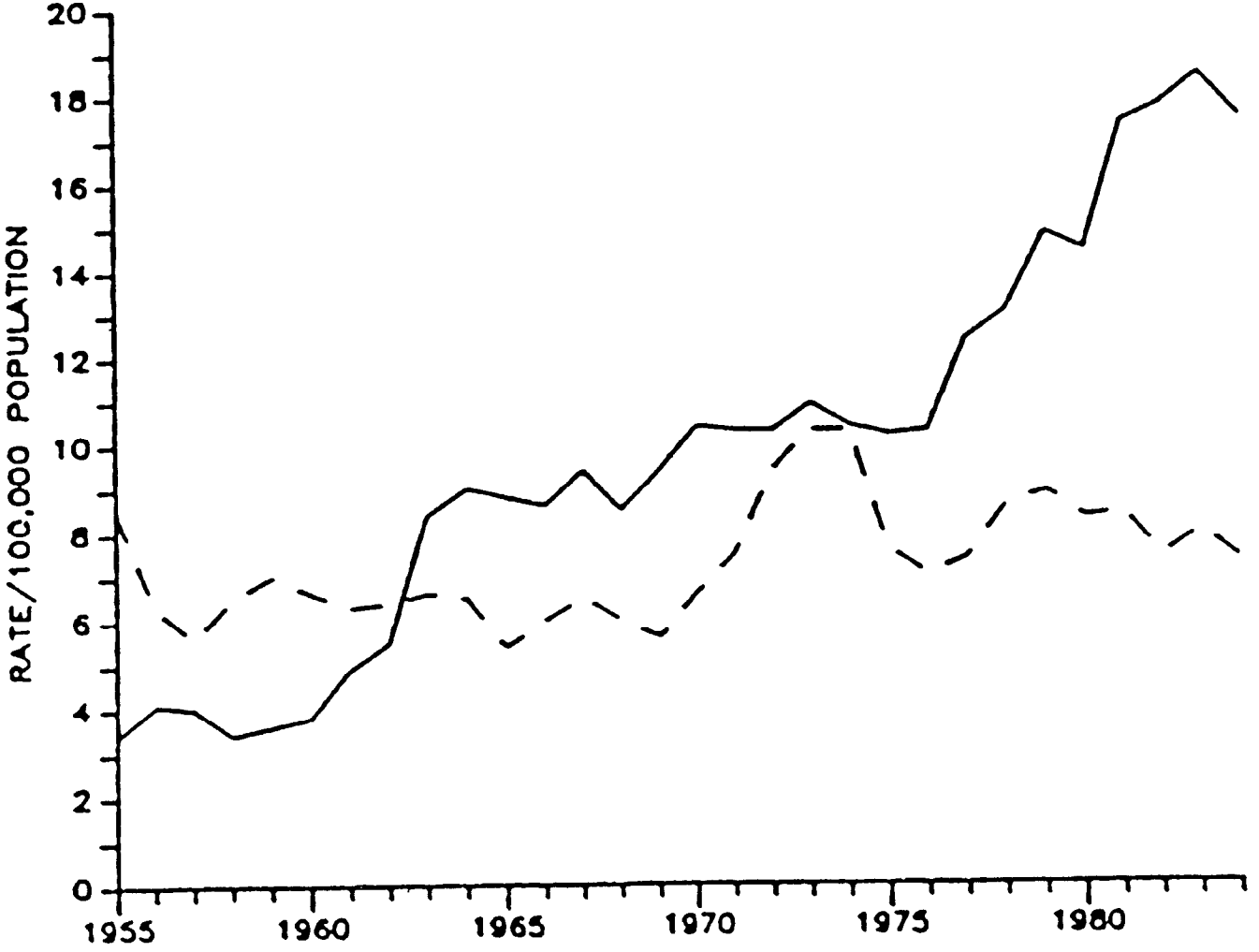

FIGURE VII-2. Salmonella (-) and Shigella (--) infections reported to the Centers for Disease Control, 1955-1984. Rates are per 100,000 population in the United States. Salmonella rate excludes infections due to Salmonella typhi. Reprinted from Chalker and Blaser.[11]

confounding variables have occurred in the same interval, including the increasing use of convenience foods and prepared "fast foods." That the increase in reported cases of salmonellosis in humans over the past 30 years is not an artifact of reporting is suggested by the fact that the number of infections caused by Shigella spp. has remained fairly constant during this period (Figure VII-2). Thus, there is direct evidence of an increase in salmonellosis in humans. Some of this increase might be attributed to some of the elements shown in Figure VII-1A. Furthermore, it is difficult to determine directly the contribution of drug resistance to fatality from salmonella infection because neither the published CDC data for reported cases nor the NCHS figures for deaths from salmonellosis make note of the drug susceptibility or resistance of the pathogen.

After consideration of the concepts illustrated in Figure VII-1 and the limited data available pertaining to the issues, the committee concluded that it was impossible to arrive at a firm answer to the important question of whether or not the administration of antimicrobial agents in subtherapeutic doses to farm animals has led to an overall change in the total number of cases of salmonellosis in humans. Accordingly, the committee decided to approach the problem indirectly by devising a risk model that focuses upon one aspect of the problem, namely, the number of deaths which can be attributed to the subtherapeutic administration of antibiotics to farm animals.

STRUCTURE AND LIMITATIONS OF THE MODEL

As summarized above, questions about the size of the human risks from low-level farm uses of antibiotics cannot be answered by the direct interpretation of data on this matter. Therefore, the committee developed and adopted a conceptual approach, or model, in which some information is available at each step. In devising the model, the committee chose to deal only with salmonellosis because this was the only pathogen for which there were data available in quantity and quality that the committee could use in quantifying the risk. Nevertheless, the committee recognized that there are other infectious organisms that may account for at least as large a part of the overall problem of human illness attributable to the subtherapeutic use of antibiotics on the farm.

The model includes a sequence of five quantitative estimates, each dependent on the prior estimates. In steps 2, 3, and 5 (see below), the estimates were calculated separately for resistance to any antibiotic and for resistance to at least penicillin/ampicillin or the tetracyclines. These estimates are illustrated in parentheses by the committee's mid-range estimate for each

step with respect to resistance to penicillin/ampicillin or the tetracyclines, as in the following:

1. Annual number of cases of salmonellosis reported in the U.S. (50,000).

2. Fraction of human cases due to bacterial strains showing resistance to penicillin/ampicillin or the tetracyclines (15%).

3. Death rate (1.0%) among cases with drug-resistant salmonellosis.

4. Fraction of these deaths associated with infection by bacterial strains of farm origin (70%).

5. Proportion of this fraction resulting from subtherapeutic use of penicillin/ampicillin or the tetracyclines in animal feed (90%).

Since these estimates are linked in stepwise fashion, and each is developed to be statistically conditional on all that precede it, they can be multiplied to estimate the number of deaths. This chapter develops the risk model and explains the committee's choices of the quantitative inputs the model requires, and Chapter VIII uses the model to develop estimates of excess deaths while reflecting the uncertainty of those estimates.

FOCUS ON SALMONELLAE

The model has several limitations. One limitation is that it assesses only the hazards of infection with Salmonella spp. The major foodborne pathogens known or suspected to be transmissible to humans from farm animals or their products are Salmonella spp.; Campylobacter jejuni enterohemorrhagic strains of Escherichia coli (EHEC), especially serotype 0157:H7; and Yersinia enterocolitica. The Centers for Disease Control reported on 151 outbreaks of foodborne bacterial disease in 1982;[8,9] of this number, salmonellae caused 55 outbreaks with 2,056 cases and 8 deaths. By contrast, each of the other three species caused only two outbreaks, with between 31 and 188 cases for each species, and no deaths. However, these data are highly selected and are incomplete.

Although recent surveys[3,4,5,14,15,19,26,27,28] suggest that Campylobacter spp. may cause at least as much illness as does salmonellosis, data on infections caused by nine named or proposed named campylobacter species are not available. Laboratory-based national surveillance of campylobacter

infections in the U.S. began in 1982 with a panel of 11 states with additions in 1983 bringing the total to 31 states.[10] The committee has not critically looked at these data. Also, antimicrobial resistance is not recognized as a major issue in treatment, and no data are available to determine whether the administration of antibiotics to farm animals has contributed to antimicrobial resistance in these species.

Similarly, there are no nationwide data available to estimate the impact of infection caused by Y. enterocolitica or EHEC. However, infection caused by Y. enterocolitica is rarely recognized in the United States. Although infections may be grossly underdiagnosed, more data would be needed to establish yersiniosis as an important clinical problem in this country. Most isolates of Y. enterocolitica are susceptible to tetracycline, but ampicillin resistance is common.[6] Strains of EHEC are almost always susceptible to commonly used antimicrobial agents.[22a,23,29a]

There are over 1500 serotypes of salmonellae.[12] However, more than 70% of the infections are caused by 10 serotypes and four are dominant: S. typhimurium causes about one-third of reported infections, S. enteritidis about 10%, S. heidelberg about 10% and S. newport about 5%. The frequency of isolation of S. typhi, which causes typhoid fever, has diminished sharply since the beginning of this century; currently, there are about 500 isolations of S. typhi per year, as opposed to more than 40,000 isolations of other species of salmonellae. S. typhi is not known to infect animals or to have an animal reservoir and is thought to be spread from person to person, so it is not considered in this analysis. The committee concluded that Salmonella is the only genus for which sufficient data are available to estimate the national impact on mortality from infections caused by drug-resistant organisms transmitted from farm animals or their products to humans. The remainder of the quantitative analysis in this report pertains to infection caused by nontyphoidal Salmonella spp. Morbidity was not included in the risk calculations.

MODEL UNCERTAINTY

A second limitation is that the model itself may be incorrect. While the steps outlined above are logically appealing, other chains of critical events could be developed, such as steps in the chain of transmission or pathogenesis, and these might produce materially different estimates.

Numerous difficulties, conceptual and practical, impede the estimation of the mortality rates attributable to salmonella infections, and the committee recommends a

substantial increase in the investigation and development of conceptual models of this matter that include morbidity.

INDEPENDENCE OF ESTIMATES

Third, our use of the model requires that the parameter estimates be conditionally independent; that is, the statistical distribution of one estimate, given others that precede it in the model, depends on those values in a way that is fully specified by the model. For example, the death rate among cases with drug-resistant salmonellosis (see step 3 in Table VIII-1) refers to reported cases in the U.S.; it is presumed that there is a much larger number of unreported cases (see below). The death rate is likely to be lower for the unreported cases than for reported cases. However, it is not generally possible to validate this assumption of conditioned independence from available data for salmonella risks. Further, limitations in the data have required the use of some estimates that are not conditional, or that are less completely conditional, than the model theoretically requires. Both the numerators and the denominators needed to calculate the rates of illness and death are subject to considerable uncertainty. Further, these uncertainties are closely linked, so that the numerator and denominator must be developed together and the resulting (death) rate should be applied to other settings only insofar as the major uncertainties are appropriately correlated.

For example, the death rate of salmonella infections depends very much on whether it is for cases such as are reported to the Centers for Disease Control or for the whole of symptomatic infections in humans. Thus, the seven deaths reported among 503 patients in a 1979-1980 CDC survey yield a death rate of 1.4%.[12] Since the denominator was 503 cases reported in approximately routine fashion to the CDC, one might, with caution, estimate that about 1.4% of all such reported cases of salmonellosis might have been fatal. Because about 50,000 cases of salmonellosis are reported to the CDC annually, one could make an estimate that there are about 700 deaths in 50,000 reported cases per year. In fact, it is reasonable to assume there are many more unreported cases; however, the death rate estimate of 1.4% does not necessarily need to be changed, because, unreported cases might be less likely to be severe or fatal and to go unreported for that reason.

Similar considerations apply to other aspects of salmonella infection, such as the rate of hospitalization, the proportion of patients with "serious" rather than mild disease, and a medical decision to culture stools or other materials.

LIMITATION TO CDC-REPORTED CASES OF SALMONELLOSIS

The committee recognizes that the numbers of cases of salmonellosis reported to CDC per year, ranging from about 40,000 to 65,000, surely is an underestimate of the number of cases in the U.S.; nevertheless, the committee decided to use this range of numbers for the first step in the risk assessment. This decision was made because: 1) several other critical estimates in the risk model apply to this same population of CDC-reported case, and 2) it may be that unreported cases are milder and of lesser consequence, although this has not been shown to be the case. However, investigation of epidemics by CDC of reported cases necessarily underestimates the scope of the problem.

LIMITATION TO ESTIMATES OF MORTALITY

A fifth limitation is that the model deals only with lethal infections. Salmonellae also cause considerable, albeit temporary, personal distress (morbidity), as well as a large economic burden. The clinical manifestations range from asymptomatic colonization through mild or sometimes severe diarrhea, to disseminated and sometimes lethal illnesses, such as meningitis or osteomyelitis. However, statistical data regarding the incidence of various symptoms are minimal or lacking even for severe cases, and there is difficulty in applying such data to the U.S. as a whole.

Fatalities due to salmonella infections are clustered in the very young and in the elderly; Table VII-1 shows the age distribution of salmonella deaths reported to the National Center for Health Statistics (NCHS) for the years 1968-1985. For each end point, the group at risk must be unambiguously defined, essentially all instances in the group must be identified, and the calculated rate must be applied to other groups only when they are likely to have about the same distribution of severity of illness and only when adequate margins of error are attached to the calculated rate. These margins of error will ordinarily be substantially wider than statistical confidence limits.

The hospitalization rate for salmonellosis in the 1979-1980 survey of selected communities was 45% and the rate in the 1984-1985 survey was reportedly similar.[12] However, in a review of recent outbreaks of S. enteritidis infections occurring in the northeastern U.S., the hospitalization rate was estimated to be only 12%.[30] Thus, a range of 12% to 45% may be entertained as the estimate for the rate of hospitalizations of patients with salmonellosis. When these rates are applied to the 50,000 reported cases of salmonellosis per year, the number of patients hospitalized for this infection ranges from 6,000 to 22,500 per year.

TABLE VII-1

FREQUENCY AND PERCENTAGE OF DEATHS DUE TO SALMONELLOSIS
(By Age, for 1968-1985)

Age	Deaths	
	Number	Percent Per Year of Age
Under 1 day	1	0.1
1 - 6 days	8	0.6
7 - 27 days	30	2.1
28 - 364 days	165	11.6
1 - 4 years	42	3.0
5 - 9 years	12	0.8
10 - 14 years	11	0.8
15 - 24 years	14	1.0
25 - 34 years	30	2.1
35 - 44 years	42	3.0
45 - 54 years	104	7.3
55 - 64 years	176	12.4
65 - 74 years	314	22.1
75 - 84 years	296	20.8
85 + years	174	12.2
Unknown	2	0.1
All Ages	1,421	100.0

Source: National Center for Health Statistics.[22]

LIMITATIONS IN THE DATA

Sixth, the model is limited by the range and quality of data available for the estimates required, as summarized above. The committee has developed three estimates for each quantitative parameter: a mid-range estimate, a high estimate and a low estimate. The mid-range estimate expresses the committee's best judgment about the value that is equally likely to be too large or too small--a median, of sorts, of the committee's collective objective and subjective judgment. The high and low estimates express the committee's best judgments about the range of figures that most other experts would find plausible. These limits are not presented as statistical confidence limits (even subjectively), nor as outside bounds on possibility. For example, if three figures for some parameter were 1 and 10, an estimate outside the range 1, 3, and 10 would not be credible, the committee believes, to most other experts. We have not attempted to attach probability values to these low and high estimates, because we have no direct evidence about what limits other experts would be willing to accept as plausible.

The committee discussed at some length how these three estimates should be derived. The basic point of discussion was the extent to which our collective subjective judgment should be used to modify specific values obtained from the literature. As an example, the fraction of strains resistant to two or more antibiotics has been rising, in contrast to published results that necessarily refer to infections detected in the past. Thus, the published range of rates of resistance to multiple antibiotics will tend to be too low, but by an unknown amount. How much should the committee's judgment about this trend over time be integrated into rates obtained from the literature?

Other problems arise because of the need to use estimates that are statistically conditioned on preceding estimates, though appropriate data may not exist. For example, the fraction of infections due to multiresistant strains should refer specifically to the kinds of cases, with specific details, reported to CDC; however, not all sources meet this requirement. In the end, the committee tended to give its subjective judgment considerable weight. We have not attempted to attach probability values to these low and high estimates, because we have no direct evidence about what limits other experts would be willing to accept as plausible.

The remainder of this chapter outlines the basis for the estimates that the committee used in its risk model to assess the contribution of subtherapeutic use of antibiotics in animal feed to the presence of drug-resistant salmonellae in humans in the United States.

NUMBER OF CASES PER YEAR

The number of cases of salmonella infections per year is large, but not precisely determined. Many observers believe that it is probably 10-100 times larger than the number of confirmed cases reported to the CDC. This is because many patients with salmonellosis do not seek medical attention; when they do, stools or other specimens may not be cultured; when cultures are attempted, they may be unsuccessful in isolating the infecting bacteria, or positive results may not be reported to CDC. Still, most of the data relating to morbidity and mortality from salmonellosis in the U.S. are derived from the CDC. The CDC in turn relies on several sources for its information, including the following:

o A Salmonella Surveillance System, maintained by CDC since 1963, when several large outbreaks of salmonellosis were traced to commercial egg products. The purpose of the surveillance system is to accumulate epidemiologic data such as the age, sex, and county of residence of patients from whom Salmonella isolates are submitted to state health departments for serotyping. Data are also kept on isolates from food and animals.

o Investigations of outbreaks by state, local, and federal agencies.

o Special epidemiologic and laboratory surveillance in selected counties.

In 1979-1980 and 1984-1985, the health authorities in a stratified sample of urban and rural counties were asked to submit all salmonella isolates, together with detailed epidemiologic information, for all patients from whom isolates were obtained.[12] The communities were chosen to provide about 5% of the expected number of reported isolates.[24] Strains from known outbreaks were excluded. The isolates collected in this way were obtained through the usual CDC reporting channels with no specific efforts at case-finding (R. Tauxe, CDC, 1988, personal communication). The isolates were tested for susceptibility to antimicrobial agents and sometimes for their plasmid DNA content. These findings have been used to provide information on the rates of antibiotic resistance, hospitalization for illness, and mortality from salmonellosis.

The salmonella surveillance system conducted by CDC has shown a fairly constant rise in the annual number and rate of reported cases of nontyphoidal salmonellosis at least since 1955 (Figure VII-2). The reasons for this rise are not clear. However, the belief that the rise was not simply the result of better case finding is supported by the observation

that there was no appreciable change in the reported rate of shigellosis over the same period. In the years from 1982 through 1986, the number of cases of salmonellosis reported per year ranged from 40,861 to 65,347.[8] Over 90% of the reported isolates were from symptomatic individuals (P. Blake and R. Tauxe, CDC, 1988, personal communication).

There is substantial underreporting of salmonellosis.[11] Indeed, it has been estimated that in several outbreaks as few as 1% of cases were reported.[2] In a telephone survey conducted recently during a massive epidemic of salmonellosis, it was found that only about 10% of symptomatic infections were reported.[25] Attempts to determine the number of cases more precisely run into the problem of defining exactly what is a case. Should one include as cases only symptomatic patients with infections? Does passage of one or two loose stools qualify? Must symptoms be severe enough to interrupt normal activities for at least 24 hours? Must symptoms be severe enough to require medical attention? The numbers might vary by one or two orders of magnitude, and no answer is inherently correct. Each investigator in the field must develop a conceptual definition that is meaningful and useful for a specific study. This must then be translated into operational terms: How can one collect and interpret data so as to estimate both the number of cases by this definition and the degree of error likely to attend the estimate?

