Chapter IV

RISKS

RISKS

INFECTIOUS DISEASES

Antibacterial-Related Risks

The increasing pool of drug-resistant bacterial pathogens is the greatest health risk posed by the widespread use of antibacterialsin animal feeds. It is also the most difficult to evaluate in terms of:

1. quantifying the pool of drug-resistant bacterial pathogens,
2. assessing the relative contribution to the pool from the use of antibacterial in animal feeds, and
3. determining causality between the use of antibacterial in animal feeds and human disease.

Hazards to the food animals themselves from such use of antibacterial are closely related to these human health concerns because the development of resistant pathogens and subsequent failure or diminished effectiveness of antibacterial therapy are problems for both human and animal health. However, this sharing of potential, long-term, adverse consequences is obscured by the predicted short-term consequences for the livestock industries of limiting the subtherapeutic uses of certain antibacterial.

On the other hand, the long-term consequences for these industries might be significantly different. With limited use, although the supply of certain food animals could diminish, shifts in supply and demand for different foods (primarily beef, pork, and chicken) may produce little change in total income for producers and modest rises in consumer prices (USDA, 1978; Headley, 1978).

The threat to human health has been the primary reason why current efforts at limiting widespread use are underway. But such widespread use poses an identical threat to the health of livestock and poultry and may even occur earlier and more visibly than the threat to human health. Present production is concentrated in high-volume, crowded, stressful environments, made possible in part by the routine use of antibacterial in feed. Thus the current dependency on low-level use of antibacterial to increase or maintain production, while of immediate benefit, also could be the Achilles' heel of present production methods.

Adverse effects on human health are possible from contact with treated livestock or the antibacterial themselves and through eating food products containing residues of animal drugs. Illness can result from poor management and sanitation practices in violation of standards, but this report is not concerned with such causes except when the circumstances show that prevention is not possible through standard-setting and compliance-monitoring. Nor is this report concerned with all disease acquired from ingesting contaminated foods but only when: (1) drug residues represent the threat or (z) antibacterial in the feed result in disease or threatened disease from antibacterial-resistant bacteria.

Human disease from contact with treated livestock or with the antibacterial(s) itself is more significant for building a case for the general risk of such uses than it is for concluding that direct disease transmission is presently a significant problem, That is, disease transmitted directly from food animals chronically fed antibacterial-supplemented feed is not of epidemic proportions, and isolated cases or limited epidemics have been so infrequently detected that they are newsworthy items even in the scientific community, But taken together with the growing num-

ber of research findings of asymptomatic spread of drug-resistant organisms from food animals to humans, they are significant in documenting the risk to human health from drug-resistant pathogenic bacteria.

Risks From Infectious Diseases

A large body of literature shows that certain infectious diseases are transmitted directly from animals to humans. Such diseases are not limited to bacterial etiologies but cover the whole spectrum of infections—e.g., tapeworms, trichinosis, psittacosis, tuberculosis, etc. Some diseases are occupational hazards in the meat industry and carry common names such as "pork infection," "swine erysipelas," and "fish poisoning."

While the causal chain between food animals or their edible products and disease in humans may not be completely demonstrated in any given case, each step in the chain has been documented repeatedly. With the added criterion that the infectious agent in the animal or its edible product be shown to be caused by antibacterial supplements in the feed, the causal chain is even more difficult to prove in any specific case. Thus there is disagreement over the interpretation of any specific case but no real disagreement over the overall conclusions that food animals are the source of some infections in humans and that the use of antibacterial in feeds is one cause of the growing pool of drug-resistant pathogenic bacteria. Instead, the disagreement is over the exact risk from this enlarging pool and the contribution of antibacterial in animal feeds to the problem.

The proliferation of antibacterial-resistant bacteria is encouraged by the presence of such drugs. Sensitive bacteria are killed or inhibited, allowing resistant strains or spontaneous mutations of sensitive-to-resistant bacteria to grow into the vacated environment. The situation is complicated because some resistant strains can transfer the resistant genes to other bacteria. Antibacterial resistance (and other properties of bacteria) are sometimes carried on bits of DNA that function independently of the organism's chromosomes. These extrachromosomal pieces of DNA are called plasmids.

Resistance plasmids (R-plasmids) may code both for antibacterial resistance and for the ability to transfer to other bacteria, or the two functions may be separate on different plasmids. Resistance to multiple antibacterial is frequently found on an R-plasmid. As in the case of single resistance, multiple resistance may or may not be transferable depending on whether the transfer code is associated with the resistance code—i. e., resistance to a specific antibacterial may be transferred alone or along with resistance to one or more other antibacterial.

Gram-negative and gram-positive bacteria also differ in plasmid-mediated transfer. R-plasmid transfer has not been found to occur between these two types of bacteria. The R-plasmid of gram-positives are not so freely transferred as those in gram-negative species, nor are linked, multiple resistances so frequently found in them.

For any antibacterial there may be: (1) no known plasmid-mediated transfer of resistance in either gram-positive or gram-negative bacteria, (z) transferred resistance only in one bacteria type but not in the other, or (3) varying degrees of linkages with other antibacterial resistances, For example: (a) bambermycin has no known effect on gram-positive or gram-negative bacterial resistance patterns; (b) tylosin and virginiamycin select for erythromycin-resistant staphylococci and streptococci (gram positives), but their effect on the resistance patterns of gram-negatives is essentially nonexistent; and (c) tetracycline selects for resistance not only to itself but also for resistance to other antibacterial linked to it in both gram-positive and gram-negative bacteria (Falkow, 1978).

Resistance can be transferred from nonpathogenic as well as from pathogenic bacteria. Although resistant nonpathogenic bacteria will not cause disease, they may be able to transfer antibacterial resistance to bacteria that can cause disease but which were previously responsive to antibacterial therapy. Thus a growing pool of plasmid-mediated resistance in nonpathogenic bacteria, even though of no direct clinical significance, poses a large threat because of the transfer of that resistance to pathogenic bacteria.

Plasmids have also been identified where drug resistance and pathogenicity are linked. Since the first reports on drug resistance and enteropathogenicity linkage in swine, similar occurrences have been found in human toxigenic E. *coli*. In E. *coli* that cause diarrheal disease by the production of an exotoxin, it has been shown that the intestinal toxin involved is often encoded by plasmids (Ent-plasmids) that can be transferred from strain to strain (Smith and Linggood, 1972; Gyles et al., 1974; So et al., 1975). A second plasmid-coded gene product, the K-antigen, which enables the organism to adhere to the wall of the intestine, is also required for pathogenicity of the organism by exotoxins. Although the K-antigen shows some species specificity, the Ent-plasmid does not.