An extensive analysis using three independent methods to derive these estimates for the annual incidence of salmonellosis produced estimates ranging from 800,000 to 3,700,000 infections.[11] A mean estimate of 1.9 million infections in 1984 would imply that about 2.5% of infections (about 50,000 cases) had been reported to the CDC.[11] Therefore, an annual incidence of 50,000 reported cases of nontyphoidal salmonellosis in the United States is a highly conservative estimate. A more probable figure is on the order of 800,000 cases per year, and the upper limit could be as high as 3,700,000 cases per year.[11]

Because many of the estimates considered critical for use in the model were based on data derived by the CDC from cases reported to that agency, the committee used the number of isolates reported as the starting point for the model (see "Limitations in the Data," above). In the estimation of risk, the committee used the following figures for low, mid-range, and high-estimates for the number of reported cases of salmonellosis per year (U.S. only): 40,000, 50,000 and 65,000, respectively.

ANTIBIOTIC RESISTANCE OF SALMONELLA

The proportion of salmonella isolates from humans with

resistance to at least one antimicrobial agent was 16% in the 1979-1980 CDC survey and 24% in the 1984-1985 survey.[20] The proportion with resistance to two or more drugs, i.e., multiresistant strains, increased from 12% to 15% during these same years.[20] These surveys avoided the counting of multiple isolates from the same outbreak or the same patient.

Changes in drug-resistance rates vary among the different salmonella serotypes. In the 1979-1980 study, the rate of resistance was high for S. heidelberg, but low for S. typhimurium; in the 1984-1985 study, the rate of resistance of S. heidelberg decreased, and that of S. typhimurium increased.[20]

There were also changes in resistance to different antibiotics. The rate of resistance to ampicillin rose from 8% to 9% between the two study periods, and the rate of resistance to tetracycline rose from 8.6% to 13%; by contrast, the rates of resistance to chloramphenicol and trimethoprim-sulfamethoxazole were each 2% or less for both study periods.[20] At the request of this committee, the CDC provided additional information which allowed the committee to calculate that 19 of 485 strains (3.9%) were resistant to both ampicillin and tetracycline, whether or not they were also resistant to other agents. In a collection of 2,826 strains isolated from humans from Massachusetts during 1979-1980 the prevalence of resistances was as follows: ampicillin, 5.1%; tetracycline, 8.7%; tetracycline and ampicillin, 3.3%; tetracycline or ampicillin, 10.3%. The overall prevalence of resistant strains in the Massachusetts collection (see Table V-1) was low, compared to other sources, perhaps because of the high proportion of generally susceptible S. enteritidis (31%).

For the estimation of risk, the committee chose the rates for occurrence of antibiotic resistance shown in Table VII-2. The high estimates for resistance were chosen to account for the apparent increase in resistance rates over time, because the reported rates may underestimate the current prevalence of resistance.

MORTALITY RATE FOR INFECTION BY RESISTANT STRAINS OF SALMONELLA

In concept, the number of deaths from salmonella infections should be only the number of infected persons who died, and who would not have died in the absence of these infections.

This concept encounters serious problems in application, because the causes of some deaths are difficult to determine in ordinary conditions of medical practice, and the asserted cause of death on a death certificate often is unproved. Some diseases or conditions act jointly to cause death,

TABLE VII-2

RESISTANCE OF SALMONELLAE TO ANTIMICROBIALS

Rate of resistance of salmonellae to:	Low Estimate	Mid-Range Estimate	High Estimate
At least one antimicrobial	16%	24%	31%
At least penicillin/ampicillin or tetracycline	10%	15%	20%

Source: Adapted by the committee, from data in Table V-3.

although neither alone has resulted in death; in such a case, what is the underlying, "cause of death"? In some instances, a severe infectious disease is almost incidental to a severe underlying terminal condition; shall we count such a death if the salmonella infection only advances the time of death by a month, a day, or an hour? A decision is needed about whether to count as a salmonella death the death of a person who was severely debilitated from other causes but whose uncontrolled salmonella infection contributed to the death.

The committee recognizes that the U.S. National Center for Health Statistics, like other offices of vital statistics, has consistent and well-developed rules for deciding how to report these types of deaths. The committee understands the need for well-defined and consistent statistical data, especially for identifying differences among populations and changes over time. However, the committee emphasizes that these rules deal with the underlying conceptual problems in consistent reporting without solving them in a way that is useful here.

Table VII-3 summarizes recent data on reported death rates of patients with salmonellosis. The CDC study of 1979-1980 identified seven deaths among 503 patients with nontyphoidal salmonellosis, for a death rate of 1.4%; there was a similar rate in the 1984-1985 survey.[12] However, these were all deaths among patients reported to have salmonellosis, regardless of specific causes of death. The committee asked for additional information regarding the role of salmonellosis in causing death. CDC was not able to provide such data for patients who died in the 1979-1980 study, but did provide additional information on the 8 deaths among about 600 patients included in the 1984-1985 survey. According to the committee's interpretation of those data, salmonellosis played an unknown role in three of the deaths and no role in four deaths, and it clearly contributed to the

TABLE VII-3

RECENT MORTALITY RATES FROM NON-TYPHOIDAL SALMONELLOSIS

Prospective CDC Surveillance Data[12]	Community-Acquired Infection			Nosocomial Infection			Specified/or Combined Community Nosocimial Source of Infection			Comments
	Suscep	Multi-Res	Not Spec	Suscep	Multi-Res	Not Spec	Suscep	Multi-Res	Not Spec	
1979-1980									1.4% (7/503)	Role of Salmonellosis in causing deaths not specified.
1984-1985									1.4% (8/600)	Only one death clearly attributable to Salmonellosis (see text).
1984-1985 (recalculated)									0.2% (1/600)	
Outbreaks										
US outbreaks 1971-1983[18]							0.2% (4/1912)	4.2% (13/312)		
US Outbreaks 1971-1980[17]	0.2% (3/1321)	3.4% (7/205)		1.0% (2/202)	11.7% (30/256)					Most of the data base was the same as for 1971-1983.[18]
N.E. USA[30]	0.5% (11/2119)									S. enteridis, presumable drug-susceptible (not stated) via grade A eggs in Northeastern USA.
United Kingdom									0.3% (40/12,000)	See Chapter 6 for discussion of cases.
Midwest USA[25]		0.1% (14/12,624)								14 deaths probably or possibly related to Salmonellosis; S. typhimurium in pasteurized milk resistant to ampicillin, the tetracyclines, carbenicillin, and sulfisoxazale.

Source: Adapted by the committee, from data by Cohen and Tauxe,[12] Holmberg,[17,18] Ryan,[25] and St. Louis.[30] U.S. Outbreaks 1971-1980[17] includes Puerto Rico.

death of only one patient, in whom the organism was isolated from blood (P. Blake and R. Tauxe, CDC, 1988, personal communication). Accordingly, in only 1 patient of about 600 (0.2%) did salmonellosis clearly contribute to the death of the patient, although the rate may have been as high as 4 in 600 (0.7%).

In a CDC review of outbreaks of salmonellosis by Holmberg et al.,[17] encompassing the period 1971-1983, the overall death rate from drug-susceptible strains was 0.2% and from drug-resistant strains was 4.2%. Of the 13 deaths caused by multiresistant strains, 8 were in elderly persons in the community, and 5 occurred among 18 infants in a single hospital nursery.[18] The basis on which it was determined that salmonella infection caused or contributed to death was not stated.

More recently, Holmberg et al.[17] reviewed both nosocomial and community-based outbreaks that were investigated by CDC and that occurred in the United States between 1971 and 1980. These outbreaks were caused by various species of bacteria. All but one of the outbreaks of salmonellosis reported in this review had already been reported in the earlier paper by this group.[18] In 10 of the community-based outbreaks of salmonellosis that were identified as being caused by drug-susceptible strains, salmonellae caused the death of three persons among 1,321 persons infected, or 0.2%. By contrast, the death rate in four community-based outbreaks caused by multiresistant strains was 7 of 205, or 3.4%.[8] The death rate in seven of the nosocomial outbreaks that were caused by drug-susceptible salmonellae was 1.0% (2 of 202 patients) but in nine of the nosocomial outbreaks caused by multiple-drug-resistant salmonellae, the death rate was 11.7% (30 of 256 patients).

In both the community acquired nosocomial outbreaks just cited[17,18] the data do not allow comparison of the ages of the individuals with salmonellosis. This comparison might be important in view of the greater number of deaths due to salmonella infections reported at the two extremes of age (see Table VII-1). Among the nosocomial outbreaks reported[17], 11 of the 30 deaths due to multiresistant salmonellae occurred in patients in neonatal intensive care units, whereas the 2 deaths due to antimicrobial-susceptible strains occurred in general hospital wards in patients whose ages were not specified. The committee consulted the CDC for additional details on this issue but no further information was available (S. D. Holmberg, 1988, personal communication). Thus, if more outbreaks due to antimicrobial-resistant strains had involved infants and the elderly than outbreaks due to susceptible strains, the higher mortality associated with resistant strains might reflect such a difference in the population at risk.

A review of 65 outbreaks of S. enteritidis infection that occurred between January 1985 and May 1987 in the northeastern United States showed a death rate of 0.5% (11 deaths among at least 2,119 cases).[30] Ten of these deaths occurred among 130 residents in nursing homes. Although antimicrobial susceptibility data were not given, strains of S. enteritidis nearly always have been found to be susceptible to commonly used antibiotics. Grade A eggs or foods containing eggs were implicated in 77% of the outbreaks in which a source of the infecting bacteria could be found.[30]

The two papers on CDC's surveys of outbreaks are summarized by Holmberg et al.[17,18] (Table VII-3) and show a higher death rate from infection due to multiple-drug-resistant strains than from infection due to susceptible strains of salmonellae. The more recent paper summarized data from outbreaks of infections that included bacteria other than salmonellae and reported nosocomial and community-acquired infections separately and more clearly than the earlier summary. The authors concluded that for both community-acquired and nosocomial infections, the mortality rate, the likelihood of hospitalization, and the length of a hospital stay were usually at least twice as great for patients infected with drug-resistant strains as for those infected with susceptible strains of the same species.[17] A higher mortality rate due to infection with drug-resistant strains of salmonellae could result from: (a) a greater virulence of the resistant strains, (b) a propensity for the drug-resistant strains to infect patients with diminished host defenses (see "etiologic fraction" below), or (c) the inefficacy of treatment with drugs to which the bacteria are resistant. Salmonella deaths are also reported by the U.S. National Center for Health Statistics (NCHS) in the National Death Index (NDI), which collects data from the total U.S. and tabulates causes of death using the International Classification of Disease (ICD) categories on the death certificates. The data summarized in the NDI do not provide any information on the individual cases, their location, the infecting bacterial serotypes (except that nontyphoidal salmonella infections are identified as such), serotypes or their drug susceptibilities. Therefore, these data do not have the same application for the analysis of risk as the CDC data. It is useful to compare the salmonella deaths reported by both sources as a check on the accuracy of salmonella deaths reported by each.

The NDI data are based on information given on the death certificates completed by physicians, coroners, or medical examiners. Summaries of deaths from salmonellosis are given in Table VII-4 for the years 1980 to 1985. During this period, "other (nontyphoidal) salmonellosis" (ICD-9, code 003) was reported as the underlying cause of death in 82-117 deaths per year. In addition, during this same period,

TABLE VII-4

NUMBER OF DEATHS DUE TO SALMONELLOSIS (ICD-9, NO. 003) TABULATED IN THE NATIONAL DEATH INDEX

Year	UC*	EA**
1985	117	218
1984	89	164
1983	82	154
1982	87	176
1981	104	-
1980	88	175

Source: National Center for Health Statistics.[22]

* UC = Underlying cause of death (i.e.) disease was listed as initiating cause of death on death certificate).

** EA = Entity axis (i.e., disease was listed as contributing cause of death).

salmonellosis, whether it was only contributory or was thedirect cause of death, was mentioned on the death certificate in 154-218 deaths per year.[22] These numbers of deaths, derived from sources and methods that are different from those used by CDC, are within the range of death rates cited by the CDC. For example, if salmonellosis was the underlying cause of death in 100 deaths per year (intermediate between 82 and 117 deaths per year) and these deaths are considered to occur among the 50,000 reported cases of salmonellosis per year, the death rate would be 0.2%. For the approximately 200 cases per year in which salmonellosis was at least a contributing factor, the death rate would be 0.4%.

The NDI data may underestimate the number of deaths from salmonellosis for several reasons: (1) a death certificate may be filled out by a physician or other authorized person who is not fully familiar with the illness of the deceased and may not recognize the contribution of the infection to "cardiac arrest" or some similar non-infectious process; (2) a salmonella infection may be recognized and reported as septicemia, meningitis, or other infection without identification of the causative organism; or (3) failure to take a bacterial culture from a patient with salmonellosis as a complication of a terminal illness might lead to failure to identify the organism.

The data on case studies in Table VII-3 suggest that the death rate may range from 0.2% to 0.5% in community-based salmonella infections caused by drug-susceptible strains, but could be as high as 1.4% if the CDC surveillance data were for susceptible strains, a fact which cannot be determined from the reports. The death rate may range from 0.1% to 3.4% or 4.2% in community-based outbreaks caused by multiple-drug-resistant strains; however, the two higher values for death rates from resistant strains are derived from on overlapping body of data and are not independent. The death rate was 1% in nonsociomal outbreaks caused by susceptible strains and was as high as 11.7% in nosocomial outbreaks caused by multiple-drug-resistant strains.

The death rates used in the risk assessment model are summarized in Table VII-5. The committee considered whether the low estimate for strains resistant to at least one drug should be set at 0.1% in accord with the report by Ryan et al.[25] on a very large outbreak of milk-borne salmonellosis. The committee concluded that the reported death rate in that outbreak was unusually low, perhaps because extensive publicity led to substantially above-average reporting of marginal cases (the denominator of the reported death rate). Each outbreak is thus considered a sample of one and not weighted according to the number of persons affected. No death rates were available from the literature for strains resistant specifically to at least penicillin/ampicillin or the tetracyclines, although resistant strains isolated in epidemics were frequently resistant to at least one of these drugs. Thus, the committee elected to use the same death rate of 0.2% for strains resistant to penicillin/ampicillin or the tetracyclines as to any other drug (see Table VII-5).

For the mid-range and high estimates of the death rate from strains with no resistance, the committee elected to use estimates that spanned the range of values shown in Table VII-5 for strains of this kind, whether the infections were community-acquired or nosocomial.

For strains with drug resistance, the committee believed that the plausible mid-range or high death rates were appreciably higher than those for strains with no resistance, for three reasons: (a) some patients, although probably few, would receive the "wrong" drug intentionally or inadvertently; (b) drug-resistant strains would tend to cause some infections in the "etiologic fraction" category (discussed below) in patients who might be debilitated and more susceptible to the consequences of infection; (c) the data of Holmberg et al. summarized in Table VII-3 indicate a higher death rate from resistant than from susceptible strains. As discussed earlier, there are theoretical reasons why drug-resistant strains might be more virulent than drug-susceptible strains, including the possibility that the resistance plasmids have acquired virulence genes (or that

TABLE VII-5

RANGE OF DEATH RATES (AS A PERCENTAGE) FOR SALMONELLOSIS FROM SUSCEPTIBLE AND DRUG-RESISTANT STRAINS

	Estimates		
	Low	Mid-Range	High
Susceptible	0.2	0.5	1.0
Resistant to at least one drug	0.2	1.0	4.0
Resistant to at least penicillin/ampicillin or tetracycline	0.2	1.0	4.0

Source: Prepared by the committee (see Table VIII-1).

virulence plasmids have acquired resistance genes). It might be argued that the death rate for strains resistant to atleast penicillin/ampicillin or the tetracyclines should be higher than for strains resistant to any drug, because the former would be more likely than the latter to lead to problems with the "wrong" choice of drug, or with the "etiologic fraction." However, lacking any specific data on this point, the committee chose to use the same mid-range and high estimates for these two kinds of strains. In any event, it seems unlikely that the results from the risk model will be much influenced by the use of similar values for these two kinds of strains. The reported death rates for infection by resistant strains in Table VII-3 are for multiresistant strains, whereas the designation in Table VII-5 is for strains resistant to at least one agent; the committee considers this distinction to be of minor consequence for the present analysis.

FRACTION ASSOCIATED WITH FARM ORIGIN

A critical step in the risk estimate is the determination of the source of resistant strains of salmonellae that cause infection in humans. In fact, the true "origin" of a strain of salmonellae that causes infection in humans--i.e., whether it arose from a food product, contact with another person or a pet, or some other source--is almost never known, except in outbreaks that are investigated. However, there is a common belief that for most strains of nontyphoidal salmonellae, the proximate

source is usually food animals or food products derived from animals. This belief is supported by the findings that carriage of nontyphoidal salmonellae in humans is generally brief and that a wide variety of commonly consumed foods are often contaminated by strains of salmonellae and by the evidence (summarized below) that, in most outbreaks in which the source could be traced, the source has been food products originating on the farm.

Reliance on the results derived from the analysis of outbreaks is problematical, because salmonella infections in humans are usually sporadic. Of course, if adequately detailed investigation could be done, it is likely that many "sporadic" cases would turn out to be small epidemics. There is no evidence that salmonella strains isolated from outbreaks are distinct from strains isolated from sporadic cases; the epidemiologic effects of a given strain are presumably related to the number of organisms in the infecting inoculum and the number of people exposed to this inoculum. Because of these considerations, the committee concluded that the data from outbreaks are usable in the present context. In CDC's review of 52 outbreaks of salmonellosis, food animals or their food products were implicated in 11 of 16 outbreaks (69%) caused by drug-resistant salmonellae, 6 of 16 (38%) outbreaks caused by drug-susceptible salmonellae, and in 1 of 9 outbreaks (11%) caused by salmonellae of unknown susceptibility.[18] By consensus, the committee concluded that the low estimate for the likely percentage of resistant strains that originated in farm animals or their products was 50%, and the upper limit of this estimate might be as high as 100%. For the mid-range estimate, 70% was used--a value similar to that found in the CDC's review of outbreaks cited above.

FRACTION OF ANTIBIOTIC-RESISTANT STRAINS CAUSED BY SUBTHERAPEUTIC USE OF ANTIBIOTICS

Because of the paucity of data, the most uncertain aspect of the committee's risk analysis is the estimation of the portion of drug resistance in salmonellae of farm origin that is attributable to the subtherapeutic use of antibiotics. Farmers use antimicrobials in subtherapeutic dosages in feed for two purposes: (a) growth promotion, and (b) prophylaxis (such as the prevention of atrophic rhinitis in swine or "shipping fever complex" in cattle). FDA's definition of subtherapeutic use includes use for both growth promotion and prophylaxis. The committee's objective is to develop data for this combined use of antimicrobials, as well as for use in growth promotion alone.

The committee could find no data that bear directly on the relative contributions to the development of drug

resistance caused by any of the three major dosage regimens for antimicrobials--for therapy, for growth promotion, or for prophylaxis. Indeed, the data are limited and inconclusive regarding the relative contribution of chronic low-dose administration vs. intermittent high-dose administration of antimicrobial agents in fostering drug resistance (see Chapter III). Overall, the limited data available to the committee suggest that chronic exposure to low concentrations of antimicrobials is at least as likely to foster resistance as intermittent exposure to high concentrations. Given the substantial uncertainties about the causal relationship between the type of antimicrobial dosage regimens and development of resistance, the committee adopted as its mid-range estimate of the relative contributions of the three major farm uses of antibiotics the approximate proportions (percentages by weight) of drugs administered nationwide for each of these purposes to animals (see Chapter IV).