The incidence of R-plasmids is very high among enterotoxigenic *E. coli* strains for both animals and humans (Gyles et al., 1974). The coexistence of Ent- and R-plasmids within the same cells raises the possibility of recombination between the plasmids, translocation, or independent cotransfer. Independent cotransfer has been shown *in vitro*. In an E. *coli* strain responsible for a hospital epidemic of infantile diarrhea, one plasmid was associated with the production of heat-stable enterotoxin, and another plasmid determined drug resistance against seven antibacterial. When the R-plasmid was transferred, the Ent-plasmid was also transferred to 36 percent of the drug-resistant recipients (Wachsmuth et al., 1976).

E. *coli* isolated from piglets with diarrhea have been found with Ent- and R-plasmids combined, presumably by recombination or translocation. These plasmids were conjugative (transfer by direct contact between bacterial cells) and determined the production of enterotoxin and resistance to multiple antibacterial (tetracycline, sulfonamide, and streptomycin) (Gyles et al., 1977). In addition, a conjugative plasmid encoding a K-antigen has also been found to carry resistance genes (So et al., 1976). These findings portend the possibility of a complete plasmid in the sense of combining conjugative, enterotoxigenic (Ent), adhesive (K), and resistance (R) properties in one package for transfer to completely nonpathogenic, gram-negative bacteria.

Humans and other animals are hosts to many of the same bacteria. It is now widely accepted that E. *coli*, some of the most ubiquitous gram-negative bacteria, are not composed of stably differentiated strains that are specific colonizers or pathogenic for separate animal species. Cross-colonization studies, serotyping, drug-resistance patterns, and plasmid types show extensive overlapping sets of human and animal organisms (Linton et al., 1977a, 1977b; Howe et al., 1976) Cross-colonization may be enhanced by plasmid-mediated factors. Colicin is a substance that kills E. *coli* except for the type producing it, thus giving competitive advantage to the producer. Oral administration of two colicin-positive bovine E. *coli* strains resulted in 100-percent replacement of the resident coliform flora in humans, whereas *colicin-negative* derivatives of these two strains were unable to colonize the same humans (Smith and Huggins, 1976).

Conjugative, colicin-positive plasmids carrying resistance genes are known to exist (Delhalle and Gratia, 1976). The potential therefore exists for animal feeding of antibacterial to cause a direct increase in the pathogenicity of E. *coli* in humans.

A single *Salmonella* serotype, S. *typhimurium*, is the most common cause of Salmonella infection in both animals and humans (CDC Salmonella Summary Report, 1973). Six of the most common human serotypes were among the ten most common animal serotypes in a recent CDC survey (CDC Report, 1974). In addition to shared serotypes between animals and humans, plasmids from E. *coli* can be transferred to Salmonella and other gram-negative bacteria.

In Connecticut in 1976, S. Heidelberg from calves infected humans, who in turn secondarily infected three infants, The organism was resistant to six antibacterial, E, *coli* with identical resistance patterns were isolated from two infected calves and from one human, This particular resistance pattern was not seen in over 10,000 pathogenic strains of E. *coli* isolated from the Northeastern United States in the same year, including 42 strains isolated from controls in the study (Cohen et al., 1977).

In a sampling of E. *coli* from a freshwater river system and within the saltwater bay into which it emptied: (a) nearly all the freshwater sites and about half of the saltwater sites sampled contained antibacterial-resistant coliforms, and (b) 20 percent of the 194 strains tested contained R-plasmids carrying multiple antibacterial resistance transferable to sensitive E. *coli*, Salmonella *typhimurium*, and *Shigella dysenteriae* (Feary, et al., 1972),

DNA base sequence homology studies of plasmids from different parts of the world have shown striking compatibility of plasmids. Plasmids with molecular weights of 57 million, one isolated from a bovine S. *typhimurium* in England in 1972, and the other from a human S. *typhi* in Vietnam in 1974, showed 100-percent homology. Table 30 summarizes these results (Anderson, et al., 1975).

Finally, a more serious occurrence than transferred resistance between *E. coli* and Salmonella has recently appeared. R-plasmids determining resistance in newly discovered ampicillin-resistant strains of *H. influenza* (Elwell et al., 1975) and in penicillin-resistant strains of *N.* gonorrhea (Elwell et al., 1977) are identical to plasmids previously found in E. *coli*. It must be assumed that these identical plasmids have a common origin.

Magnitude of the Risk

What proportion of antibacterial resistance is caused by subtherapeutic use in food animals as opposed to therapeutic use in both animals and humans is unknown. Thus this risk is not yet possible to fully quantify. For some aspects of the problem, such as the degree of compromise in treating Salmonella infection, it is possible to arrive at some quantitative notion of the magnitude of human risk. But for the overall risk to humans from increased antibacterial resistance, not only is it unclear what the final deleterious event should be whose frequency would be estimated, but there are also complicated interactions among human and animal populations with which we must contend.

As a rule the transmission of Salmonella infection is from animals to man, and the Salmonella reservoir in animals is considered the direct source of most of the Salmonella infections in humans (Sickenga, 1964). The antibacterial-mediated reservoir of resistant *E. coli* in animals provides a source of R-plasmids that can transfer to Salmonella.

A continuous increase in antibacterial resistance has been noted among Salmonella isolated from farm animals. There has been a dramatic increase in resistance to antibacte-

Table 30[a].—Homology Between Plasmids of Animal and Human Origin

(The values indicate the degree of reassociation at 75 °C of 3H labeled plasmid DNA with unlabeled plasmid DNA relative to the reassociation both with DNA of the same plasmid (= 100) and with *E coli* K 12 chromosomal DNA (=0))

Unlabeled DNA from strains bearing plasmids			Labeled plasmid DNA											
	Human (H) or animal (A)	Plasmid	FII			I,		N		H,			H,	
Group	origin	no	240	R1.19K.	TP1 66	TP102	Δ	TP120	TP1 58	TP123	TP153	TP15>	TP1 16	TP167
FII	H	240	100	57	64	14	13	0	0	3	—	4	o	3
	H	R1 -19K.	-	100	93	—	—	—	—	17	—	—	—	—
	A	TP166	64	85	100	9	10	10	–	17	6	5	9	4
I,	H	TP102	13	–	11	100	75	7	4	2	—	3	—	—
	A	Δ	15	–	11	65	100	1	0	—	—	4	o	o
N	H	TP120	1	—	4	0	1	100	94	5	—	0	0	2
	A	TP158	1	—	3	1	0	101	100	10	–	–	–	—
H,	H	TP123	10	17	24	0	0	9	6	100	91	91	8	o
	H	TP153	–	6	12	–	—	—	3	94	100	9S	5	0
	H	TP163	10	6	9	1	0	8	4	88	96	82	7	5
	A	TP1 71	–	—	—	—	—	—	—		100		—	—
	A	TP154	6	0	8	0	0	7	12	87	92	100	o	o
H,	H	TP1 16	2	—	—	—	0	8	—	4	0	—	100	65
	A	TP167	–	—	—	—	—	—	—	—	o	—	81	100

Note—The broken line encloses reactions of labelled plasmid DNA with unlabeled plasmids from the same compatibility group
—, Not done

[a]Anderson et al., J @n Microbiol 91 376.362, 1975. table 3

rials in human Salmonella infections, A review of several studies of human infections conducted over a period of years indicates that resistance of *Salmonella* to tetracycline has increased continuously from l-percent resistant organisms prior to 1948 to more than 40-percent resistance in 1973.[1] A comparison of antibacterial resistance in *Salmonella* isolated from hospitals in 1967 and 1975 conducted by the Department of Health, Education, and Welfare's (HEW) Center for Disease Control showed overall resistance to at least one antibacterial increased from 41.1 to 69.4 percent, Multiple resistance to six or more antibacterial increased from 0,8 to 9.2 percent (table 31).