Even this seemingly straightforward approach proved difficult, because of the problems in obtaining reliable estimates of the amounts of the various antibiotics used for each of the three main purposes (see Chapter IV). As a starting point, it appears that, overall, about 12% of antibiotics sold for veterinary use is used for therapeutic purposes and about 88% is used in subtherapeutic dosage regimens (see Table IV-9 for the source of these percentages).

Based on the tonnage ratios shown in Table IV-9, some two-thirds of the drugs used for subtherapeutic purposes is given for prophylaxis and one-third growth promotion; this would result in a partition of the 88% into about 60% for prophylaxis and 28% for growth promotion. However, in the judgment of committee members the fraction used for prophylaxis is probably about three-fourths. This would result in a partition of the 88% used subtherapeutically into 66% for prophylaxis and 22% for growth promotion. The arithmetic means of these estimated percentages are 63% (60-66%) for prophylaxis and 25% (22-28%) for growth promotion, as shown in Table VII-6. Accordingly, on the simple assumption that the contribution of any drug used on the farm to the development of drug resistance would be in linear proportion to the amount used for each of the three purposes, the committee chose a mid-range estimate of 12% for the contribution of <u>any drugs</u> used for therapeutic purposes and a mid-range estimate of 88% for the contribution of <u>any drugs</u> used subtherapeutically (63% for prophylaxis and 25% for growth promotion).

The committee recognizes that there may not be a linear relationship between selection of antibiotic resistance and the total amounts of antibiotics used in farm animals. By consensus, the committee chose the plausible low estimate for

TABLE VII-6

ESTIMATED PERCENTAGE OF ANTIBIOTIC RESISTANCE IN STRAINS OF FARM ORIGIN CAUSED BY SUBTHERAPEUTIC USE OF ANTIBIOTICS IN ANIMAL FEED

Use	Proportion of Tonnage for Specified Use	Contribution of growth-promotion use to resistance: Low Estimate		Mid-Range Estimate		High Estimate	
Any Resistance Caused by Any Drug							
Therapeutic	12	15		12		8	
Subtherapeutic							
Prophylaxis	60-66 (63)	80	85	63	88	42	92
Growth promotion	22-28 (25)	5		25		50	
	100	100		100		100	
Estimated Percentage of Penicillin/Ampicillin Or Tetracycline Resistance Caused by Administration of Penicillin/Ampicillin or the Tetracyclines							
Therapeutic	10	14		10		6	
Subtherapeutic							
Prophylaxis	60	81	86	60	90	34	94
Growth promotion	30	5		30		60	
	100	100		100		100	

Source: Table prepared by the committee.

any drug given for growth promotion of 5% and the high estimate of 50%. These estimates were chosen bearing in mindthat the most plausible figure for the actual tonnage (mid-range estimate) of drugs used for growth promotion was 25%. The remaining percentages of any drugs used therapeutically or prophylactically were partitioned with the same ratio as for the mid-range estimates--i.e., about 1:5--to fill out the data base for the low and high estimates.

The lower half of Table VII-6 presents the committee's estimates of the contribution of penicillin/ampicillin or the tetracyclines to the development of drug resistance when used in the feed of farm animals for one of the three majortreatment purposes. These were derived in an analogous manner. However, for these antibiotics the committee assumed that a higher percentage was used for growth promotion than shown for any drug in the upper half of Table VII-6. In particular, it was considered that the addition of the tetracyclines to swine feed was predominantly for the purpose of growth promotion; therefore, the mid-range estimate of 25% for the contribution of drugs given for growth promotion was raised to 30% for strains resistant to penicillin/ampicillin or the tetracyclines. The other two values in the mid-range estimate for penicillin/ampicillin or the tetracyclines were reduced as follows: for prophylaxis, 63% to 60%; and for therapy, 12% to 10%. The ratio of therapeutic to prophylactic use thus became 1:6, which corresponds to the proportional amounts given to animals for these purposes. Thus, by committee consensus, 5% was chosen for the low estimate and 60% for the high estimate of the proportional use of antibiotics for growth promotion. These estimates are somewhat higher than those for resistance to any drug in light of the extensive use of the tetracyclines for growth promotion. The remaining estimates for therapeutic and prophylactic use were partitioned in a ratio of about 1:6.

Thus, all estimates have been adjusted slightly so that the total for each of the three types of use in each column is 100%. This assumes that essentially all the antibiotic resistance found in salmonellae encountered on the farm is related to the amount of antimicrobial drug in the aggregate, used for each of the three major types of application in the aggregate.

PREVENTABLE CASES OF SALMONELLOSIS: "ETIOLOGIC FRACTION"

The approach used in the risk model has been to estimate the number of persons who die each year as a result of infection with drug-resistant strains of salmonellae that originated on the farm (i.e., salmonellae isolates from meat or animal food products, eggs, or milk) and for which the drug resistance was selected by the subtherapeutic doses of

antibiotic drug in animal feed. However, at the beginning of this section the committee acknowledged that it cannot estimate the total number of cases of salmonellosis, the profile of drug susceptibilities, or the source of the bacterial strain that would likely occur in the United States if subtherapeutic doses of any antibiotics, or of penicillin/ampicillin or the tetracyclines, were not administered to farm animals.

It might be argued that, if all subtherapeutic use of penicillin/ampicillin or the tetracyclines were stopped, deaths due to infection by strains of drug-resistant salmonellae would be replaced by a like number of deaths caused by drug-susceptible salmonellae. However, there are at least three ways in which drug resistance itself might contribute to a higher total number of deaths from salmonella infection: (a) by leading to a "wrong" choice of drug for treatment (presumably an uncommon event), (b) by being intrinsically more virulent and hence lethal (a possibility for which there is some evidence, but which might in turn relate to wrong choice of drug or to etiologic fraction), or (c) by causing some infections that would not have occurred but for the resistance of the infecting bacterial strain to the antimicrobials administered. Infections caused in the third way have been called the "etiologic fraction," and the resulting cases of illness are termed "excess cases." The concept of the "etiologic fraction" arose from two observations: first, the ingestion by salmonella carriers of drugs to which the salmonellae were resistant in occasional instances appeared to provoke the development of clinical salmonellosis;[12,23] second, in one outbreak of salmonellosis, the association between ingestion of penicillin or other antimicrobials and the development of infection was so striking that it led to the initial suspicion that the drug was contaminated with salmonellae.[16] Subsequently, controlled studies have documented repeatedly that antimicrobial ingestion does enhance the likelihood of infection by drug-resistant salmonellae in epidemic situations. The hypothesis, for which supporting evidence exists in animals[5a,5b,20a,20b,20c] is that the antimicrobial drug suppresses the drug susceptible competing fecal flora, and enhances the opportunity for the pathogen to become implanted or, in carriers, to proliferate and cause disease. The effect is to lower the size of the inoculum needed to cause infection. For purposes of calculation, the etiologic fraction is determined by multiplying the relative strength of association between the recent ingestion of an antimicrobial agent and the likelihood of development of salmonellosis--i.e., the "odds ratio"--by the proportion of patients with that risk factor. In a recent review by Cohen and Tauxe[12] of various outbreaks, the proportion of cases in the "etiologic fraction" ranged from 16 to 64%. Whether

patients in the "etiologic fraction" are more susceptible to infection with salmonellae and whether they are more likely to die of this infection is not known, i.e., the relative death rates in the "etiologic fraction" are not known. As the proportion of bacterial strains that are drug-resistant increases, cases belonging to the "etiologic fraction" should constitute a larger and larger proportion of all infections. Table VII-7 summarizes quantitative evidence from six recent studies about the increased risk of drug-resistant salmonellosis among individuals taking antimicrobial agents.

As an illustration, the information from Adler et al.[1] is summarized in Table VII-8 where the figures in parentheses are obtained by difference from the four figures in the top line of Table VII-7. The odds ratio is then calculated from the cross products of the four "internal" cells of Table VII-8 as follows:

$$OR = (28x19) / (21x8) = 3.2$$

Other lines in Table VII-8 are to be interpreted similarly.

The odds ratios, which are close estimates of relative risks, are presented without statistical confidence limits, because confidence limits express only the uncertainty due to random error, and each of these sources is subject to considerable additional nonrandom uncertainty in any generalization to a broader population. For example, a study of a single strain (as in an outbreak) violates the basic assumption of statistical independence; controls drawn from patients on a pediatric ward may be both more vulnerable and more exposed to resistant strains than infants in general (or than the population as a whole); and household contacts of patients may tend to share patterns of antibiotic use with the patients. In addition, the sources of subjects were not always well characterized, antibiotic resistance was determined in different ways, and methods of ascertaining drug use varied. Nevertheless, the odds ratios considered in this analysis are thought to be applicable to patients who ingest penicillin/ampicillin or the tetracyclines and to salmonellae that are resistant to those drugs, in that they were derived primarily from studies in which those were the drugs involved in producing the "etiologic fraction."

Given these different kinds of uncertainty, the consistency in the elevation of odds ratios is impressive. However, because these odds ratios refer to different sorts of subjects and diseases (ill children, outbreaks vs. sporadic cases, etc.) and because one may expect such differences to affect relative risks, none of these figures alone is entirely suitable for estimating the odds ratio for the public at large or for estimating the part of the total national burden attributable to the overgrowth of resistant strains when the normal flora is suppressed by antibiotic

TABLE VII-7

SOME STUDIES OF THE FREQUENCY OF ANTIBACTERIAL THERAPY IN INDIVIDUALS INFECTED WITH ANTIMICROBIAL-RESISTANT SALMONELLA STRAINS

Senior Author	Total Subjects	Infected with Resistant Salmonellae	Recent Drug Use	Both Infected and Recent Drug Use	Odds Ratio
Adler 1970[1]	76 patients on a pediatric ward	36 multi-resistant (single strain)	49 took semisynthetic penicillin or ampicillin, time not stated	28	3.2
Riley 1984[24]	(a) 504 cases, geographically dispersed	66 resistant to 2 or >2 anti-microbials	43 took 1 or >1 antimicrobials within 4 weeks	13	3.3
	(b) 43 patients receiving antimicrobials	13 resistant strains*	25 took ampicillin, amoxicillin, or penicillin	12	15.7
Holmberg 1984[16]	(a) 21 patients with S. newport infection	10 resistant (single strain)	7 took amoxicillin or penicillin within 1 week	7	Infinite
	(b) Same 10 drug-resistant cases + 29 household contacts	Same	Same	7	Infinite
	(c) Same 10 drug-resistant cases + 27 non-S. newport	Same	Same plus ophaloridine	7	Infinite
Mac Donald 1987[20]	485 isolates, geographically dispersed	117 resistant to at least one antibiotic	63 took anti-microbials within 4 weeks	23	2.0
Spika 1987[29]	45 cases, 88 matched controls	45 epidemic multi-resistant S. newport	13 took penicillin or tetracycline within 1 month	11	13.9
Ryan 1987[25]	50 cases, 50 matched controls	50 epidemic multi-resistant S. typhimurium	Not stated	Not stated	5.5

Source: Adapted by the committee from Adler et al.,[1] Riley,[24] Holmberg,[17] MacDonald,[20] Spika,[29] and Ryan.[25]

* Not clear whether resistant to any antibiotic, to 2 or more antibiotics, or to penicillins (with or without other antibiotics).

TABLE VII-8

NUMBERS OF PATIENTS WITH RESISTANT SALMONELLA USING ANTIMICROBIALS

Resistant Salmonella	Recent Drug Use		All Subjects
	Yes	No	
Yes	28	(8)	36
No	(21)	(19)	(40)
Totals	49	(27)	76

Source: Adapted from Adler et al.[1]

use. Those studies that come closest to ideal for estimating a population-wide odds ratio are those of Holmberg et al.,[16,18] Spika et al.,[29] and Ryan et al.[25] The three estimates are: infinite (based on a small sample), 13.9, and 5.5. The committee is inclined to believe that the true population-wide odds ratio, for cases similar to those regularly reported to CDC, for oral intake of any antimicrobial in common use among humans and for salmonellae resistant to that drug (and perhaps others), may be about 5 and is probably between 2 and 20. Clearly, these estimatesare uncertain, and additional research is needed to improve them.

A population-wide estimate of the proportion of salmonella infections or deaths (the "etiologic fraction") can be derived from an odds ratio combined with an estimate of the proportion of the population taking antibiotics as:

$$EF = (OR-1)\ P/\ [1+\ (OR-1)\ P],$$

where OR is the odds ratio and P is the proportion exposed to the risk factor (here, antibiotic use). The definition of "taking antibiotics" must be close to that used in estimating the odds ratio, so the committee reviewed the studies in Table VII-7 to see whether any could be used for this purpose. There are uncertainties in each of the studies, but the closest seem to be those of Holmberg (none of 29 household contacts had taken antibiotics within one week, giving a rate of use of 0.0%) and Spika (2 of 88 matched controls had taken antibiotics within one month, or 2.3%). Clearly, the population-wide figure is larger than Holmberg's 0.0%, and it may be close to Spika's figure, scaled down from

2.3% for use in the past month to, perhaps, 0.5% per week. Thus, our best estimate here is 0.5% use within the past week, to which we attach a high estimate of 1% and a low estimate of 0.2%.

Using the low, mid-range, and high estimates of both OR and P, the committee produced nine estimates of the proportion of human salmonella infections due to this mechanism alone (the etiologic fraction):

	ODDS RATIO		
Antibiotic Use (Past Week)	2	5	20
0.2%	0.2%	0.8%	4%
0.5%	0.5%	2.0%	9%
1.0%	1.0%	4.0%	16%

Thus, the committee's best estimate is that 2% of salmonella infections of the sort reported to CDC are a direct result of the use of antibiotics by persons who harbor salmonellae resistant to those antibiotics. We think it unlikely that the high estimates of both OR and P hold or that both low estimates hold (the upper left and lower right corners of the table). The range of the estimates excluding those two possibilities is 0.5% to 9%. The committee fully recognizes the great uncertainty of the estimates here. Nevertheless, we believe that the estimates are worth presenting, partly because they embody our best judgment about the matter, and partly because they point in a clear manner to a need for additional research. The uncertainty is inherent in the limitations of available data, and other methods of analysis and presentation would simply hide the uncertainty, rather than reduce it.

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VIII

THE ESTIMATION OF RISK

RISK ASSESSMENT AND UNCERTAINTY

As noted in earlier chapters of this report, there is no direct evidence that subtherapeutic uses of antibiotics in animal feed create an excess risk of disease or death in humans consuming products from treated animals. This is not unexpected, at the present state of knowledge it would be unreasonable to expect direct evidence, even if the risk were relatively large. Attempts to collect such evidence are beset with serious methodologic difficulties; opportunities for collecting direct evidence on the magnitude of this risk are rare; and when they exist, are not likely to produce unambiguous results. There are questions about what agency has the mandate, funding, or manpower to obtain the information needed to evaluate the effectiveness of any regulatory action regarding the feed additives under consideration here. The cost of the data collection will certainly be high. There are unanswered questions about whether FDA now has the legal authority to demand the collection and submission of the types of data that would be required to show safety, in its broadest sense for human health.

The tools of risk assessment are typically applied in situations of this type where there is a need to acquire some sense of the probable size of a potential public health problem, some relevant data are available, but no means exists to obtain a direct measure of risk.[1]

Because risk estimates produced by such means are based on assumptions and limited data, they should be interpreted and used with caution. Such estimates are best seen as scientific hypotheses about the possible extent of a problem. This does not mean they are "hypothetical" in the weak sense that they are based on speculation. Rather, they are hypotheses that are consistent with all available information and scientific understanding, but that have not been verified by traditional scientific methods. All the estimates presented in this report should be viewed in this perspective.

An essential part of any risk assessment is the characterization of the associated uncertainties. In most cases, including the present one, risks are presented as numerical estimates (for example, as deaths per year) or as

ranges. We caution that such numerical estimates are incomplete descriptions of risk and should not be used without citation of the associated uncertainties, many of which can not be expressed quantitatively.

METHOD OF RISK CALCULATION

Chapter VII explained the risk model, provided a view of the uncertainties associated with each parameters used by the committee and presented the committee's low, mid-range, and high estimates of these parameters required by the model. The estimates of risk presented in this chapter are limited to infections with antibiotic-resistant strains (due to subtherapeutic uses of antibiotics in animals) of Salmonella (other infectious bacteria have been mentioned, but risks are not calculated for them) and further limited to annual numbers of deaths from these infections (risk due to morbidity and from non-lethal cases were not calculated).

Annual numbers of deaths are estimated by a straightforward multiplication of combinations of parameter estimates, as illustrated for the mid-range estimates alone at the opening of Chapter VII. The committee could not select a single "best estimate" of any of the parameters for use in describing risk. All possible combinations of the parameters were thus used to produce a range of possible risks. The various combinations selected for estimating different risks are described in the text to follow. In each case the complete set of possible risks for each combination was calculated, and the results were converted to percentiles and plotted on graphs as cumulative distributions (Figures VIII-1 through VIII-12).

The committee attaches some importance to the 5th, 50th (median) and 95th percentiles as descriptions of the whole set of estimates. However, the 5th and 95th percentiles are not to be interpreted as confidence limits, because they do not reflect any underlying probability distribution. A given percentile simply describes the fraction of risk estimates that fall below it. Thus, for example, if a specific risk is based on multiplication of five different parameters, and there are three different estimates of each parameter (low, mid, and high-range), then there are 243 different possible estimates of risk (3^5). The lowest five percent of these 243 estimates falls below the 5th percentile, etc. The committee believes that it is unlikely that all (or nearly all) of the low or high estimates hold simultaneously. Thus, combinations of parameters that are all (or nearly all) low, or high, are highly implausible. This is based, in part, on the improbability of being consistently wrong in the same direction, and in part on the committee's attempt to set the low and high estimates at the bounds of general plausibility.

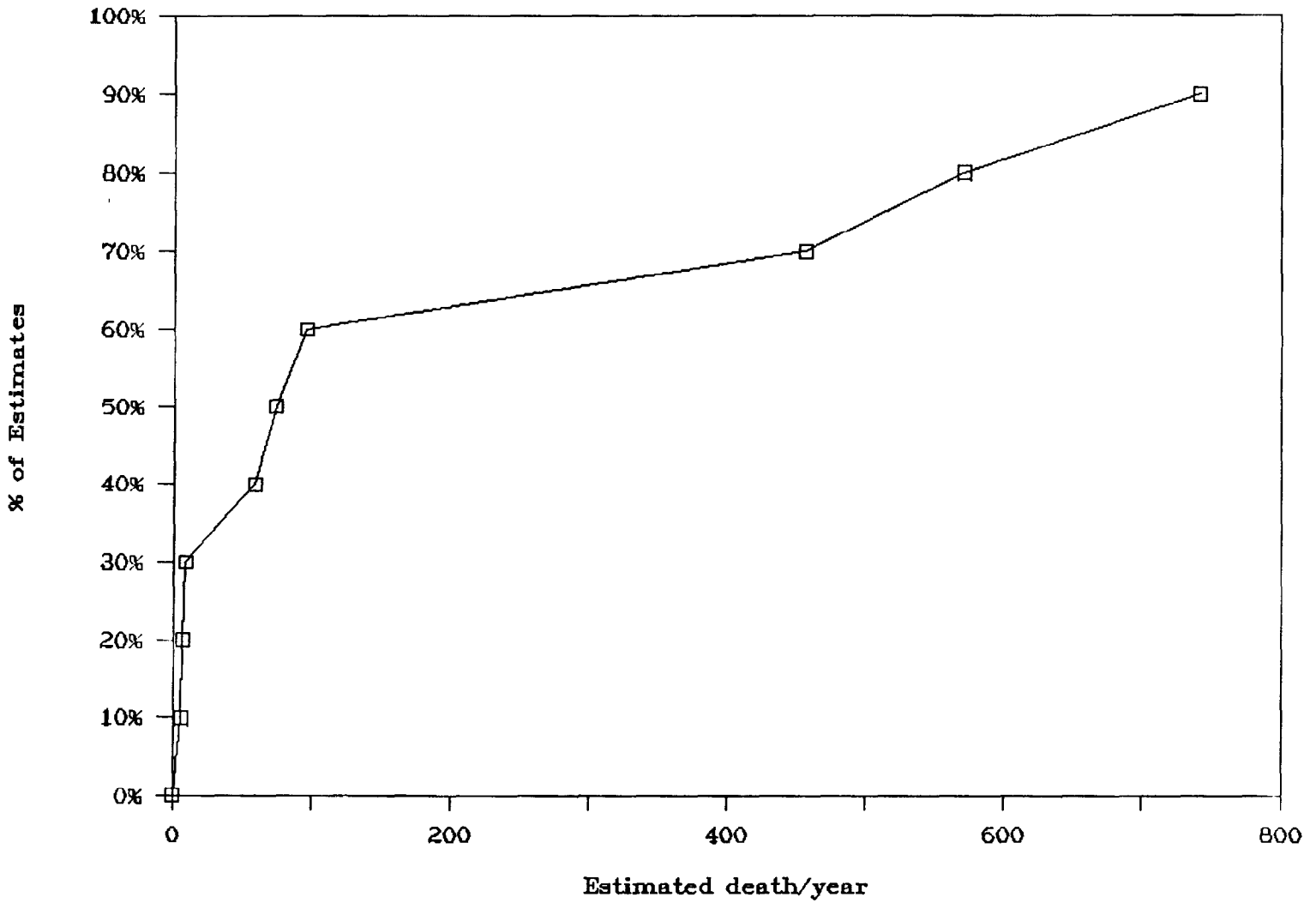


Figure VIII-1. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 2[a], 3[b], 4, 5[a] of Table VIII-1).

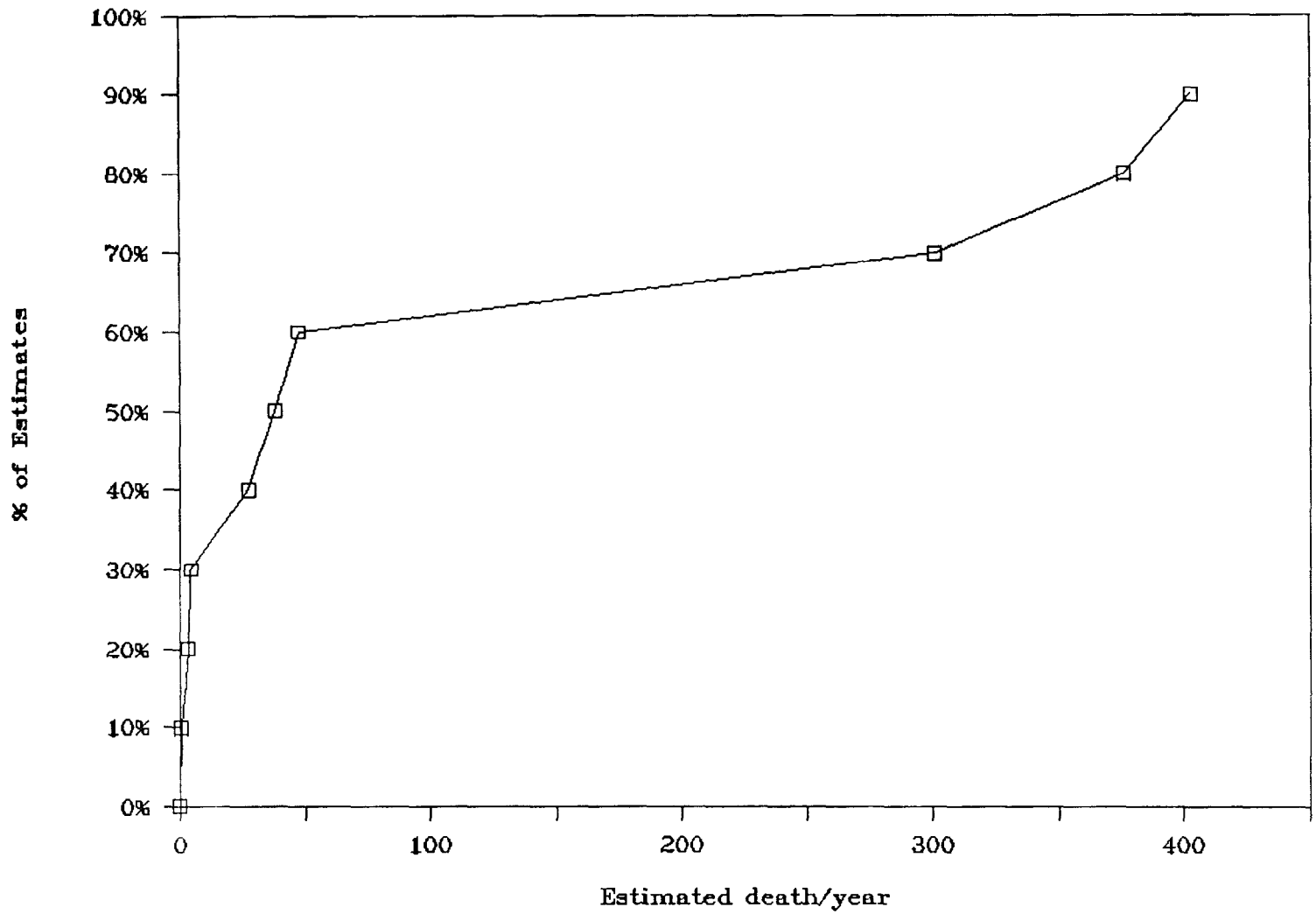


Figure VIII-2. Estimates of annual numbers of deaths from subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2[b], 3[c], 4, 6[a] of Table VIII-1).

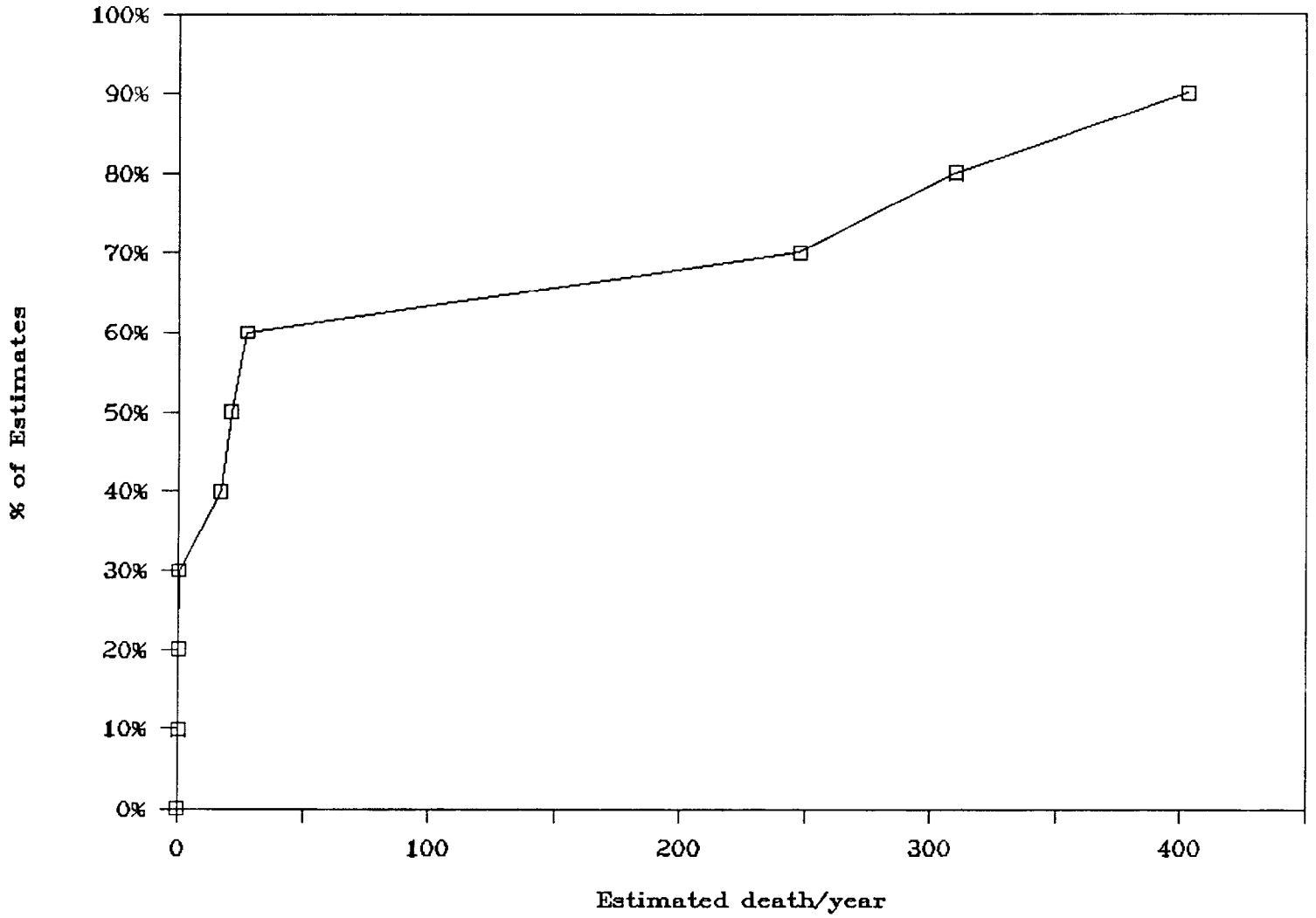


Figure VIII-3. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2[a], 3[b], 4, 5[b] of Table VIII-1).

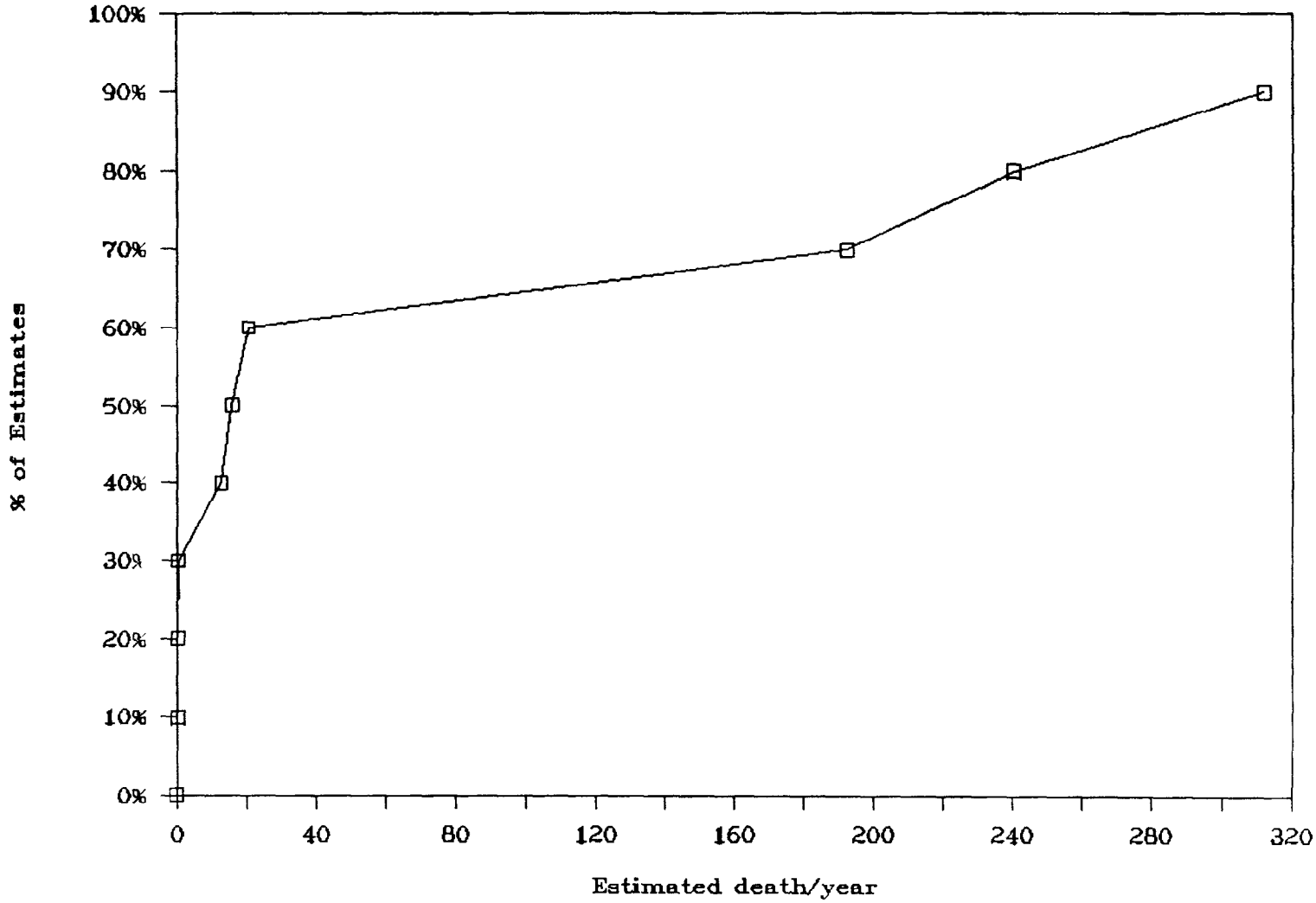


Figure VIII-4. Estimates of annual numbers of deaths from subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^{b}, 3^{c}, 4, 6^{b} of Table VIII-1).

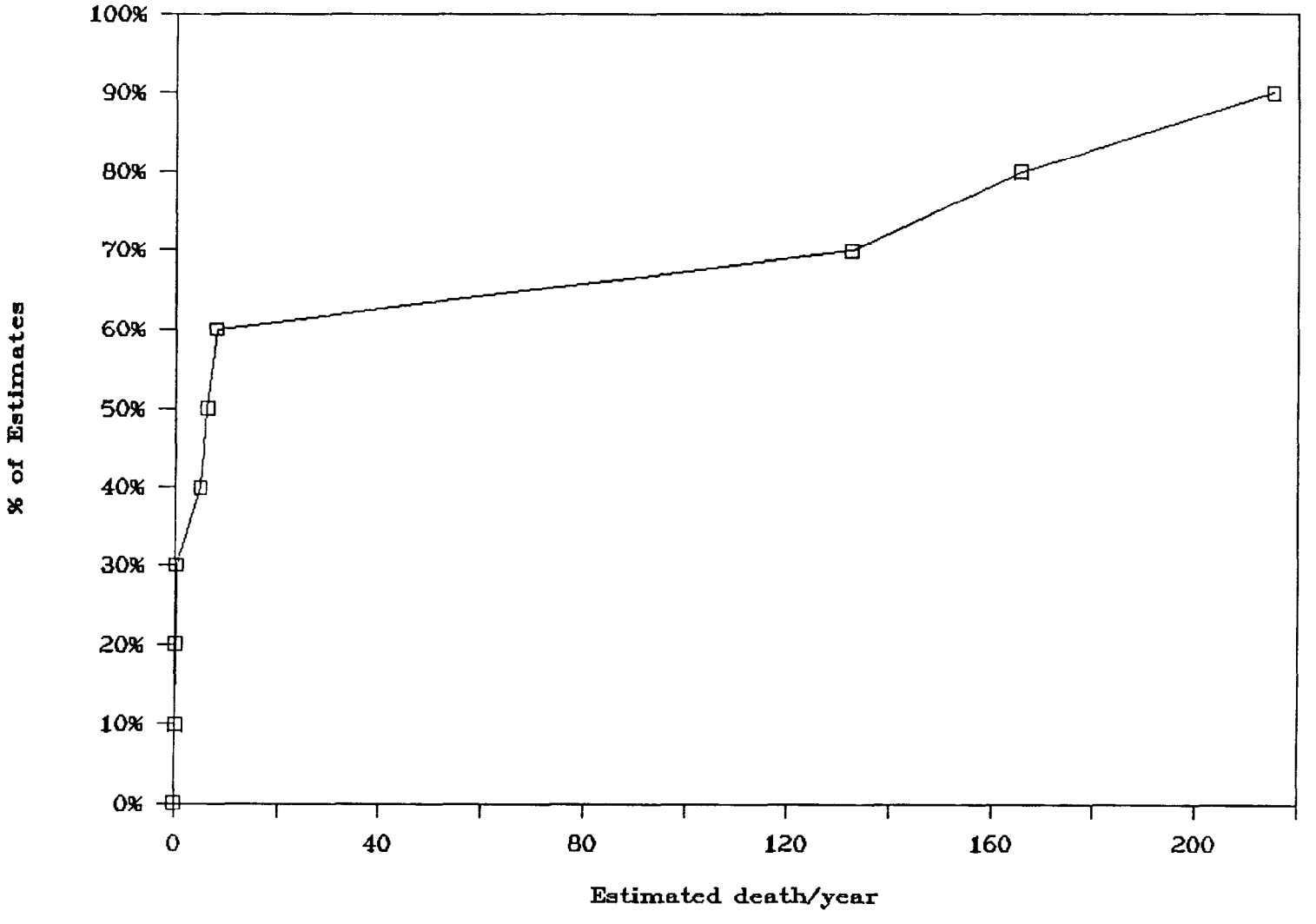


Figure VIII-5. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic use of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^{b}, 4, 5^{a} of Table VIII-1).