FDA estimated that there are 2.5 million cases of Salmonella infection in the United States each year; about 30 percent were severe enough to be seen by a physician, and approximately 1 percent of these develop life-threatening septicemia where appropriate antibacterial therapy is critical. In 27 percent of the cases treated, the first antibacterial chosen for treatment proves to be ineffective because the disease was caused by antibacterial-resistant bacteria.[2] Thus there are currently about 2,500,000 x 0.3 x 0.01 x 0.27 = 2,025 Americans who annually contract a life-threatening *Salmonella* infection that requires antibacterial treatment and for whom treatment is compromised to some extent by antibacterial resistance of the infecting Salmonella strain. Additionally, there is an even larger number of people with nonsystemic infections who are treated with antibacterial and for whom treatment is also compromised.

Table 31. –Antibiotic Resistance in *Salmonella* Isolated From Hospitalized Patients

	1967 400 *strains*	1975 754 *strains*
Resistance to one or more antibiotics		
S typhimurium	41 1%	69 4%
Other serotypes	15870	**43** 9%
All strains	22 2%	**49** 7%
Resistance to two or more antibiotics	15 0% (60 strains)	**26 5%** **(200** strains)
Resistance to six or more antibiotics	**O 80/0** **(3** strains)	9 2% **(69** strains)

SO URCE 42 F R 56272 Oct 2 ' 1977

All Salmonella infections cannot be ascribed to antibacterial use in food animals, but the risk is also not a static situation. If the risk were static, the lifetime risk of contracting systemic Salmonella infection and the subsequent treatment being compromised by antibacterial resistance could be approximated as (2,025 people/year x **70** years)-200,000,000 = l/1,411, But in view of the rapid rise in multiple resistance, it must be assumed that both the degree and extent to which treatment is compromised are increasing at a rapid rate.

The risk from resistance plasmids of animal origin is not quantifiable even by the rough estimates made for Salmonella infections. The majority of resistance in human bacterial populations is probably caused by widespread use of antibacterial in humans (some of which is unnecessary), but the enormous pool of R-plasmids as now exists in animals, together with the ability of an R-plasmid to be promiscuously transferred among bacterial species, must be regarded as a threat to the therapeutic value of antibacterial in the treatment of both human and animal diseases.

In assessing the risks to humans from the use of antibacterial in animals, the cumulative nature of these risks cannot be overlooked nor the importance of understanding the time rate of change of these risks. Although penicillin and tetracycline have both been used for over 25 years in animal feeds without seriously compromising the effectiveness of these drugs in the treatment of human disease, it cannot be assumed that there will be no problems in the future. Both the acquiring of resistance in animals and the passing of resistance from animals to humans are cumulative processes, and perhaps the point has not been reached, but will be at some future time, where significant deleterious effects will be observed in humans.

[1] **42** F.R. 56272, Oct. 21, 1977.
[2] Ibid.

Types of Risk

Monitoring of drug residues focuses on the direct harm to humans from consumption of edible animal byproducts. There are two types of risks that are addressed: carcinogenic residues and residues with other health effects. As discussed earlier, there can be no residue of carcinogenic substances, with "no residue" determined by a method prescribed or approved of by the Secretary of HEW. Criteria by which the acceptability of a method will be judged have not been finalized, but the Food and Drug Administration (FDA) continues to proceed with regulations that would avoid the problem of always "chasing zero" that results from the increasing ability to measure extremely minute amounts of substances. There are approved drugs that are regulated on a "no residue" basis because they are known or suspect carcinogens. There are official methods for these drugs, although there is disagreement on whether or not these methods are adequate when reviewed by current scientific standards.

Residues of noncarcinogenic drugs do not have to meet the "no residue" requirement applicable to carcinogens but are governed by safety factors as described in the law. These factors include the probable consumption of the drug and of any substance formed in or on food, the cumulative effect, other safety factors deduced by scientific experts from animal experimentation data, and whether the conditions of use are reasonably certain to be followed in practice.[3] In practice, FDA sets specific tolerances for residues based on these safety factors and the availability of a practical analytical method to determine the quantity of residue. Chronic studies are required to support a finite tolerance. Acute toxicity studies of 90 days' duration are minimally required for a negligible tolerance. If it is determined that negligible residues will probably not occur, no tolerance is required. And if the drug may be metabolized and/or assimilated in such form that any possible residue would be indistinguishable from normal tissue constituents, no tolerance is required.[4]

The treatment of carcinogens differs from that of noncarcinogens in that: (1) if finite residues are present, carcinogens are banned and noncarcinogens are not, the latter contingent on establishment of a tolerance, and (2) if it is not possible to determine whether residues will be present (a) for carcinogens, the manufacturer fails the burden-of-proof test for safety and the drug is not approved or withdrawn, whereas (b) for noncarcinogens, negligible tolerances or no tolerances are set, based on a showing that residues are expected to be below a level of potential toxicological significance. The judgments are not made without toxicological data. For carcinogens, even this distinction is somewhat artificial because in the case of either measurable or unmeasurable residues, the drug is not approved or withdrawn. FDA's current attempt to extrapolate from animal test data to man, so that "no residue" would be defined by risk, would be one method of regulating carcinogens on a more rational basis.

Noncarcinogenic Risks

Tolerances for noncarcinogenic drug residues are determined by the general criteria for safety enumerated earlier and by the requirement that the residue level cannot be set higher than that reflected by the permitted use of the drug. When a specified level of residue is determined to be safe through toxicological data, a withdrawal period prior to slaughter of the animal may be required before the drug can be approved. Most approved drugs require a withdrawal period only because they are approved on a negligible-tolerance basis instead of on a finite-tolerance basis, Because the safety factor applied to establish a negligible as opposed to a finite tolerance is very large, withdrawal periods are necessary for residues to deplete below-tolerance levels. The withdrawal period for a specific drug may vary for different animal species or production classes and also may

'21 U.S.C. 360 b[d]2].

'21 CFR 556.1.

vary depending on its combination with other drugs.