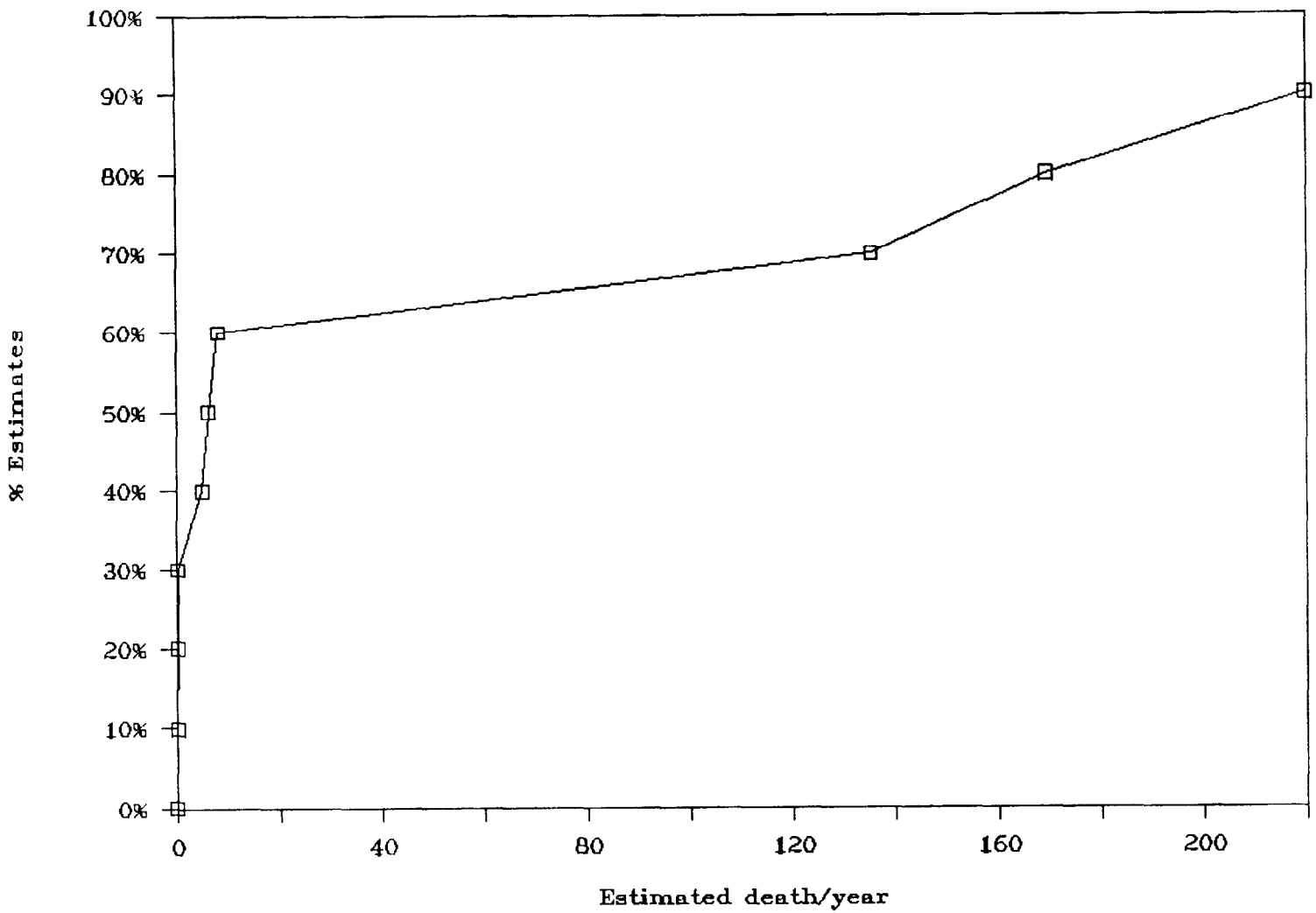


Figure VIII-6. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of penicillin /tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^{c}, 4, 6^{a} of Table VIII-1).

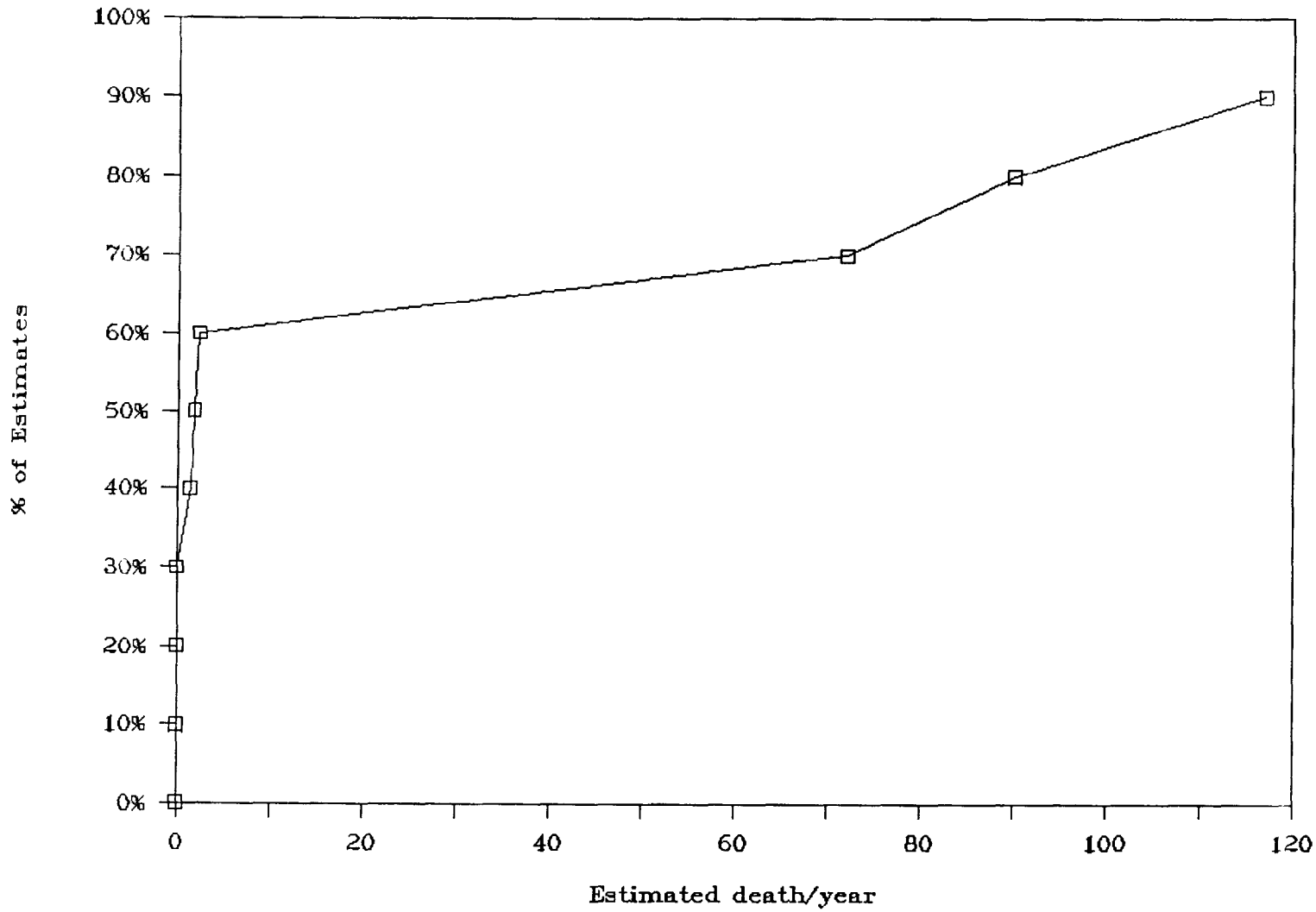


Figure VIII-7. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 7, 3[b], 4, 5[b] of Table VIII-1).

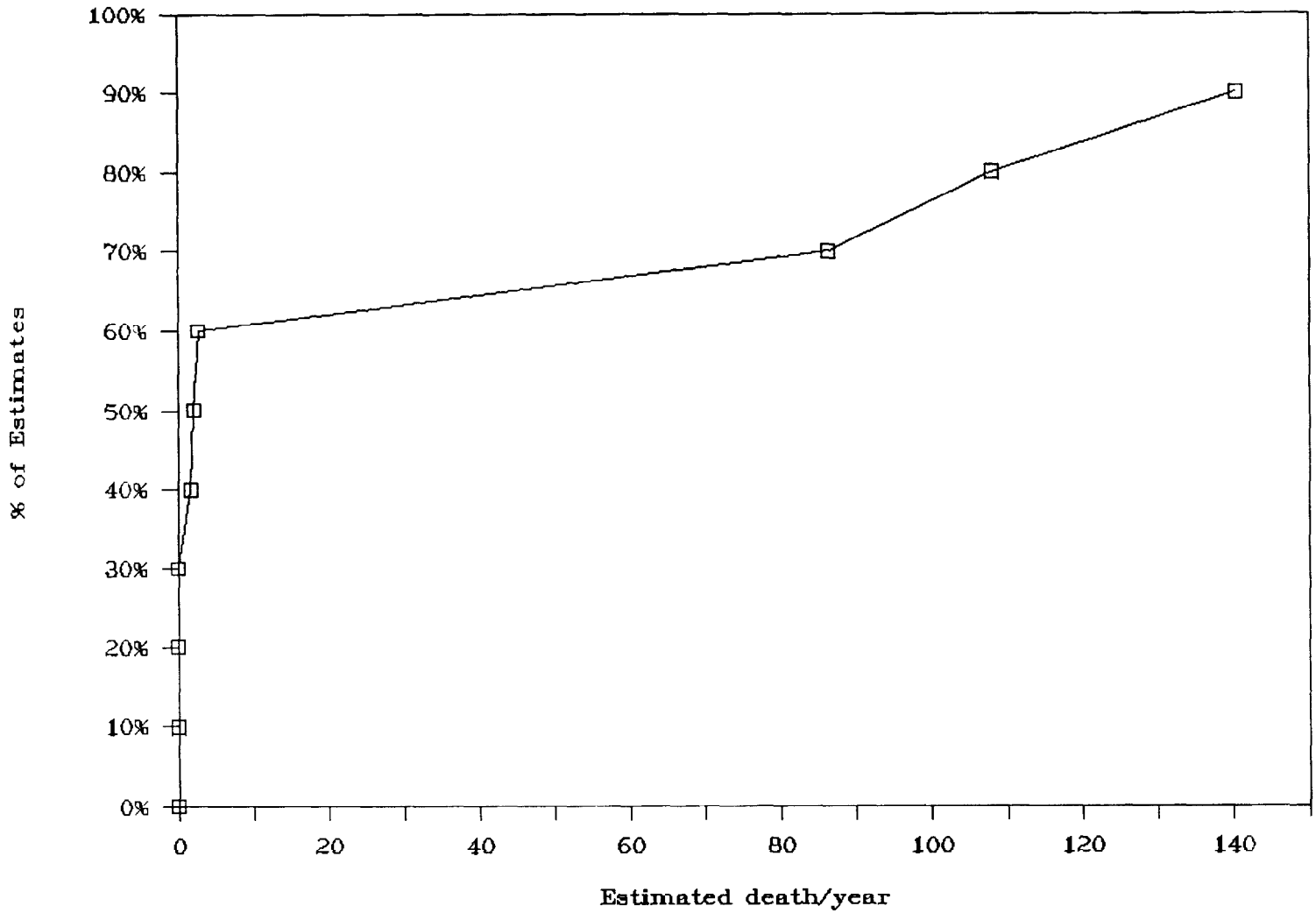


Figure VIII-8. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 7, 3[c], 4, 6[b] of Table VIII-1).

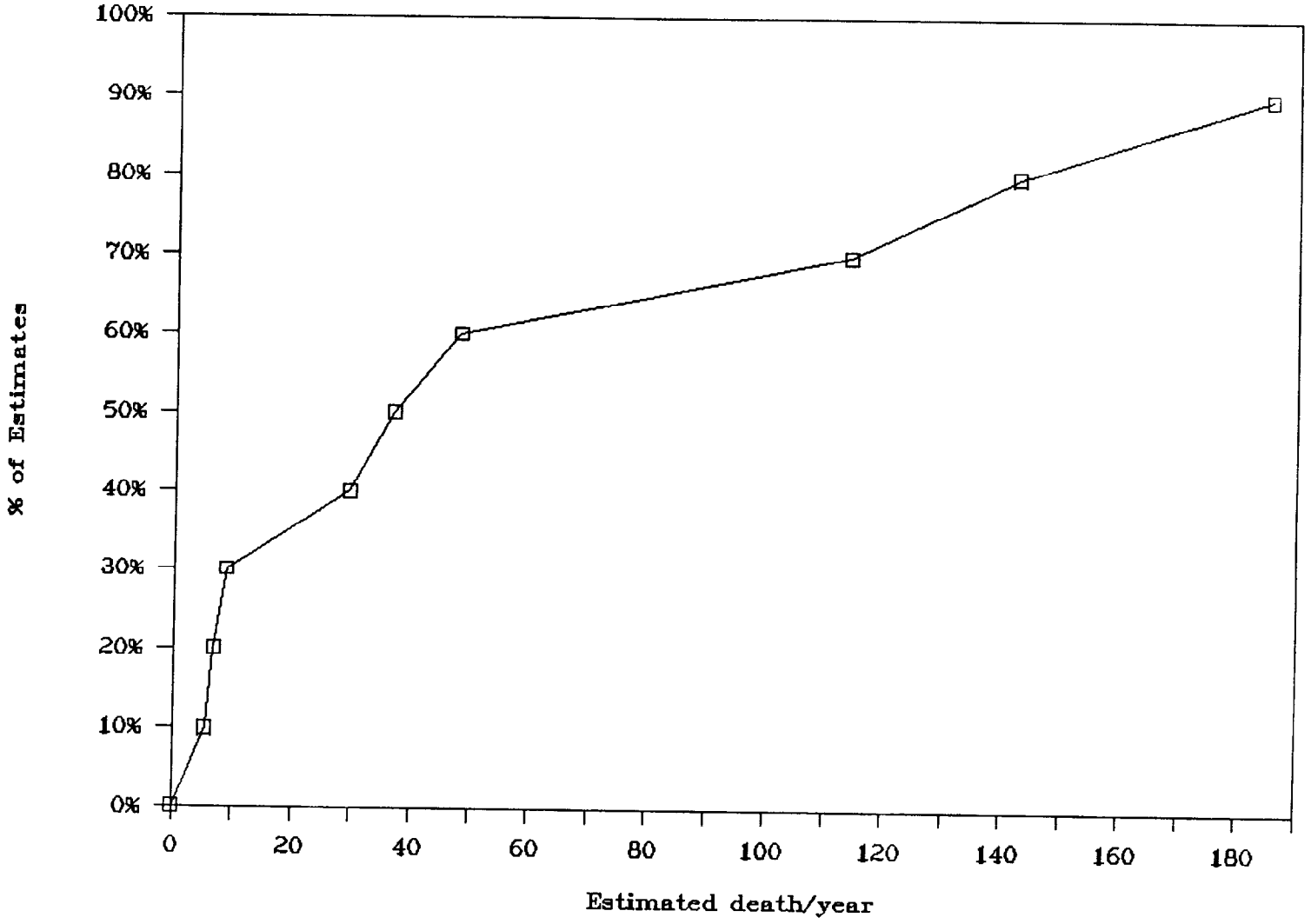


Figure VIII-9. Estimates of annual numbers of deaths arising because of higher death rate and increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of times 1, 2^{a}, (3^{b}-3^{a}), 4, 5^{a} of Table VIII-1).

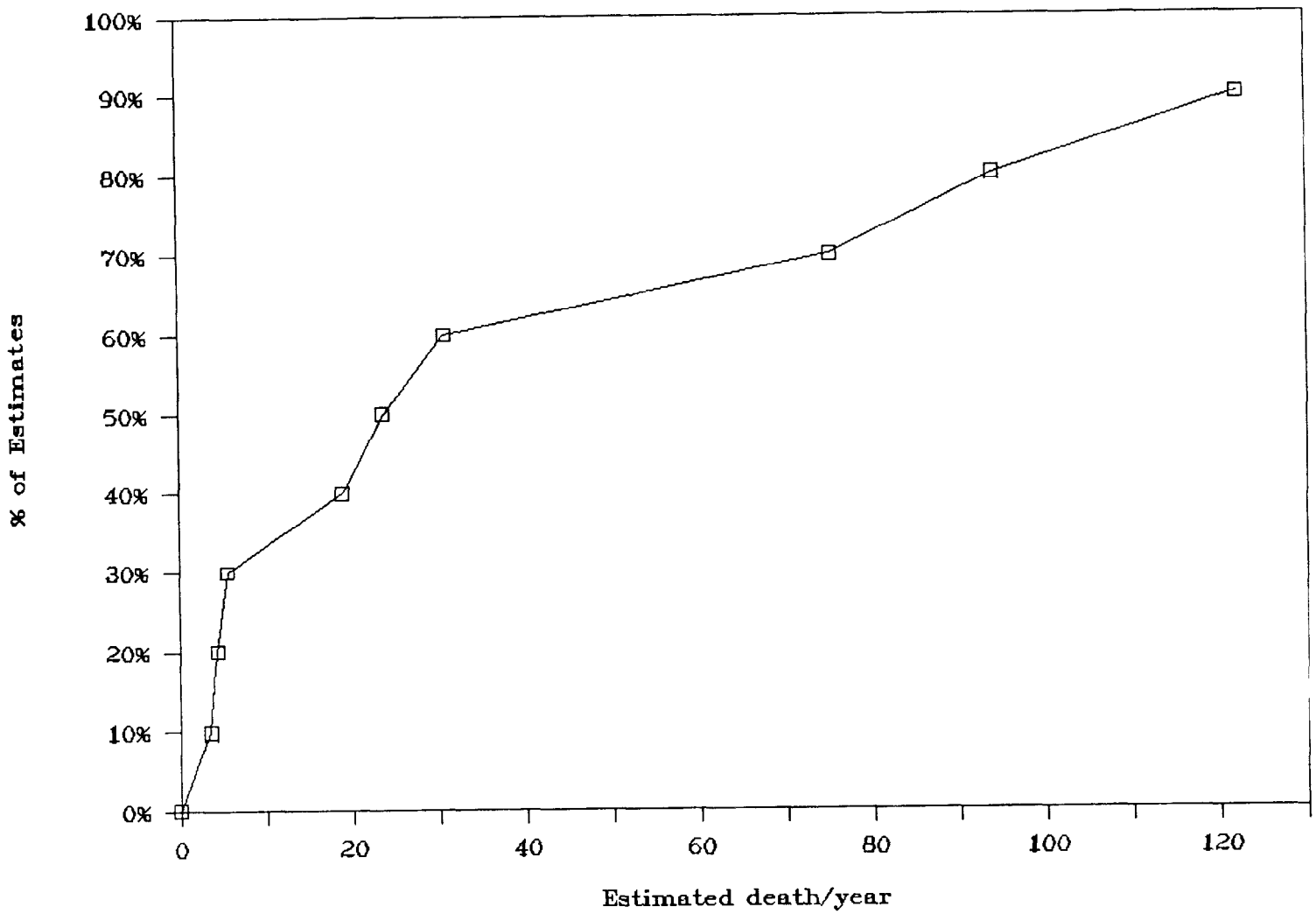


Figure VIII-10. Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^{b}, (3^{c}-3^{a}), 4, 6^{a} of Table VIII-1).