Sulfamethazine residues in swine have caused the greatest problem in this area, with tissue residues in excess of the 0.1 ppm limitation averaging 13.1 percent of the samples tested in the latter half of 1977. As explained earlier, more than half of these violations were probably caused by contamination of the withdrawal feed. If so, then increasing the withdrawal time will have little effect on violation rates without parallel action in decreasing cross-contamination of feeds,

FDA subsequently did increase the withdrawal period for all uses of sulfamethazine to 15 days and was nearing completion of a proposal to set action levels for cross-contamination at the end of 1978. Prior to this action, the withdrawal period had been 5 days when in combination with tylosin and 7 days in combination with penicillin and tetracycline. These withdrawal periods had been established prior to new regulations issued in 1975 that established a 10-day withdrawal period for sulfonamides not already subject to regulation.' The 10-day period was set because the judgment was made at that time that 10 days would probably be adequate to assure that residues would be below 0.1 ppm and because of the degree of thyroid response to sulfonamide drugs,

Other sulfonamides have not been affected by the new withdrawal period. The withdrawal period for sulfathiazole, also used with tetracycline and penicillin in swine feed, remains at 7 days.[6] Sulfaethoxypyridazine is used for therapeutic purposes in swine and cattle for use by or on the order of a licensed veterinarian. The withdrawal period remains 10 days.' Sulfamerazine is used in trout, with a withdrawal period of 3 weeks. [8]

Residue violations from other antibacterial have not been significant. Most of the residue problems result from therapeutic and not from feed-supplement use. The incidence of violations for some antibacterial is summarized in table 32.

[5]21 CFR 510.450.
"21 CFR 558.155,
[7]21 CFR 558.579.
[8]21 CFR 558.582.

Antibacterial residues were previously considered important in the development of antibacterial-resistant bacteria because of ingestion by humans, but this is now considered the least likely contributor. However, the evidence that violative residues of sulfamethazine were caused largely by contamination of the withdrawal feed may bring a new perspective to this issue, As previously discussed, the contribution of antibacterial-supplemented feed to the growth of drug-resistant bacteria comes primarily from selection and promotion of resistant strains of the micro-organisms in animals, not humans. So antibacterial residues in edible animal products are the wrong indicator of this potential problem if the level of such residues does not reflect dependably the antibacterial contamination.

Cross-contamination of feeds may also be occurring for other antibacterial, particularly penicillin and tetracycline, because they are widely used and mixing is not limited to certified feed mills or under the direction of a licensed veterinarian. The sulfamethazine problem was detected because contamination led to violative tissue residues. For other antibacterials, cross-contamination may be occurring but may not be reflected in increased concentrations of tissue residues. Thus, reliance on tissue residues as an indicator of cross-contamination of feed may not be appropriate, and direct monitoring of supposedly antibacterial-free feeds would have to be undertaken to eliminate cross-contamination as a possible significant contributor to the development of drug-resistant bacteria. A limited amount of this feed monitoring is presently conducted by FDA.

Carcinogenic Risks

General Considerations

Current reliance is on testing in small animals for both cause and effect and quantitative extrapolation to humans, All substances demonstrated to be carcinogenic in animals are regarded as potential human carcinogens. No clear distinctions exist between those that cause cancer in laboratory animals and those that cause it in humans. However, the accurateness of extrapolation from

Table 32.—Incidence of Violations Among Different Antibiotics in Kidneys From Food Animals, Food Safety and Quality Service, Residue Monitoring Program, 1973-77

Year and specie	Samples analyzed	Total violations		Penicillin		Streptomycin		Neomycin		Tetracycline		Chlortetracycline		Oxytetracycline		Erythromycin		Unidentified microbial inhibitor	
	No.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
1973:																			
Steers/heife s	0	0	0	0	o	0	0	0	o	0	o	o	o	0	o	0	o	0	0
Cows	1,594	44	2.8	3	o 2	31	1.9	7	o 4	0	o	o	o	0	o	0	o	3	1.9
Calves . . .	1,889	152	8.0	5	o 3	45	2.4	24	3	7	o 4	o	o	5	o 3	2	o	64	3.4
Swine . . .	834	15	1.8	0	o	2	0.2	0	o		1	o	o	0	o	0	o	4	0.5
Chickens .	665	4	0.6	0	o	0	0	0	o	o	o	o	o	0	o	0	o	4	0.6
Turkeys . .	176	1	0.6	0	o	0	0	0	o	o	o	o	o	0	o	0	o	1	0.6
1974:																			
Steers/heife s.	35	o	0	o	o	0	o	0	o	0	o	o	o	o	o	o	o	0	o
Cows	1,301		1.2		o 1	12	o 9	1	o 1	0	o		o	o	o	o	o	0	o
Calves . . .	2,849	94	3.3	7	o 1	3	2	7	o 6	7	o 6	o	o	o	o		o 3	21	o 7
Swine . . .	292	7	2.4		o 3		0	0	o	1	o 3	o	o	o	o	o	o	2	o 7
Chickens . :.	296	2	0.7	o	o	o	o	0	o	o	o	o	o	o	o	o	o		o 7
Turkeys . .	218	2	0.9	o	o	o	o	0	o		o 5	o	o	o	o	o	o		o 5
1975:																			
Steers/heifers. . .	222	0	0	0	0	0	o	0	0	0	0	o	o	o	0	o	o	0	o
Cows	236	5	2.1	0	0	2	o 8	1	0.4	0	0	o	o	o	0		o 4	1	o 4
Calves	2,13	155	7.3	5	0.2	2	3	58	2.7	29	1 4		o o4		0 04	o	o	33	5
Swine	15	4	2.7	0	0	o	o	0	0	2	1 3	o	o	o	0	o	o	2	3
Chickens	17	5	2.8	0	0	o	o 5	0	0	4	2 3	o	o	o	0	o	o	0	o
Turkeys	49	17	3.5	1	0.2		o 2	5	o	6	2	o	o	o	0	o	o	4	o 8
976:																			
Steers/he ıe s	187	0	0	o	o	0	0	0	0	0	o	o	o	o	0	o	o	0	o
Cows . . .	353	9	2.5		o 3	6	1.7	0	0	0	o	o	o	o	0	o	o	2	o 6
Calves	1,378	88	6.4	o	o	22	1.6	44	3.2	9	o 7	o	o		0 07	o	o	2	o 9
Swine	247	3	1.2		o 4	1	0.4	0	0	0	o	o	o	o	0	o	o	1	o 4
Chickens . .	155	1	0.6	o	o	0	0	0	0	0	o	o	o	o	0	o	o	1	o 6
Turkeys . . .	258	0	0	o	o	0	0	0	0	0	o	o	o	o	0	o	o	0	o
977:[a]																			
Steers/heifers.	198	1	0.5	0	o	0	o	0	o	1	o 5	o	o	o	o	o	o	o	o
Cows	755	17	2.3	1	o 1	8		4	o 5	3	o 4	o	o	o	o	o	o		o 1
Calves	566	28	4.9	2	o 4	1		9	6	5	o 9	o	o	o	o	o	o		o 2
Swine	211	2	0.9	0	o	1	o	0	o	1	o 5	o	o	o	o	o	o	o	o
Chickens	177	0	0	0	o	0	o	0	o	o	o	o	o	o	o	o	o	o	o
Turkeys	204	2	1.0	0	o	0	o	0	o		o 5	o	o	o	o	o	o		o 5

[a] 1977 data are from January to June
SOURCE: USDA, 1978, table 3.

laboratory animal results to humans to quantify the effect is less certain (Cancer Testing Technology and Saccharin, 1977).