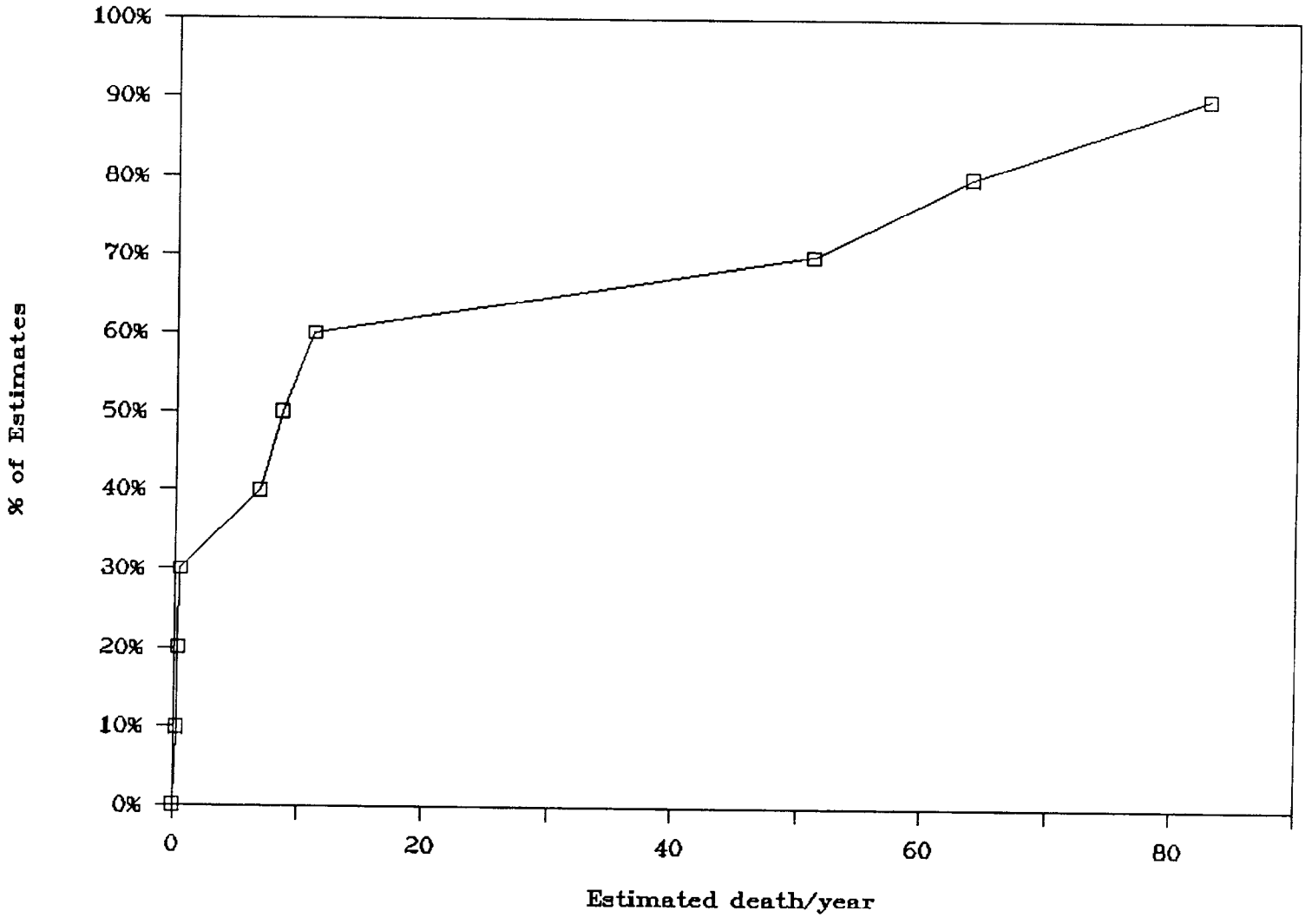


Figure VIII-11. Estimates of annual members of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, (3^b-3^a), 4, 5^b of Table VIII-1).

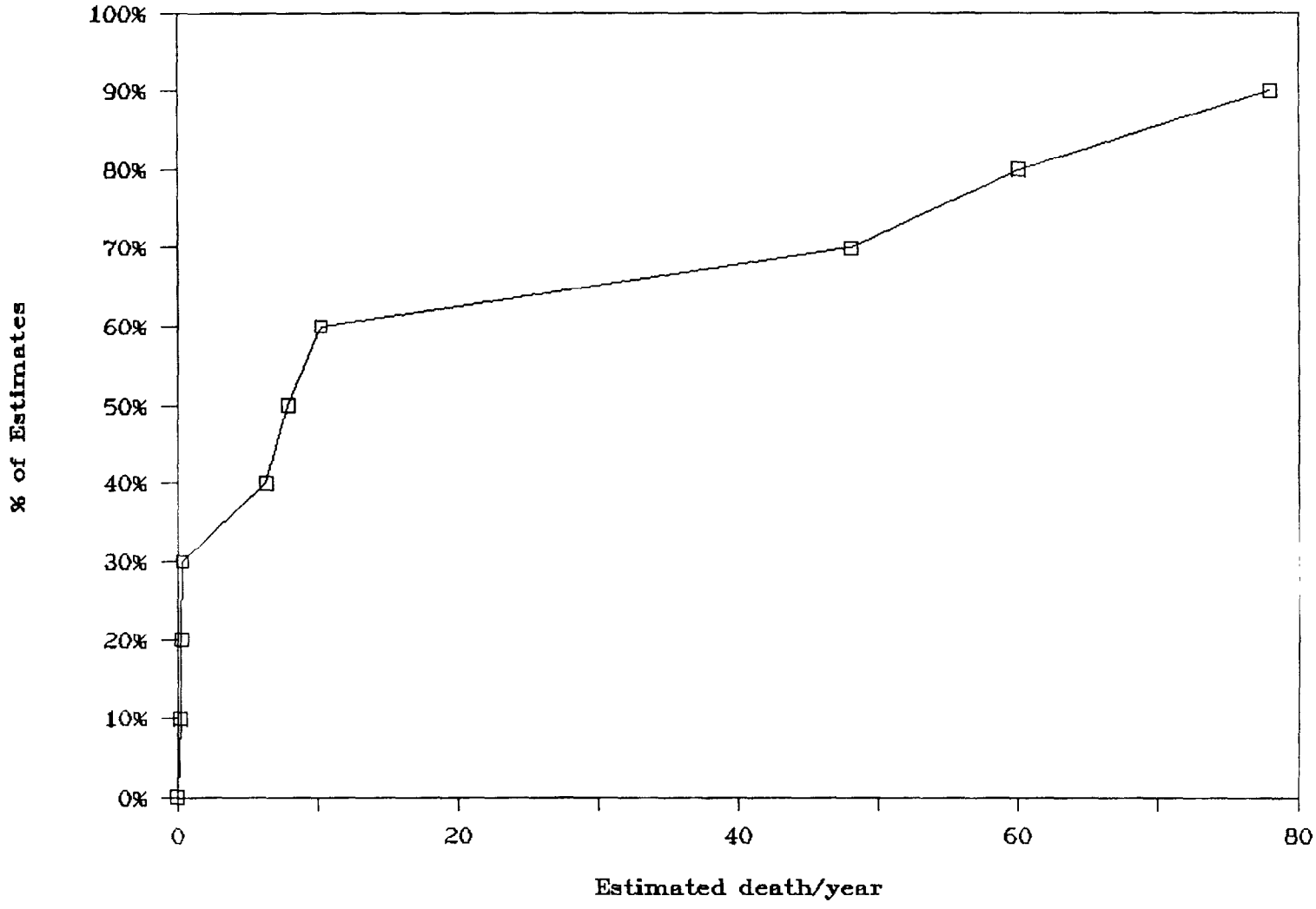


Figure VIII-12. Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^{b}, (3^{c}-3^{a}), 4, 6^{b} of Table VIII-1).

The committee thus tends to place greatest reliance on estimates near the median value.

ESTIMATION OF RISKS

Chapter VII, "The Risk Model", provides the data on which the Committee has based its estimates of human health risk that may be associated with the subtherapeutic use of antibiotics in animal feed. Table VIII-1 gives the parameter estimates used in assessing the whole of the problem of excess human salmonellosis deaths that might be attributable to any low-level farm use of antibiotics. These estimates are taken directly from the text and tables in Chapter VII. Various combination's of these parameters are used to estimate different types of risk. Twelve different sets of risk estimates were produced from these data; these estimates are presented graphically in Figures VIII-1 through VIII-12, and are summarized in Table VIII-2. The basis and meaning of the twelve different sets of estimates that are shown in Figures VIII-1 through VIII-12 are described in the following text.

MAXIMUM POSSIBLE NUMBERS OF EXCESS DEATHS

Figure VIII-1 shows the cumulative distribution of the 243 estimates of annual deaths generated for drug resistance of any type (Table VIII-1, multiplication of parameters from lines 1, 2^a, 3^b, 4, 5^a). Figure VIII-2 shows the corresponding distributions for resistance to penicillin and/or tetracycline specifically (Table VIII-1, multiplication of parameters from lines 1, 2^b, 3^c, 4, 6^a). Estimates presented in Figures VIII-1 and VIII-2 represent subtherapeutic use of antibiotics for both growth promotion and prophylaxis.

Figures VIII-3 and VIII-4 are similar to Figures VIII-1 and VIII-2 except that the estimates are for farm use of antibiotics for growth promotion only, rather than for all subtherapeutic uses on the farm. Figure VIII-3 describes risks for use of any antibiotic and is based on multiplication of lines 1, 2^a, 3^b, 4, and 5^b of Table VIII-1. Figure VIII-4 describes risks for use of penicillin and tetracycline only and is based multiplication of parameters from lines 1, 2^b, 3^c, 4, and 6^b in Table VIII-1.

Readers are cautioned that these are not necessarily "excess deaths" in the sense that the total number is increased by the quantity indicated; they are rather estimates of the annual numbers of deaths attributable to salmonellosis of the specified origin. These may, to some extent, overlap or replace deaths (in these patients or

TABLE VIII-1

DATA USED TO ESTIMATE ANNUAL DEATHS FROM SUBTHERAPEUTIC USES OF ANTIBIOTICS IN ANIMAL FEED

	Estimates		
	Low	Mid-Range	High
1) Reported Salmonella per year	40,000	50,000	65,000
2) Resistance of Salmonella to antimicrobials			
a) Resistance to any antibiotic	*0.16	0.24	0.31
b) Resistance to penicillin/ tetracycline	0.10	0.15	0.20
3) Death Rate -- infection by strains			
a) Not resistant to any antibiotic	0.002	0.005	0.01
**b) Resistant to any antibiotic	0.002	0.01	0.04
**c) Resistant to penicillin/ tetracycline	0.002	0.01	0.04
4) Fraction of those deaths associated with strains of farm origin	0.5	0.7	1.0
5) Fraction of antibiotic resistance of farm origin caused by subtherapeutic use of any antibiotic in animal feed			
a) Prophylaxis and growth promotion	0.85	0.88	0.92
b) Growth promotion only	0.05	0.25	0.50
6) Fraction of antibiotic resistance caused by subtherapeutic use of penicillin/tetracycline in animal feed			
a) Prophylaxis and growth promotion	0.86	0.90	0.94
b) Growth promotion only	0.05	0.30	0.60
7) Etiologic Fraction	0.005	0.02	0.09

Source: Table prepared by the committee. The bases for all estimates are provided in Chapter VII. Note: penicillin/ tetracycline = penicillin, ampicillin or tetracyclines.

* Decimal fraction or proportion.

** The committee could not find evidence sufficient to justify the use of different death rates for strains resistant to one drug rather than another, or for multiresistant strains vs. strains resistant to only one drug.

TABLE VIII-2

SUMMARY OF THE VARIOUS ESTIMATES OF ANNUAL NUMBERS OF DEATHS FROM SUBTHERAPEUTIC USES OF ANTIBIOTICS

Source of Estimates	Best Estimate[a]	Range[b] (median)
Figure VIII-1[c]	70	5-700
Figure VIII-2[d]	40	1-400
Figure VIII-3[e]	20	1-400
Figure VIII-4[f]	15	1-300
Figure VIII-5[g]	06	1-200
Figure VIII-6[h]	06	1-200
Figure VIII-7[i]	02	0-100
Figure VIII-8[j]	02	0-100
Figure VIII-9[k]	40	3-200
Figure VIII-10[l]	20	2-100
Figure VIII-11[m]	08	1-100
Figure VIII-12[n]	08	1-100

Source: Adapted by the committee from Table VIII-1 and Figures VIII-1 through VIII-12.

[a] 50% of estimates fall below this figure (rounded to one significant figure).

[b] 5% of estimates fall below lower end of the range; 95% of estimates fall below upper end.

Estimates of annual numbers of deaths:

[c] from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 2^a, 3^b, 4, 5^a of Table VIII-1).

[d] from subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^b, 3^c, 4, 6^a of Table VIII-1).

[e] from subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, 3^b, 4, 5^b of Table VIII-1).

[f] from subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^b, 3^c, 4, 6^b of Table VIII-1).

[g] in the etiologic fraction attributable to subtherapeutic use of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^b, 4, 5^a of Table VIII-1).

[h] in the etiologic fraction attributable to subtherapeutic uses of penicillin /tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^c, 4, 6^a of Table VIII-1).

[i] in the etiologic fraction attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 7, 3^b, 4, 5^b of Table VIII-1).

[j] in the etiologic fraction attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 7, 3^c, 4, 6^b of Table VIII-1).

[k] arising because of higher death rate and increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of times 1, 2^a, $(3^b\text{-}3^a)$, 4, 5^a of Table VIII-1).

[l] arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^b, $(3^c\text{-}3^a)$, 4, 6^a of Table VIII-1).

[m] arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, $(3^b\text{-}3^a)$, 4, 5^b of Table VIII-1).

[n] Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^b, $(3^c\text{-}3^a)$, 4, 6^b of Table VIII-1).

others) from salmonellosis that would have occurred anyway with some other strain. Conversely, it is possible that these estimates underestimate the real number of excess deaths if, for example, resistant strains tend to be more virulent than drug-susceptible strains, or if the estimates in successive lines of Table VIII-1 are not independent, (see Chapter VII regarding independence).

ESTIMATES OF DEATH BASED ON ETIOLOGIC FRACTION

As explained above, the estimates for deaths from all Salmonella strains with drug resistance attributable to low-level farm uses of antibiotics are not necessarily estimates of the excess number of salmonellosis deaths from such use. A fraction of the excess can, however, be estimated--the "etiologic fraction" discussed in this section and the death of farm origin "harder-to-treat fraction discussion in the following section. These two fractions may overlap (e.g., figures for the etiologic fraction may reflect some increase in the difficulty in providing effective treatment) and, further, these two fractions do not necessarily account for the whole effect of farm use of subtherapeutic levels of antibiotics (e.g. there may be a difference in virulence).

Estimates for deaths attributable to the etiologic fraction--that is, cases of salmonellosis that would simply not have occurred in the absence of resistance--require some modification in approach. Parameter estimates are given in Table VIII-1. The odds ratios in Table VII-7 are calculated for the whole population of exposed persons; of these, some proportion harbor resistant strains. The estimated odds ratios would be larger--perhaps substantially larger--if they were calculated to express the risk in persons who harbor such resistant strains. Use of the odds ratios in Table VII-7, therefore, already incorporate a reducation factor to express the risk in the population as a whole. Furthermore, this automatically reflects the actual proportion of persons who have resistant strains (perhaps in addition to susceptible strains) and does not depend on the kind of estimate in line 2 of Table VIII-1, which deals with proportions of strains rather than with the whole set of resistant strains that may inhabit one person. This approach ignores the likelihood that persons within families, within hospital wards, or otherwise in proximity may tend to carry the same strains of salmonellae, but no data on this seems to be available for use here. Because of the frequency distribution of resistant strains already incorporated into the odds ratios (line 7), no further adjustment for resistance (line 2) is needed or appropriate.

Perhaps future research studies can estimate odds ratios for the "etiologic fraction" of cases among persons who are hosts to one or more resistant strains. The odds ratios are likely to be substantially higher, but will be reduced by the (then appropriate) inclusion of such factors as those in line 2. Until this kind of additional information is available, we believe that our present analytic approach to the etiologic fraction is correct. In addition, the committee is concerned that death rates in the "etiologic fraction": (see line 3) may be above average, because some persons who receive antibiotics do so because of conditions related to immunosuppression, general debility, or other illnesses that may damage normal body defenses. In the absence of data, however, the committee has chosen to apply the death rates in line 3 of Table VIII-1 to the "etiologic fraction".

Two sets of estimates are presented for the "etiologic fraction" component of salmonellosis. Figure VIII-5 presents the 243 estimates for deaths in the etiologic fraction attributable to subtherapeutic farm use of any antibiotic for both prophylaxis and growth promotion (multiplication of parameter in lines 1, 7, 3^b, 4, 5^a, of Table VIII-1), and Figure VIII-6 presents similar estimates for penicillin/ampicillin and/or tetracyclines uses only (multiplication of parameters in lines 1, 7, 3^c, 4, and 5^b of Table VIII-1). Figures VIII-7 and VIII-8 present similar estimates for growth promotion alone; Figure VIII-7 concerns farm use of any antibiotic and Figure VIII-8 concerns uses of penicillin/ampicillin and/or tetracyclines only.

EXCESS DEATHS DUE TO INCREASED DIFFICULTY OF TREATMENT

While few or no strains of salmonellae are resistant to all clinically useful antimicrobials in the modern therapeutic armamentarium, some individual drugs are potentially toxic, have unwanted effects in particular groups of patients, may require parental administration, and some are very expensive. Further, critical time is required to determine patterns of resistance of bacterial isolates in specific infections. Thus, it would be medically inappropriate, to treat each suspected case of salmonellosis with the whole combination of antimicrobials that could conceivably be effective. More selective therapy is medically appropriate, but it has the unfortunate effect in some cases of delaying or replacing treatment by the optimum drug or drug combination, and as a result death rates may be higher in salmonellosis with resistant strains than with susceptible strains.

Whatever the reason(s), it has been commonly observed that infections with resistant strains of salmonellae more often end in death than infections with susceptible strains,

suggested by lines 3^a, 3^b, and 3^c of Table VIII-1. The difference between these lines can be interpreted as an index of the increased difficulty of providing effective therapy in cases of resistant salmonellosis. Because the estimates in lines 3^a, and 3^b and 3^c are so closely linked, the committee simply worked with the three differences (at low, mid-range, and high levels) rather than the 9 possible combinations.

Estimates of the size of this effect for all subtherapeutic uses of any antibiotic are presented in Figure VIII-9 (multiplication and parameters in lines 1, 2^a, (3^b-3^a), 4 and 5^a, Table VIII-1) and similarly in Figure VIII-10 for resistance to penicillin/ampicillin and/or tetracycline antibiotics (lines 1, 2^b (3^c-3^a), 4, 6^a).

Similar figures, but limited to drug use for growth promotion, are given in Figure VIII-11 (lines 1, 2^a,(3^b-3^a), 4, 5^b) for any drug resistance and Figure VIII-12 (lines 1, 2^b, (3^c-3^a),4, 6^b) for penicillin or tetracycline resistance.

SUMMARY OF NUMERIC RESULTS

Each of the figures in this chapter presents a range of risks, reported as annual numbers of deaths. This procedure was used because the committee had no basis for selecting any single "best" estimate. This procedure produces, for the data in each figure, a total of 243 estimates. The committee believes that the best single estimator is the median of the 243 estimates, and that the range from the 5th to 95th percentile is quite likely to contain the unknown true value. Because of the way these estimates were developed they do not provide ordinary statistical confidence limits (as explained above), but they should in practice provide even greater certainty than, say, 90% or 95% confidence limits. The committee believes that the minimum and maximum estimates presented in the figures are <u>not</u> scientifically plausible because they would require that the mid-range estimates for the parameters (Table VIII-1) all be consistently or nearly consistently wrong by a large margin and all be in the same direction.

Figures are presented to one decimal to emphasize that they are estimates, not counts. Data from the twelve figures are summarized in Table VIII-2. The following is an illustration of how the figures and data in Table VIII-1 are to be read:

<u>Figure VIII-1</u>. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion. Figure VIII-1 is read as follows:

(i) Five percent of the estimates fall below 5 to 6 deaths per year, and 95% fall below 700 deaths per year. Thus, the committee believes that the true number is very likely to be between 5 and 700 deaths per year.

(ii) The likeliest estimate is 70 deaths per year. This is the committee's best single estimate for mortality in this category.

The estimates in Table VIII-2 are derived from Figures VIII-1 through VIII-12, and each range is based on different assumption's regarding uses (e.g., any antibiotic vs. penicillin/ampicillin and, or tetracyclines only, on prophylaxis and growth promotion uses vs. growth promotion only). The ranges also differ with regard to other assumptions (e.g., inclusion of "etiologic fraction", consideration of increased difficulty of treatment). The specific meaning of each set of estimates is indicated by the Figure headings, that are reproduce at the foot of this table.

INTERPRETATION OF RESULTS

The various estimates of risk presented in Table VIII-2 are based on somewhat different assumptions and have different meanings, as indicated in the foregoing text and as summarized in the Figure headings. For each set of estimates the committee places greatest reliance upon the 50th percentile figure, which has been termed the "likeliest estimate" in Table VIII-2. The range shown in Table VIII-2 almost certainly encompasses the true figures.