Short-term tests are developing as aids in evaluating the potential of substances to cause cancer. Short-term tests are based on the presumption that cancer is related to cellular DNA changes and that detection of such changes is predictive for a substance's potential carcinogenicity. These tests examine the capacity of a substance to cause mutations or other genetic alterations. Several hundred known animal carcinogens and noncarcinogens have been tested in the *Salmonella*/Ames test, which at this time is the most extensively studied short-term test. About 90 percent of the known carcinogens are positive in this test, in contrast to positive results in about 10 percent of substances that are not carcinogenic in animal tests (McCann et al., 1975; Purchase et al., 1976; Sugimura et al., 1976).

Typically, animal experiments use on the order of 100 animals at each experimental dose. If a particular experimental dose causes a lifetime increase in cancer risk of 1/10, this increase can be measured with a small degree of accuracy using 100 animals. If background or spontaneous carcinogenesis is present, even larger numbers of animals will be required. On the other hand, the extra human risk that we may want to estimate resulting from environmental exposure is usually much smaller than 1/100 for any given chemical, perhaps on the order of $1/10^6$. Clearly, it would not be practical to conduct an experiment with enough animals to measure directly an increase in risk this small.

For these reasons the procedure has been developed of conducting lifetime animal-feeding experiments using, in addition to a control dose of zero, several doses at which the projected extra cancer risk may be 1/10 or larger. The high-dose data are used to estimate a dose where the risk may be no larger than, say, $1/10^6$. That is, the high-dose data is used to estimate the risk at dose levels considerably below a level at which effects could be discerned from practical experimental feeding studies. An equally important variant to this problem is the calculation of the so-called "safe" dose, for which there is some measure of statistical assurance that the extra risk at that dose is no more than, say, $1/10^6$. These problems are often referred to collectively in the literature as the "low dose extrapolation problem.

The most common animals used are rats and mice, and these species, as well as different inbred strains of them, often vary in their sensitivity to the substance being tested. Also, the number of animals used in these experiments is a compromise between large enough numbers of them to detect positive effects and the costs and time of conducting these experiments. Rats and mice live for 2 to 3 years, and when this time is added to the time needed to set up the experiment, examine tissues, write up the results, etc. a typical experiment takes about 4 years. And statistically speaking, the law of probabilities tells us that positive results cannot be expected all of the time even when the substance being tested is carcinogenic.

For these reasons, when both positive and negative results are obtained in different experiments and there is no known reason for the discrepancy, more weight is given to the positive results. Scientists would agree that statistically positive results obtained in at least two animal species by appropriate tests are reasonably conclusive evidence that the substance is likely to be a human carcinogen. Clearly positive results in one valid and appropriate animal species are also considered by a majority of scientists to be a sufficient basis for labeling a substance a carcinogen. In addition, short-term tests may be helpful in predicting that a substance is genotoxic and may, therefore, aid in the identification of a substance's carcinogenic potential.

Carcinogens may act in a variety of ways, ranging from a genotoxic interaction of the agent with the cell genome to the enhancement of the expression of tumorigenesis initiated by other known or unknown agents. Science is progressing rapidly in the elucidation of the mechanisms of carcinogenic action, but it is seldom possible at this stage to be certain by what mechanisms an individual agent acts.

The concept of a threshold below which a carcinogen may be ineffective has been the subject of debate. It is not possible to determine by experiment whether such a threshold exists because of the vast numbers of animals and the consequently large facilities and reservoir of trained personnel required. Nevertheless, there is substantial evidence that the lower the exposure to a carcinogen, the lower the risk of developing cancer. This established fact is the justification for attempts to extrapolate from the effects of carcinogens at high doses to their postulated effects at much lower doses. And even if threshold issues were resolved—i. e., for a given substance there is or is not a threshold—how to determine the threshold with a high degree of confidence would remain as a major issue.

In the absence of contrary data, it is prudent in extrapolating from the results of animal experiments to humans to give the most weight to the results of the most sensitive animal experiments. The general rationale is to err on the side of safety. Laboratory animals are deliberately inbred to have uniform characteristics so that confounding factors relating to individual animal variability are minimized within a specific experiment. Generally, these experiments attempt to introduce only one variable—the substance to be tested—so that causality can be deduced between it and the resulting carcinogenic effect.

When the risks from animal experiments are extrapolated to expected incidence in humans, the results are usually expressed in risks per lifetime exposure, the usual exposure period of present animal tests. Yet lifetime exposure is not a necessary precondition for carcinogenesis, since even single exposures to potent carcinogens can produce cancer. Lifetime exposure is intended to elicit the maximum response to a particular concentration of the tested substance.

Lifetime exposure to large doses by experimental animals and the use of these findings to extrapolate to low doses in humans are often misunderstood. The usual misunderstanding is to equate the concentrations used in the experiments with that consumed by humans. For example, in announcing the results of positive carcinogenic tests on saccharin and its intention to ban it as a food additive, the initial press release from FDA made the statement that "The dosages of saccharin fed the rats in the Canadian study were in excess of the amount that a consumer would receive from drinking eight hundred (800) 12 oz. diet sodas daily over a lifetime" (FDA Press Release, Mar. 9, 1977).

These misunderstandings leave the impression that animal experiments predict unrealistically high carcinogenic effects in humans. Yet these experiments are conducted in carefully controlled conditions where other carcinogens are not present, in contrast to the conditions of human exposure. There is a rough similarity between (I) the correlation of experimental conditions with environmental exposure to which humans are subject and (2) the correlation of experimental with field results on the effectiveness of antibacterial for growth promotion and feed efficiency in food animals. In the latter, the quantitative effect in the field is greater than under controlled, experimental conditions, though the precise mechanisms are not known. Perhaps a similar result might be hypothesized for carcinogenic effects, but at the minimum, the conditions are not so radically different that in carcinogenic testing the opposite result should be expected. That is, there is no strong argument that animal data overstate the risk to humans.

Quantification of Risk

It is not scientifically possible to determine the slope of the carcinogenesis dose-response curve for any carcinogen at low exposure levels. Therefore, performing a low-dose extrapolation involves the choice of a mathematical function to model the dose-carcinogenic response relationship and the choice of statistical procedures to apply to the mathematical function. The choice for this mathematical function turns out to be extremely crucial to the outcome of low-dose risk estimation. If the assumed relationship between tumor occurrence and dose does not apply in the regions to which the extrapolation is being made, a serious overestimate of the "safe" dose may result (Mantel and Bryan, 1961). For example, a comparison of five standard dose-re-

sponse models showed that they could differ by many orders of magnitude at low dose levels for which extra risks are on the order of $1/10^6$ (Chand and Heel, 1974).

It is theoretically possible to discriminate among the various potential dose-response functions on the basis of experimental data; however, two different dose-response functions can often fit experimental data equally well but still differ by several orders of magnitude at very low doses. Moreover, even if a particular dose-response function were to give a significantly better fit to data than several others, this would still not furnish assurances that this function would necessarily correlate in any way with the true dose response at very low doses where it is not feasible to measure the true extra risk directly. As a consequence of the great disparity of dose-response functions at low doses, the dose-response function should reflect known or at least plausible information regarding the biological mechanisms through which a chemical induces or promotes cancer and not solely on the basis of how well it can be made to fit "experimental data. "

For genotoxic carcinogens probably the substance itself or a metabolize acts directly at the cellular level and produces a heritable change that eventually leads to tumor formation (Crump et al., 1977). Carcinogens that are carcinogenic by reason of their mutagenicity should fall into this category. Therefore, carcinogens that test positively in the *Salmonella*/Ames mutagenicity screening test are very likely to be genotoxic. As 90 percent of the known carcinogens tested have been found to be mutagenic, most known carcinogens are probably genotoxic.

A partial solution to the low-dose extrapolation problem for the case of genotoxic chemical carcinogens has been given (Crump et al., 1976; Guess and Crump, 1976; Pete, 1977). The key result is that, at least as long as background carcinogenesis is present, the dose-response curve should not be expected to be absolutely flat at zero dose. What this means is simply that when risk is plotted against dose response on ordinary linear scales, the tangent line to the dose-response curve at zero dose should have a positive slope. When a dose-response function has this property, it is *linear* at *low* dose. This simple property can have far-reaching consequences on low-dose extrapolation. For example, consider the two potential dose-response functions (1) $0.1\ [(99/999) \times d + (900/999) \times d^2]$ and $0.1 \times d^2$ for the dose interval $0 \leq d \leq 3$. Both of these curves give a risk of 1/10 at a dose of $d = 1$ and are practicably indistinguishable at higher doses. However, at a dose of $d = 1/10^3$ (1) predicts a risk of $1/10^5$ and (2) predicts a risk of $1/10^7$, a difference of two orders of magnitude.

One explanation of why the dose-response function should be linear at low dose when background is present is that the cellular mechanism through which the test agent produces cancer should already be operative in producing background tumors. When this is true, the effect of the test agent is to add to an already ongoing process (Crump et al., 1976; Pete, 1977). If background carcinogenesis is allowed for by positing an effective background dose, the wide range of risks obtained using different models effectively disappears. This does not imply that the dose-response curve is not expected to be linear at low dose in the absence of background carcinogenesis (Crumpet al., 1976; Watson, 1977).

The evidence for low-dose linearity given above applies mainly to genotoxic carcinogens. A nongenotoxic carcinogen might cause some gross physiological change such as suppression of ovulation, which could predispose the subject to cancer. For such carcinogens the shape of the dose-response curve at low dose is highly speculative. There could possibly be a threshold dose below which the agent has no carcinogenic effect at all on an individual. However, even if a threshold mechanism is operative, there is likely to be considerable variation in individual thresholds in a large population. Consequently, the dose-response curve for the entire population could still exhibit a linear trend at risks as low as $1/10^6$ or lower,

The effects of metabolic activation and detoxification on carcinogenic dose response have been recently considered through a kinetic model that encompasses free toxic substance, metabolize, deactivator, and the inter-

actions of these substances (Cornfield, 1977). Only a steady state situation is studied in that variation over time of the concentrations of these agents is not considered. The model predicts a threshold dose below which there is no carcinogenic risk under the assumption that the deactivator is 100 percent efficient in deactivating the carcinogen. However, in a naturally occurring process it is likely that deactivation would not be perfect and would be less than 100 percent effective in always combining with 100 percent of the carcinogen before an amount of the active metabolize reaches a cancer target site. Any of a number of modifications to the model to allow for nonperfect deactivation would rule out a threshold and would lead directly to a model for which carcinogenic response varies linearly with dose at low doses. Cornfield's own modification of perfect deactivation, that of allowing the deactivating reaction to be reversible, leads, as Cornfield points out, to a model that is linear at low dose. This occurs regardless of how slowly the reverse reaction takes place, as long as the possibility is not eliminated entirely. Furthermore, even in the extremely unlikely case of perfect deactivation, an otherwise realistic model should still imply low-dose linearity, since the theoretical time required for perfect deactivation would not be zero and would likely be infinite.

For most, perhaps all, carcinogens, the mechanisms through which cancer is produced are not sufficiently understood so that the shape of the carcinogenic response curve can be predicted with certainty. As pointed out earlier, experiments of sufficient size cannot be conducted that would permit direct experimental investigation of the dose-response curve at low doses. There are plausible arguments that the dose-response curve is linear at low dose for many carcinogens. In view of these uncertainties, it would seem reasonable to base estimates of added risk of cancer on a mathematical model that encompasses low-dose linearity unless the mechanism through which the carcinogen operates is sufficiently understood so that low-dose linearity can be conclusively ruled out. Once the principle of low-dose linearity is accepted, the problem of estimation of risks at low doses is nearly solved. This is because the disagreement between the upper statistical confidence bounds on risk at low doses based on a model that incorporates low-dose linearity, and one that does not is typically several orders of magnitude; whereas the corresponding disagreement between two reasonable models, both of which incorporate low-dose linearity, is usually much less than this.

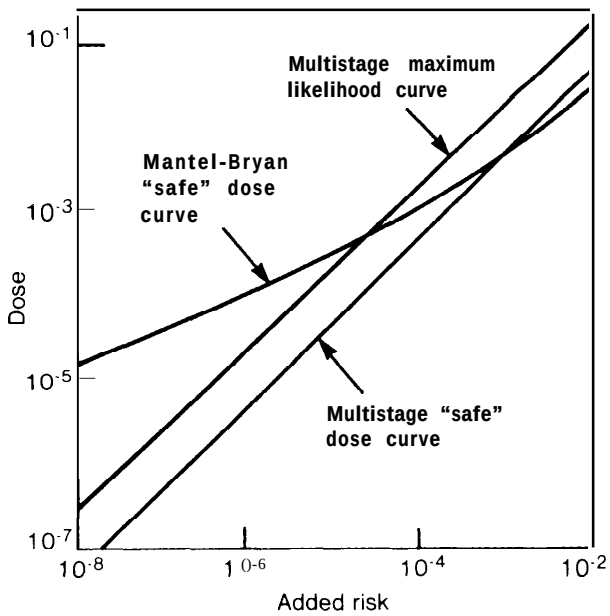
The new procedures and criteria for evaluating the assays for carcinogenic residues in edible products of animals that FDA is attempting to implement (see chapter II) originally adopted the Mantel-Bryan mathematical model (Mantel and Bryan, 1961; Mantel et al., 1975) to quantify the residue level corresponding to a risk of $1/10^6$ in test animals. This residue level, or S_0, is expressed as a fraction of the total diet—i.e., in parts per billion (ppb). This level is adjusted to account for the respective portion of the human diet that is represented by the various food products containing residues of the carcinogen being tested, the transfer from animals to man being made on a fraction-of-total-diet basis. The resulting dose level, S_m, is "the level of total residue of carcinogenic concern that can be operationally defined as satisfying the no-residue requirement of the Act for specific tissues. "[9] The dose level S_m represents the upper bound to the lowest limit of reliable measurement that an approved assay method must satisfy. The specific mathematical model chosen is thus an integral part of defining "no residue. "