The committee is not able to assign a numerical probability to the likelihood that the estimates shown are correct. As noted earlier, none of these estimates has been verified by traditional scientific methodologies (i.e., experimental or well-controlled field studies), and thus should be interpreted as scientific hypotheses about the possible extent of the problem that are consistent with all available scientific information. The committee knows of no direct evidence to support these estimates. They should be considered as having scientific support roughly comparable to that available for estimates of low dose carcinogenic risk associated with chemical carcinogens subject to regulation.

The estimates of death presented in Table VIII-1 can be placed in the context of other types of risk estimates. FDA, for example, generally holds that, for carcinogenic drugs used in animals that leave toxic food residues, lifetime risks of cancer (presumed to be equivalent to lifetime risks of death), of around 10^{-6} or less are of insignificant public

health consequence. Using this yardstick, and assuming the entire population of the United States to be potentially exposed to such residues, the numbers of excess annual cancers (i.e., deaths), assuming the risk to be accurately known, can be estimated for one such drug as follows:

$$240 \times 10^6 \text{ persons} \times 10^{-6} = 240 \text{ lifetime deaths, or}$$
$$= 3\text{-}4 \text{ deaths per year.}$$

The total number of deaths due to carcinogenic residues depends on the number of such drugs in use.

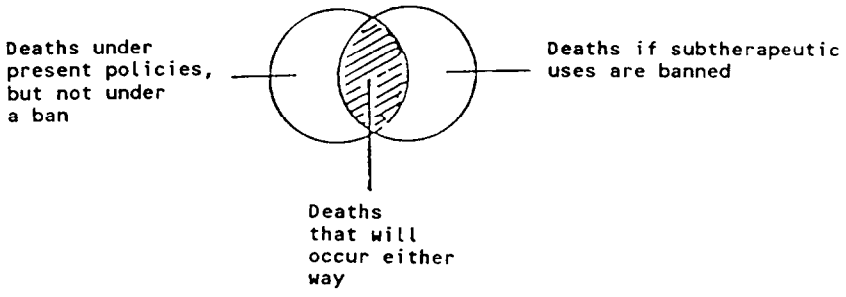
Actual numbers of deaths are probably much lower than these figures indicate, because actual residue levels rarely approach the maximum allowable and because it is unlikely that most of the population is exposed to these drugs on a continuing basis. Moreover, the risk estimation method used for carcinogens is designed to overstate risk. That is, the procedures used to estimate excess cancer risk include adoption of upper 95% confidence limits on the dose-response curve and several other assumptions about interspecies and high-to-low dose extrapolation that almost guarantee that the actual numbers of deaths will be less than those shown above. In fact, actual risk may be zero. The above figures are helpful nonetheless, because they reflect the hypothetical number of excess deaths that might be considered of negligible public health consequences.

The estimates of annual numbers of excess deaths presented in Table VIII-1 are derived by a method that is not strictly comparable to that used by FDA for carcinogenic drug residues, so care must be taken in comparing these two sources of risk. However, no better basis for comparison is known to the committee, and, with the appropriate qualifications, the drug-residue cancer risks, apparently considered acceptable by FDA, do provide a moderately useful yardstick against which the risks in Figure VIII-1 can be measured. Moreover, the committee does not mean to suggest that the risks considered acceptable by FDA for carcinogenic animal drug residues are necessarily applicable to the determination of acceptability of the risks that are the subject of this report. Whether the risks presented in Table VIII-1 are to be considered acceptable or unacceptable depends on many factors that fall outside the scope of the committee's charge. Such determinations of acceptability are risk-management decisions and thus are properly left to FDA.

EFFECTS OF DISCONTINUATION

Will the number of deaths from salmonellosis and its complications be reduced or otherwise altered by the discontinuation of the use of the subtherapeutic doses of antibiotics in farm animals, or by discontinuation of use specifically for growth promotion? The committee, in the discussion that follow below, is inclined to think that the total number of deaths due to salmonellosis would decline. However, these matters are not at present subject to scientific proof.

The committee did not deal with the conversion of drug-susceptible bacterial organisms to drug-resistant clones (by plasmid transfer) in situ, but with the reduction in numbers of bacteria of drug-susceptible strains and the subsequent overgrowth with the more drug-resistant strains to fill the vacated ecologic niche. In this context, it may be useful to consider a simple diagram with two circles, one with deaths at some future time if no discontinuation of antibiotics is instituted, and one if discontinuation has been put in place as follows:



A ban then, would remove deaths in the left-hand lunule in this figure and replace them with by deaths in the right-hand lunule. The committee might pose the question about whether this shift is worth making. The committee has not attempted any risk-management policy analysis (it was not part of the committee's mandate), but believes that the following comments are within its mandate. The left-hand lunule alone is approximated, for various facets of the problem, by Figures VIII-1 through VIII-6, and certain aspects of the net difference of the left-hand minus the right-hand lunule (the best benefits of discontinuation of antibiotics) should be approximated by Figures VIII-7 through VIII-12. The committee believes that overall there would be a net benefit in reduced mortality (thus, the right lunule might be smaller than the left), but this cannot be proved with mathematical certainty, nor can the size of the net benefit be estimated with precision.

Critical to consideration of a ban is the likelihood of a long-term reduction in the proportion of salmonella strains

with resistance to antibiotics. The genie is out of the bottle; will it return? Resistant strains appear to have no survival advantage in the absence of challenge by antibiotics (otherwise they would have driven out susceptible strains long before the modern era), but there is little evidence that they have a survival disadvantage either. Further, other uses of antibiotics will continue including therapy of infections in both humans and animals, and compliance with a ban on subtherapeutic uses might be incomplete. Thus, it may be that a ban would retard the increase in proportion of resistant strains, but not stop or reverse the increase.

REFERENCES

1. National Research Council, Committee on the Institutional Means for Assessment of Risks to Public Health. Risk Assessment in the Federal Government: Managing the Process. Washington, D.C.: National Academy Press, 1983.

IX

DISCUSSION

The use of tetracycline and penicillin in subtherapeutic concentrations in animal and poultry feeds has aroused concerns about the possibility of a risk to human health. There are good reasons for concern: the known properties of transferable resistance plasmids and transposons among bacteria, the powerful action of antimicrobial drugs in selecting for antimicrobial-resistant bacteria, and the high levels of antimicrobial resistance found in E. coli and salmonella isolates from farm animals and humans. In addition, it is now possible to detect clonally salmonella strains from various sources in the food production chain (from farm to consumer) and so establish linkages between isolates from humans and from farm animals (or animal food products). This report deals mainly with the magnitude of the human health hazard and with whether sufficient data are available to assess the risk.

There is no direct evidence to quantify the human health hazard from antibiotic-resistant pathogenic bacteria created by the use of subtherapeutic amounts of penicillin or the tetracyclines in animal feed. Using the available indirect evidence shows these antibiotics in subtherapeutic concentrations do present a hazard to human health and may contribute to a percentage (see Figure VII-2) of the approximately 500 deaths annually in the United States from salmonellosis. Although the focus in the analysis of risk has been only on deaths attributed to salmonellosis, there are the same concerns about risk due to E. coli (and other Enterobacteriaceae) and to other pathogens (both gram-negative and gram-positive) known to be drug resistant that might infect both animals and humans. Human exposure to enteric organisms (pathogens and commensals) of animal origin is extensive. In food-animal processing plants, the incidence of bacterial contamination has been reported as high as 34% for chickens, 74% for beef, and 84% for pork.[10] Figures reported for comparable E. coli contamination range from 73% for beef carcasses, 81% for chicken and 97% for pig carcasses. The E. coli contamination presumably is from fecal sources. In studies from Great Britain,[7,8] 38% of E. coli in calf feces were resistant to one or more antimicrobials, and other studies showed values of 49% for pigs and 83% for poultry. In the state of Washington in surveillance for enteric pathogens in a poultry processing

plant, 47% of poultry were contaminated with Campylobacter jejuni and 4.7% were contaminated with Salmonella species.[13] Contamination with Campylobacter spp. was found in 22% of poultry from retail sources and 3.5% with Salmonella spp. Contamination with C. jejuni was observed in 0.4% of beef samples, but no salmonella contamination was found. Salmonella contamination was 2.7% in pork products. In this study, 30% of the salmonella isolates from retail poultry were resistant to tetracycline. These findings show that E. coli, Salmonella spp., and C. jejuni commonly are found on meat and poultry products. Human ingestion of these bacteria might result from contamination of hands during food preparation or consumption of inadequately cooked animal food products. Colonization of the human intestine by antibiotic-resistant E. coli, in the absence of antibiotic use, has occurred following handling of commercially prepared chicken carcasses in the kitchen.

The ability of particular E. coli strains to colonize the intestinal tract both of humans and various species of animals depends on the presence of colonization factors and specific cell surface characteristics common to both, because many of the O-serotypes of E. coli found in poultry, pigs, and calves also have been found in humans.[7,8] Therefore, it is likely these E. coli are from a common pool.

It has been shown that E. coli strains from the alimentary tract of humans and chickens are identical by O, H, K serotyping, by antimicrobial resistance patterns, and by plasmid restriction endonuclease profiles.[7,8] Also, serotype identity among E. coli strains of one specific serotype (02:K1) have been identified commonly in human urinary tract infections and neonatal meningitis and in animal disease (bovine mastitis, and chicken septicemia).

Recently, a group of such strains of both human and animal origin was submitted to clonal analysis by comparison of outer membrane protein (OMP) patterns, lipopolysacehharide patterns, electrophoretic mobilities of enzymes, biotyping, etc.[1] Human isolates were found to fit into two clonal groups, poultry isolates belonging to one and bovine isolates to the other. Human isolates of one clonal group were distinguishable from poultry isolates of the same group by their plasmid content; human isolates of the second clonal group were distinguishable from bovine isolates of that group by a minor alteration in the OMP pattern and by their plasmid pattern. Whether these differences in plasmid pattern (or in the OMP pattern, in the case of bovine isolates) indicate that the populations of human and animal isolates are not overlapping, even though very similar, is unclear.

In view of the exchange of E. coli and Salmonella spp. that can occur between food animals and humans, movement of antimicrobial-resistance genes from the intestinal flora of animals to the flora of humans may occur by carriage of

plasmids and transposons. Such movement of antimicrobial-resistance genes may follow movement to and persistence in the human alimentary tract of the foodborne enteric bacteria or from subsequent conjugative transfer of the plasmid to a resident constituent of the human intestinal flora. Conjugative transfer of R plasmids can be detected in the human intestinal tract in the presence of an antimicrobial that allows an increase in the number of R^+ donor cells and other cells that have received the R plasmid.[2] However, such transfer might not occur commonly in the absence of antimicrobial selection[12] in humans, although it occurs quite efficiently in the rumen of sheep after 24 hours of starvation.[14]

The foregoing suggests that the populations of enteric bacteria of animal and human origin might be considered as a common pool of antimicrobial-resistance genes (transposons, R plasmids, and chromosomal genes) capable of being amplified through antimicrobial exposure and subsequent selection.

DISSEMINATION OF RESISTANCE GENES AND GENOMES

Use of each new antimicrobial agent introduced over the past half-century has caused the emergence and global dissemination of bacterial genes encoding resistance to the agent. Growing prevalence of genes that encode resistance to older agents has prompted development and use of new ones, which have caused succeeding rounds of emergence and spread of new resistance genes. Dissemination of a resistance gene incurs different kinds of costs as it proceeds. When the resistance is not recognized or when optimal medical skills, laboratory services, or newer antimicrobial agents are not promptly available, the health burden is treatment failure with prolonged morbidity or death. When optimal support is available, which is rare everywhere at first and seldom in poorer regions, costs shift towards the expense of the support and the costly new agents and the toxicity of some of the agents.

Recognition of the emergence and spread of resistance and of its costs initially raised fears that the activities encoded by the emerging and spreading resistance genes would exceed our ability to develop new agents. However, nearly all of the target sites in bacteria that are exploited by existing agents were exploited in the first-quarter century of the antimicrobial era. The finding of few new target sites in the second-quarter century suggested that these sites were an unreplenishable resource--one increasingly endangered by proliferating resistance genes that prevented intact antimicrobial molecules from reaching the target sites.

The fear that we would run out of effective antimicrobial agents altogether was greatly diminished in the past decade by the introduction of many new agents that evaded the effects of existing resistance gene products and reached and inhibited the old target sites. Within the past year, however, a number of new resistance genes have been detected in different parts of the world that inactivate many of the largest class of the newer agents, the third-generation cephalosporins.

It was recognized early that use of antimicrobial agents was the major force driving the emergence and dissemination of resistance genes, and that such use should therefore be reduced to its essential minimum. What was not clear earlier, however, was the interrelatedness of what might be called a global system of antimicrobial resistance and the consequent effect of use in one area upon resistance in another. New evidence for this has been developing from both molecular and epidemiological work.

The molecular studies show that resistance genes and the plasmids that carry them constitute intricate assemblages of multifunctional modular components with the size and complexity but not the packaging of viruses. For such a genome to have arisen de novo in a patient or his neighbors in an intensive care unit would be the equivalent of spontaneous generation. Each must have had a lengthy evolutionary history. Studies in molecular biology and molecular genetics are beginning to suggest some of that evolutionary history. Individual resistance genes evolve from ancestral genes, are moved to other genomes or transposons or by site-specific recombination, acquire promoters, become linked to genes under different selection, are transferred on conjugative plasmids to other strains occupying other niches in bacterial ecosystems, and are carried in bacteria to other bacterial habitats. Each such event in the evolution of a resistance gene or its plasmids may initiate a new stage in its dissemination by extending its range or persistence. And the chance of occurrence of each such event would be greatly enhanced by antimicrobial use, which amplifies at every step the prevalence of the gene and its genome and hence the chance that something new will happen to them. Besides showing that resistance genes and their plasmids must have extended lineages, the molecular work is now also beginning to trace some of those lineages. Genetic maps of the large transposon Tn 21 suggest that it carried mercury resistance as well as resistance to several of the early antimicrobials before being included in the first recognized resistance plasmids (in shigellosis in Japan in the late fifties). This transposon has subsequently turned up in plasmids in many parts of the world, including most of the varied plasmids that first brought gentamicin

resistance to German medical centers and plasmids carrying a variety of different β-lactamase genes.

A corollary of the extended lineages of resistance genes and plasmids is that the resistance observed in the bacterial populations of one patient or one medical center is a consequence of prior use of antimicrobials, not just there but in other bacterial populations as well, including others that may have been remote in time and place.

This growing understanding of the interrelatedness of the resistance observed in the world's interconnecting bacterial populations intensifies earlier concerns about antimicrobial agents as animal feed additives. Animals get nearly half of the antimicrobials used in the United States, and the pool of resistance genes and genomes in their flora may be much greater than that in humans. Bacteria of animal origin are not a remote and separate population, moreover, but enter most households continually on slaughtered animal carcasses. If use of antimicrobials in one bacterial population affects prevalence of resistance in other bacterial populations more than slightly, then antimicrobial additives in animal feeds would contribute significantly to resistance in human flora.

Epidemiologic observations in the last few years has added to these examples of specific resistance plasmids that are found in isolates of bacteria from both animals and humans in the United States, and has in some cases reconstructed a path of spread from animal to human. These examples have thus far been observed in isolates of Salmonella spp., thus their elaborate serotyping by a network of medical and veterinary laboratories made them peculiarly traceable and particularly suited at this stage to risk assessment modeling. It should be emphasized, however, that salmonellae are a small part of the aerobic flora in the gastrointestinal tract of animals, and an even smaller part of that of humans; and these salmonellae represent less than 1% of the bacteria against which therapy in humans is directed and in which resistance may be a problem. The concerns outlined above, although now best exemplified by salmonellae, apply to all bacteria that infect humans.

PAUCITY OF DIRECT EVIDENCE

There is little evidence directly linking subtherapeutic use of penicillin and tetracyclines in animal feeds to human infections with pathogenic bacteria. As summarized in Chapter V, there is good evidence from only two studies that non-salmonella enteric organisms in which antimicrobial resistance was induced by the subtherapeutic administration of antimicrobial agents might be spread from animals to humans.[5,6] Two other studies[4,10] failed to show that

multiple-drug-resistant strains of animal origin cause infection in humans exposed to these strains, but it is possible that the power of these studies to detect such infections was too low to make a negative result meaningful.

A number of studies have attempted to demonstrate that outbreaks of drug-resistant salmonellosis in humans may be attributed to the administration of antimicrobial agents in subtherapeutic dosages to farm animals; however, in all but one instance there has been some defect in the proof of the chain of transmission (see Chapter V). A convincing case was an outbreak of infection due to chloramphenicol-resistant S. newport in which infections in humans were attributed to the ingestion of ground beef from animals medicated with chloramphenicol (a drug not approved by regulation for use in beef animals);[15] evidence for identity of the strains through the chain of transmission was based both on the unusual pattern of antimicrobial resistance and on plasmid analysis.

In summary, whereas the theoretical basis for concern that the subtherapeutic administration of antimicrobial agents to animals may foster infections by drug-resistant pathogens in humans is immense, the direct evidence of such an effect is sparse and generally indirect. There are many possible reasons why such an effect might be difficult to detect:

- The degree to which antimicrobial agents are administered for subtherapeutic purposes is generally unknown, varies from farm to farm and from time to time, and is not generally monitored; likewise, the proportion of drugs given for subtherapeutic, as opposed to therapeutic, purposes is not well defined.

- The relative influence of subtherapeutic use (growth-promotional and prophylactic administration) and therapeutic use on the prevalence of drug-resistant strains is not known with certainty and may differ among drugs.

- The prevalence of salmonellae, both susceptible and resistant, in various food products is not routinely monitored, except in special circumstances, such as outbreaks; even in special circumstances, it is generally assessed only retrospectively, when the situation may be quite different from that at the start of the outbreak (when samples of suspect food products are not available).

- Most cases of salmonellosis are unidentified, and in only rare outbreaks is an effort made to identify the source of the infecting organism; no effort to identify a source is made in sporadic cases.

o There are many sporadic cases of salmonellosis which create considerable "background noise" for the investigator attempting to define the chain of transmission of a specific salmonella strain; precise, rapid, and efficient molecular techniques for the identification of unique isolates (i.e., to prove the clonal identity of isolates) have only recently become available.

There is no direct evidence that establishes the proportion of human multiple-drug-resistant salmonellae that is of animal origin or the proportion due to person-to-person transmission. Only a small proportion of multiple-drug-resistant salmonellae in humans occurs as part of a recognized outbreak or epidemic. When such outbreaks have been detected and investigated, CDC surveillance data have indicated that foods of animal origin are implicated in almost 70%.[3]

It is reasonable to speculate that sporadic cases of human salmonellosis caused by multiple drug-resistant salmonellae may occur as part of undetected outbreaks, and still others are undoubtedly of indirect animal origin, resulting from person-to-person spread. There is not an extensive body of data bearing on this issue. Some investigators believe that the number of cases of person-to-person spread of salmonellosis has been underestimated, and that contamination of food products by human carriers, as well as animal sources, of multiple-drug-resistant salmonellae must be considered in the estimates of cases. Some multiple-drug-resistant salmonellae, notably Salmonella wein, (uncommon in the U.S.) have no apparent animal source.

Nevertheless, there is a general parallelism between the prevalence of multiple-drug-resistant salmonellae in animals and in humans, and numerous investigators in the United States believe, therefore, that the majority of human multiple-drug-resistant salmonellae are, directly or indirectly, ultimately of animal origin.

It must be emphasized again, however, that food processing techniques are designed to prevent contamination and transmission of animal pathogens to humans via the food chain. In the majority of outbreaks of multiple-drug-resistant salmonellae in humans, it has been possible to demonstrate flaws or defects in food processing techniques that allowed the contamination with salmonellae. Such defects have nothing to do with whether the salmonellae are fully susceptible to antibiotics or are multiple-drug-resistant. Ultimately, therefore, the farm animal-to-human chain of transmission, of all salmonellae will be interrupted more reliably by careful attention to accepted techniques of food processing and preparation than by any other public measure that could be contemplated.

EFFECT OF DRUG RESISTANCE ON HUMANS

The committee has been asked whether drug resistance of salmonellae caused by subtherapeutic administration of antibiotics in feed causes an increase in the number of cases of salmonellosis in humans or complicates treatment of these cases. These questions are difficult to answer, although they are obviously fundamental to the assessment of risk. Drug-resistant Salmonella spp. infectious to both humans and animals could cause an increase in morbidity in humans in four ways:

o By increasing the overall prevalence of these pathogens (both resistant and susceptible strains) in animals or their food products, could increase the potential for exposure of humans to salmonellosis. Whether or not this increase occurs is unknown. The prevalence of resistant strains in animals might be increased by the subtherapeutic administration of antimicrobial agents and the subsequent suppression of the normal gut flora of the animals; that would be analogous to the "etiologic fraction" in humans. However, the prevalence of susceptible strains might be reduced concomitantly, with an overall effect that is difficult to predict.

o By increasing the virulence of drug-resistant pathogens (see Chapter III). It is unclear whether virulence is increased; some evidence suggests that virulence may be increased, other evidence, possibly less convincing, suggests that the opposite result may occur. To the extent that the epidemiologic behavior of other resistant species is a guide to the effect of the widespread use of antimicrobial agents on the prevalence of pathogens in the environment, it cannot be determined that such resistance will decrease the prevalence or virulence of the resistant species. Overall, the incidence of reported cases of salmonellosis in the United States has continued to rise, slowly but steadily, over recent decades concomitantly with evidence of increasing prevalence of drug resistance in the isolates. Whether or not the increase in reported cases of salmonellosis is related to the subtherapeutic use of antibiotics in animal feed is not clear, of course; but certainly it cannot be said that the incidence of this infection in humans has been decreasing while the subtherapeutic administration of antimicrobials to animals has been steadily increasing. However, many other confounding factors make it difficult to determine the cause-and-effect relation between the subtherapeutic administration of antimicrobials to animals and the increasing number of cases of salmonellosis caused by both susceptible and resistant isolates. Among these

confounding factors is the increasing prevalence of "fast food" in the American diet; these foods are prepared often in large batches wherein a small amount of contaminating bacteria may have a magnified impact.

o By evoking the effect of the "etiologic fraction." Evidence clearly indicates that in some individuals infected with drug-resistant strains of salmonellae the infection is sustained because, prior to infection, they were ingesting antimicrobial agents to which the bacterial strains were resistant. It is believed that these individuals would not have been infected had the strains been susceptible.

o By interfering with the efficacy of antimicrobial treatment. There are some patients infected by drug-resistant strains of nontyphoidal salmonellae for whom antimicrobial treatment is ineffective because the pathogens are resistant. The committee believes that such instances are rare at the present time.

SUBTHERAPEUTIC VS. THERAPEUTIC DOSES

The presence of antimicrobial agents in the environment obviously causes the selection of microorganisms that are resistant to those agents. The clearest example of this phenomenon is seen in the *in vitro* determinations of antimicrobial resistance of isolates of bacteria, which are performed daily in clinical microbiology laboratories. With the rare exception of chromosomally mediated drug resistance, most such resistance is due to transferable resistance factors, or R plasmids. By definition, the minimal inhibitory concentration (MIC) of an antimicrobial agent for a given bacterium is the lowest concentration that inhibits completely the growth of the organism. At sub-MIC concentrations there can still be measurable, dose-dependent growth inhibition that is not complete.

In determining the relative effects on drug resistance of subtherapeutic vs. therapeutic concentrations of antimicrobial agents, several considerations must be evaluated: (1) how often and for how long does the concentration of the drug reach or exceed the MIC? (2) how quickly do the resistant organisms grow during this period? (That is related to evaluating the relative growth advantage of the resistant vs. the susceptible organisms.) (3) at concentrations of drugs below the MIC, is there a dose-related effect on the efficiency of R-plasmid transfer?

Virtually all work to date on drug resistance involves the study of clonally pure single strains of bacteria. Thus, the important issue of spread of drug resistance via R plasmids from resistant to susceptible strains, particularly

of different species, has not been fully explored at a range of drug concentrations that would shed light on the differential efficiency of drug-resistance selection.

It is nevertheless possible to speculate on the effects. Assume the simple case of two strains in the environment at equal inocula; one strain possesses a transferable R plasmid and is drug-resistant, the other strain lacks an R plasmid and is drug-susceptible, but can acquire drug resistance on acquisition of the R-plasmid by conjugative transfer. Consider the effects on the environment of several different concentrations of drugs. At high, super-MIC, drug levels, only the resistant strain survives. There is a net increase in drug resistance, as a consequence of continued growth of the resistant strain, but there is no spread of resistance. All resistance increase is from clonal expansion. At low enough drug levels (i.e., sub-MIC) there is no selective effect of the antibiotic. At MIC (bacteriostatic, but not bactericidal), there is selection and expansion not only of the original R-plasmid-containing strain, but also of the relatively rare R-plasmid conjugative recipient. Under this condition, the diversity of drug-resistant bacteria (i.e., two different strains), as well as the extent of resistance, is increasing.

Although these conditions have been neither adequately modeled for potential analysis nor appropriately tested in an in vitro situation that would reflect actual forces in vivo, the theoretical considerations raise concern that subtherapeutic concentrations of drugs may be doing as much harm as therapeutic concentrations, if not more, particularly in view of their more continuous use. Veterinary studies discussed above lend credence to this concern.

A complete chain of direct evidence linking human disease caused by multiple-drug-resistant organisms to subtherapeutic use of penicillin and the tetracyclines in animal feeds does not exist. Such evidence as does exist is limited to outbreaks of multiple-drug-resistant salmonellosis. Conclusive direct evidence of such a linkage would include full characterization of the infecting salmonellae based on available techniques of plasmid analysis; epidemiologic evidence implicating a particular food; isolation of the infecting organism from the implicated food and proof of its clonal identity; epidemiologic evidence linking the contaminated food with a particular farm service; isolation of the infecting organism from the implicated animals or poultry with proof of its clonal identity; and documentation of the subtherapeutic use of penicillins or tetracyclines in feeds consumed by the implicated animals or poultry. It might still be argued that multiple-drug-resistant salmonellae were present before subtherapeutic use of penicillins or tetracyclines was initiated, but most scientists would accept the outlined chain of epidemiologic

and microbiologic evidence as providing direct and conclusive proof of a cause-and-effect association.

In only one outbreak, reported by Spika et al.,[15] was this chain of transmission fully documented; the antibiotic used in this instance was not penicillin or tetracycline, but rather chloramphenicol. The use of chloramphenicol as a feed additive has never been approved by FDA in the United States, although in this instance it was used therapeutically. All other reported outbreaks that implicated multiple-drug-resistant salmonellae from an animal or farm source fall short in their provision of evidence that conclusively links the source of the drug-resistant organism with subtherapeutic use of antibiotics in animal feeds. Those reports did not document fully the chain of transmission, prove identity of the infectious salmonellae with those from the implicated farm source, and document the type or amount of antibiotic used in the animal feeds. The last has been particularly difficult to ascertain in most of the disease outbreaks, i.e., to establish retrospectively the precise antibiotics or the amount used in feed.

Thus, the studies of outbreaks of multiple-drug-resistant salmonellosis in humans, although they are the best evidence available, have not provided direct evidence of the human health risks due to subtherapeutic use of penicillin or the tetracyclines in animal feeds.

LOSS OF DRUG RESISTANCE

Upon cessation of drug use, there should be a measurable and continuous decline in the concentration of drug in the environment. At a point at which this concentration is significantly below the MIC of the susceptible strains, those strains should manifest a growth advantage over otherwise identical bacteria that in addition possess R plasmids. This advantage should be in direct relationship to the amount of diverted energy and raw materials the cell needs to keep the R plasmid on board (i.e., new DNA, RNA, and protein synthesis) and may be subtle. In sharp contrast to the drug-loaded environment, where the effect of the drug on the population of susceptible bacteria is seen within hours or days (because of the enormous growth advantage of the resistant bacteria), the effect of antimicrobial removal may take months or even years to be manifested fully. The more subtle the growth advantage of susceptible bacteria in the drug-free environment, the longer the period before the outgrowth of susceptible (i.e., R-plasmid-free) bacteria can be seen. For these reasons any analysis of the effects of drug removal from the environment must be extended past the immediate postwithdrawal period.

The prospective CDC studies of salmonellosis in selected urban and rural counties showed that the overall frequency of resistance to one or more antimicrobials had increased from 16% to 24% between 1979 and 1984.[3,9] However, in one serotype, S. heidelberg, the frequency of resistance declined from 67% to 35% during the 5-year period. Poultry was a common reservoir of S. heidelberg; from 1979 to 1983, CDC reported 69% of the nonhuman isolates of S. heidelberg from this source. By the late 1970s, most poultry producers had stopped using penicillin and tetracylines as growth-enhancers; for 1979-1982, only 4% of broiler-chicken producers were reported as using low doses of the tetracylines in feed. The decline of resistance in this salmonella serotype associated temporally with the decrease in use of penicillin and the tetracylines as growth-enhancers suggests that decreased antimicrobial resistance might follow reduced use of these drugs in subtherapeutic dosages. However, whether the use of penicillin and the tetracylines for disease prevention also decreased during 1979-1982 is unclear. The number of isolates of S. heidelberg studied was small; a much larger group of isolates should be examined to establish the validity of this interesting preliminary observation.

In practice, the indications for the subtherapeutic use of antimicrobials for disease prevention appears to the committee to be interpreted broadly. The goal of such use might be to halt the spread of overt disease that has appeared in a few members of a herd. It appears to be used at certain periods in the rearing of farm animals when they are considered to be particularly vulnerable to various infections (e.g., shipping-fever complex when cattle are moved into feedlots, and respiratory diseases in pigs). Often, subtherapeutic dosages are employed in feed for long periods without clear indications. In the case of swine, they are used regularly at specific stages of production: starter, grower, lactation, breeding, and gestation. Some farmers may be using antimicrobial-containing feeds without being aware of it. Mixing procedures may be such that the concentrations achieved may exceed those targeted.

Although distinctions have been made between use of subtherapeutic doses of antimicrobials in feed for growth promotion and for disease prevention the value of distinguishing between these two uses is rendered uncertain by many aspects of current practice. It seems most reasonable, therefore, to continue to categorize both uses as subtherapeutic, as they are currently viewed by the FDA. Better defined guidelines for use of subtherapeutic concentrations of antimicrobials for disease prevention would be of benefit.

FOREIGN EXPERIENCE IN BANNING ANTIBIOTICS AS FEED ADDITIVES

The Swann committee report of 1969 in England addressed the issue of feed antibiotics (subtherapeutic use) and their effects on the selection of strains of bacteria resistant to antimicrobial drugs.[13] It recommended that all antimicrobials used in humans be prohibited from use for growth promotion in animals. It also stated that antimicrobial drugs used for humans could be used in treating animals for disease or prophylactic indications when prescribed by a veterinarian.

In subsequent years, the central veterinary laboratory regularly conducted antibiotic-susceptibility testing on strains of salmonellae submitted to it. Its intention was to determine if the Swann committee mandates influenced the antimicrobial susceptibility patterns. It collected data on trends of drug-resistance patterns over the years 1972, 1974, 1976, 1977, 1984, 1985, and 1986. Two major observations were based on these data. First, resistance patterns persisted throughout the period; rarely was there any decrease. Second, one group of related phage types of *S. typhimurium* (204C was the predominant type) appeared in calves in 1979. These strains are multiple-drug-resistant and are responsible for the increase in resistance patterns detected during this period. In 1985, 204C constituted 62% of salmonella strains isolated from cattle. Almost all strains (more than 89%) were resistant to tetracyclines, ampicillin (and related penicillins), trimethoprim, and chloramphenicol. Resistance to gentamicin has gradually increased. *S. dublin* strains during these surveys were the second most common isolates after *S. typhimurium*. Most *S. dublin* were isolated from cattle.

In 1985, less than 1% were resistant to tetracycline, ampicillin, trimethoprim-sulfonamide, and chloramphenicol. Streptomycin and sulfonamide resistance was more common--66.7% and 28.6%, respectively. Most other salmonella serotypes isolated from cattle were susceptible to these antibiotics.

In poultry, selected antimicrobial drugs demonstrated failure to inhibit growth of salmonellae; 24% of strains were resistant to streptomycin, 8% to tetracycline, 72.8% to sulfonamide at 50 μg, and 11% at 500 μg sulfonamide, and 0.8% to chloramphenicol.

It is clear that the phage type 204C of *S. typhimurium* is an example of an unusual strain that can periodically cause epizootics. In 1964-1965, an outbreak of *S. typhimurium* phage type 29 occurred in cattle. By 1969, this epidemic was largely over. That strain may have been selected through antibiotic pressure. The persistence of it and phage type 204C appears to be related to biologic

properties that permit intestinal colonization and ability to induce disease. Strain 204C has the propensity to acquire plasmids. It probably became a problem in calves because of multiple exposures associated with the many times when these animals were transported from broker to broker. The great mobility of calves among brokers was different from the situation in the years before the Swann committee recommendations.

The use of antibiotics in animal husbandry in England has not decreased, but rather has continued to increase. This increase is due to prophylactic and therapeutic uses. Penicillin and the tetracyclines continue to be the most widely used drugs. This fact suggests that they have not lost their effectiveness for treating animal diseases. Although nonprescription uses of antimicrobials have been documented by the British Veterinary Association, the higher concentrations of antimicrobials in prescription-authorized therapeutic and prophylactic uses are blamed for selecting resistant strains of salmonellae and other bacteria in animals. The short life span of the food animals and the apparent rapid decline in the number of resistant strains once the antimicrobial drugs are withdrawn are thought to be relatively effective barriers to a more widespread dissemination of these potential pathogens.

The incidence of salmonellosis in humans in England has shown a persistent yearly increase from 1970 to 1980. In the subsequent four years, the incidence appeared to increase rapidly presumably because of increased numbers of S. typhimurium cases. During these years other serotypes appeared, increased to a peak, and then usually subsided to low numbers. Reasons for these variations are unknown. The isolates of S. typhimurium phage type 204C from humans have not had the same high incidence of resistance to antimicrobial drugs as those from animals. In 1985, 207 human isolates were tested and more than 92% were susceptible; in the same laboratory, of 1,050 bovine isolates, only about 20% were susceptible. The 207 human isolates represented 4% of all S. typhimurium isolated in 1985, while 50% of the bovine strains were of phage type 204C. Strain 204C may be in the food chain, but it has not evolved in the same fashion as have the bovine strains; it is less common and has not developed the same high incidence of resistance to antimicrobial drugs.

The data indicate that the Swann committee recommendations have not had a significant effect on the number of resistant strains of salmonellae. This may be an unfair assessment, because there is no organized data base from before the recommendations with which to compare data collected later. Furthermore, some changes in agricultural practices have occurred which have enhanced the spread of salmonellae.

Although annual mortality rates in humans associated with salmonellosis in England were not available to the committee, there were reports of deaths in various outbreaks, but details were not obtained. However, there is no evidence of an increasing mortality rate, as might be anticipated with an increasing incidence of infections.

THE RISK MODEL

The committee learned that a similar risk model had been used by the National Resources Defense Council (NRDC) in its petition dated 20 November 1984 to the Secretary of Health and Human Services asking for suspension of the approval of the new animal drug applications for subtherapeutic use of penicillin and the tetracyclines in animal feeds.[16] The NRDC alleged that the use of these drugs presented an imminent hazard to the public health. The committee's risk model and the parameter estimates used in it are summarized in Table VIII-1. The NRDC used "best estimates," while the committee used three estimates: low, mid-range, and high and applied these to five distinct parameters in the risk model. In comparison, the NRDC estimate of the number of deaths per year due to salmonellosis associated with subtherapeutic use of penicillin and the tetracyclines was 116 deaths, that corresponds to this committee's mid-range estimates in Table IX-1 of 30 deaths--a rather similar result in the face of so much uncertainty. The second NRDC estimate, 264 deaths per year, is based on a different method that starts from an estimated 1,000 deaths per year due to salmonellosis, a figure we believe to be too high.

The Food and Drug Administration (FDA), a constituent agency of the Department of Health and Human Services (DHHS), carefully analyzed the NRDC petition and recommended that the Secretary of DHHS deny the petition on the grounds that an "imminent hazard" had not been demonstrated.[17] The petition was in fact denied. The FDA's analysis concluded that the NRDC had not shown in its petition that antibiotic resistance caused by the subtherapeutic use of penicillin or the tetracyclines in animal feed had a significant impact on the outcome of a significant number of cases of salmonellosis and thus, that no "imminent hazard" had been demonstrated.

The FDA's analysis first discussed the difficulty of treating infections by resistant salmonellae. It did not accept arguments about increased difficulty in treatment, because most infections with salmonellae are uncomplicated and resolve without treatment (so "antibiotic treatment is not recommended in patients with the uncomplicated diarrheal type of salmonellosis," and for those cases occurring outside the intestine the drug of choice is chloramphenicol, to which

only about 0.7% of salmonellae are resistant and for which alternative drug therapies exist).

The FDA also concluded that the data then available did not demonstrate any alteration in virulence and contended that some salmonella deaths (such as from heart attacks caused by dehydration and stress due to salmonellosis) are unrelated to antibiotic therapy.

The FDA then considered what we call the "etiologic fraction," as well as inappropriate therapy for infections not at first recognized as salmonellosis, and concluded that neither had been shown to present a major problem.

Finally, the FDA commented on the NRDC parameters (see Table IX-1) and took special issue with the estimated death rate of 4.2%, on the grounds that it was subject to a number of potential biases and limitations, including lack of documentation that salmonellosis was the primary cause of the reported deaths. (The largest difference between our mid-range estimate and the NRDC estimates is in the death rate. We queried the CDC, as noted above, and found that some deaths were indeed not due to salmonellosis and that others were questionable; we reduced our mid-range estimate accordingly). The FDA also concluded that the NRDC's estimate of 69% of resistant salmonellae traceable to animal sources was based on a very limited sample and that such deaths caused by subtherapeutic use of penicillin or the tetracyclines (estimated by NRDC as 50%) could not be estimated accurately from the available data. The committee has considered these objections carefully, in light of advances in scientific understanding since 1984 and the whole body of data available at the time that it worked on this matter (the first half of 1988). The committee has not tried to judge the merits of either the NRDC's petition or the FDA's response. The committee believes, however, that some estimates can be made, as shown in Figures VIII-1 through VIII-12. These estimates are still highly uncertain, as indicated in the figures themselves.

EVIDENCE SUGGESTING THE PRESENCE OF HAZARD

The estimates presented here have wide margins of possible error, as reflected in the ranges from the 5th to 95th percentiles (percentile is the scriptor for fraction of estimates falling below it and are not confidence limits) in Figures VIII-1 through VIII-12. This is a direct reflection of the compounding of estimates of component factors that themselves have substantial ranges from the lowest plausible to the highest plausible estimate. If our model is to be adopted for future use, we urge that the responsible authorities promote the appropriate research to produce the data needed to narrow each of the ranges of estimates shown