Crump (1978) discusses the Mantel-Bryan model, simple linear extrapolation models to low doses (Heel et al., 1975; Brown, 1976), and multistage dose-response models (Guess and Crump, 1976, 1978; Crump et al., 1977; Hartley and Sielkin, 1977). In the Mantel-Bryan procedure, the mathematical model used for the dose-response model is what is termed the "probit" function. The Mantel-Bryan procedure, as was to be applied in the FDA regulations, rules out linearity at low dose in favor of a "flatness property" at low doses. This results in "safe" dose estimates that are considerably higher than those obtained using the multistage model because it

[9] 42 F.R. 10422, Feb. 22, 1977.

assumes away linearity at low dose, an assumption that is probably unwarranted for the majority of carcinogens, which appear to be genotoxic. Figure 1 illustrates this difference. Note that for the data reflected in

Figure 1.—Comparisons of "Safe" Doses Computed From the Mantel-Bryan Procedure and From a Procedure Based On the Multistage Model[a]



Source Crump K S 1977 Response to OpenQuery Theoretical Problems in the Modified Mantel. Bryan Procedure *Biometrics* 33 752 755

this graph, the Mantel-Bryan "safe" dose lies above the multistage "safe" dose at values of added risk below 5×10^{-4}, When the Mantel-Bryan method predicts that a cancer risk is no greater than $1/10^{6}$ the true risk could easily be one or two orders of magnitude higher, or between $1/10^{4}$ and $1/10^{5}$ (Crump, 1978).

FDA has now proposed that linear extrapolation be used, rather than the Mantel-Bryan procedure, because, among other reasons, linear extrapolation is least likely to underestimate the risk.

Since extrapolation based on the multistage model will often be linear at low doses, the question arises as to how different the result will be from simple linear extrapolation. For some data the difference will be minimal, but for other data sets the difference could be considerable, For example, from DES data in C3H female mice (Gass et al, 1964), the "safe" dose based on linear extrapolation is lower than the "safe" dose based on the multistage model by a factor of about five and there are doubtless cases where the difference could be greater than an order of magnitude (10 x).

Diethylstilbestrol (DES)

DES has been shown to be carcinogenic in both animals and humans. The association between the use of DES by women during pregnancy and the appearance of clear-cell adenocarcinoma of the vagina or cervix in their exposed daughters was recently reviewed by a task force at the National Institutes of Health (DES Task Force Report, 1978). The main conclusions were as follows:

1. **DES daughters.** A clear association between in *utero* exposure to DES and clear-cell adenocarcinoma of the vagina or cervix is established. Estimates are that the incidence will be between 0.14 to 1.4 per 1,000 through age 24 among the exposed daughters. Cancers of this type and histological sites were almost unknown in women of that age group prior to this discovery. (The eventual incidence as these women grow older obviously is unknown.)
2. DES mothers. A relationship between DES during pregnancy and risk of cancer in the mothers is unproved. However, existing studies indicate that this population, like others exposed to high levels of estrogens, may in the future develop excessive incidence of specific tumors.
3. DES sons. Until recently, DES effects on exposed sons had not been reported. Recent studies clearly show an excess of genital abnormalities in these individuals. As yet, there is no definitive information on the fertility implications of these findings nor firm evidence of an association between DES exposure in sons and an increased risk of testicular cancer.

The animal data currently available on carcinogenic dose response to DES consists primarily of mice data (Gass et al., 1964; Gass et al., 1974), although new experiments at the

[a] 44 F.R. 17070, Mar. 20, 1979.

National Center for Toxicological Research in Jefferson, Ark., are nearing completion. DES was given in the diet beginning from 4 to 6 weeks after birth and continued throughout their lives. (Note that this is less than maximum lifetime exposure, so the cancer response might have been greater.) The 1964 micedata are summarizedin table 33.

Crump (1978) analyzed these data sets according to specified methods for analyzing animal carcinogenicity data (Crump et al., 1977) to estimate the risks to mice at dose levels comparable to those encountered by humans through DES residues in beef. Because suppression of appetite at the two highest doses was reported, the data at 500 ppb and 1,000 ppb were omitted from analysis.

The average food intake of Americans is about 24 lbs a week (Riedman, 1971), about 2.3 lbs of which is beef (CAST report, 1977). The lowest limits of reliable measurement of the FDA-approved mouse uterine method for measuring DES residuesis 2 ppb. The concentration of DES residues in liver is about 10 times that in beef muscle (U.S. Congress, 1971). Tested livers cannot exceed 2 ppb; otherwise DES would be detected and the present "no residue" test would be violated. Thus the average DES residue in beef muscle mightbe 0.2 ppb. This gives an average dose of DES to Americans from DES residues in beef muscle to be:

$$\frac{\text{(2.3 lbs a week)}}{\text{(24 lbs a week)}} \times \text{(0.2 ppb)} = 0.02 \text{ ppb}$$

Table 34 summarizes the estimates of extra cancer risks at the dose of 0.02 ppb based on applying the multistage model and related statistical theory to these mice data. The most sensitive mice data predict a risk of I/13,000, and the least sensitive a risk of 1/82,000. As explained earlier, when the Mantel-Bryan model predicts a risk of $1/10^6$, the true risk could easily be between $1/10^4$ and $1/10^5$, or within the same range as summarized in table 34.

Assuming that the population at risk is 200 million people, lifetime exposure to DES in meats at 0.02 ppb would result in 15,385 extra cancers as derived from the most-sensitive mice strain (200 million x 1/13,000), and 3,390 or 2,439 extra cancers as derived from the less-sensitive mice results from table 34. These estimates should be compared with 200 extra cancers, which would be the "no residue" level of the $1/10^6$ target risk from the proposed FDA regulation.

Table 35 summarizes the doses derived from these same experimental data that result in an added carcinogen response of $1/10^6$, the target "no-residue" level of the proposed FDA regulation. In contrast to the 0.02 ppb estimate exposure to DES from present consumption of food, these doses are in the range of 0.001 to 0.0003 ppb, or 1/20 to 3/200 the estimated exposure.

The evidence for DES's carcinogenicity points to a nongenotoxic mechanism, so an effect at low doses can be disputed (Weisburger, 1977). In addition, the response might be

Table 33. –Occurrence and Latent Period of Mammary Carcinoma in Mice Fed Varying Concentrations of DES in the Diet (Gass et al., 1964)

	C3H females			*C3H males*			*Strain A castrate males*		
DES in diet ppb[a]	*No. of mice*	*Percent with tumors*	*Latent period in weeks*	*No. of mice*	*Percent with tumors*	*Latent period in weeks*	*No. of mice*	*Percent with tumors*	*Latent period in weeks*
0	121	330	49.12	115	0	—	136	0	—
6.25	56	482	49.96	59	0	—	78	0	—
125	60	433	46.57	58	17	–	78	1.3	6200
25	60	433	51.07	62	0	—	70	2 9	48,50
50	68	529	45.19	62	4 8	66.00	77	3.9	69,66
100	64	656	42.19	60	5.0	4467	74	8.1	61.33
500	59	847	30.66	60	38.3	3995	52	13,5	5400
1,000	58	86.2	31.40	71	42.3	36.03	76	19.7	4780

[a]Mice consumed approximately 2 5 to 36 g Of food per day animals receiving the two highest concentrations consumed slightly less due to estrogenic suppression of appetite ppb = parts per billion
SOURCE Crump 1978

Table 34.–Extra Risk of Mammary Tumors at a Dose of 0.02 ppb (Mice Data, Gass et al., 1964)

M/cc *strain*	*Most l/kc/y estimate*	*Upper 57 50/0 confidence bound*
C 3H females	1 / 13,000	118000
C3H males	1 82 000	1 /47 000
Str al n A castrate males	1 /59.000	1 /37 000

[illegible]

Table 35.–Estimates of Dosage (ppb) of DES Required To Effect an Added Carcinogen Response of 1/108 (Mice Data, Gass et al., 1964)

Mice *strai* "	*Most l/kc/y estimate*	*Lower* **97** *5%* *confidence bound*
C3H females	0 **000258**	**0000162**
C3H ma es	O 00164	0000944
Strain **A** castrate males	0170117	0000748

[illegible]

largely limited to females. However, in both animal experiments and from what is known about DES effects in humans: (1) cancer is known to occur even in the absence of continuous DES stimulation, and (2) effects in males have been observed, The rough estimates for the number of extra cases expected in humans are for lifetime exposure risks. If DES has a carcinogenic effect at low doses, these estimates would not be overstating the ef feet.

Nitrofurans

In 1964, in the course of conducting toxicity studies, scientists at the University of Wisconsin discovered that a substantial number of mammary tumors had developed in rats fed nitrofurazone. Subsequent studies in 1966 and 1967 showed that animals fed nitrofurans had significantly higher incidence of tumors, Since that time, Norwich Pharmacal Company has conducted four chronic toxicity studies to assess the tumorigenic and carcinogenic effects of one of these nitrofurans, furazolidone. In all of these studies, the experiments were started when the animals were about 2 months of age, and three of these studies fed furazolidone for a limited period, followed by a furazolidone-free diet until the experiment was terminated. A more pronounced carcinogenic effect might have been observed if the doses had been continued throughout the experiment.

Brief descriptions of these experiments follow:

1. **The High-Dose Sprague-Dawley Rat Study** .[11]—Four **hundred** Sprague-Dawley **rats approximately 2 months of** age were divided into four groups of 50 male and 50 female rats each. The diet of the four groups contained furazolidone in the feed in the amounts of O ppm, 250 ppm, 500 ppm, and 1,000 ppm for approximately 18 months. All groups were then maintained on a furazolidone-free diet until mortality in each group reached 90 percent, at which time the remaining animals were sacrificed.
2. The Fischer Rat Study .'[z]—This study was performed identically to the High-Dose Sprague-Dawley Rat Study except that Fischer 344 rats were used instead of Sprague-Dawley rats.
3. The Low-Dose **Sprague-Dawley** Rat Study .[13]—Three hundred and twenty Sprague-Dawley rats approximately 2 months old were divided into four groups of 40 male and 40 female rats each. The diet of the four groups contained furazolidone in the feed in the amounts of O ppm, 17.6 ppm, 87,9 ppm, and 264.4 ppm. These are average amounts since the concentrations of furazolidone in the diet were increased as the animals continued to grow. The animals were treated continuously until the experiment was terminated after 2 years.
4. The Mouse Study .[14]—Four hundred Swiss MBR1ICR mice approximately 2 months of age were divided into four groups of 50 male and 50 female mice each. The diets of the four groups contained furazolidone in the feed in the amounts of O ppm, 75 ppm, 150 ppm, and 300 ppm for approximately 13 months.

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[11] "Tumorigenesis Evaluation of NF- 180 in Sprague-Dawley and Fischer Rats, Part **1.** Sprague-Dawley Evaluation. " Nov. 9, 1973, Project No. **475.091),**

[12] "**Tumorigenesis** Evaluation of NF-180 in Sprague-Dawley and Fischer Rats, Part II, Fischer 344 Evaluation, " Jan. 31, 1974, Project No. 475.09D.

[13] "Chronic Toxicopathologic Safety Study [two years] of NF-180 in Rats," Nov. 9, 1973, Project No. 475.09C.

[14] "Tumorigenesis Evaluation [twenty-three months] of Furazolidone [NF-180] in If ice. Jan. 31, 1974, Project No. 475.09E.

The four groups were then maintained on a furazolidone-free diet for 10 additional months, at which time the experiment was terminated and the surviving animals were sacrificed.

Table 36 summarizes the tumorigenic and carcinogenic findings. There is a high rate of spontaneous tumors in all four groups. The mice results are the most sensitive. Although exposed to the lowest concentrations of furazolidone, they developed the greatest percentages of tumors, particularly when malignant tumors were separated from nonmalignant ones.

The results of various statistical tests performed on the data are given in table 37. A chi-square goodness-of-fit test of no carcinogenic-dose-related effect is significant at the 0.01 level of significance for four of the data sets. More importantly, a test of no dose-related effect versus the alternative of a one-stage effect (a multistage model) is significant at the 0.01 level for six of the data sets including all four of the data sets for mice. Thus furazolidone had a statistically significant effect in mice. A chi-square goodness-of-fit test for compatibility with the one-stage model of carcinogenesis was significant at the 0.05 level in only 2 of the 16 data sets. The two data sets for which significance was found were quite anomalous and would likely not be compatible with any dose-response function for which the risk increases with increasing dose.

Table 36.–Summary of Tumorigenic and Carcinogenic Results From Four Experiments With Furazolidone (N F-180) (Data is presented in the form "no. responders/no animals tested")

	Dose (ppm)	All neoplasms		Malignant neoplasms	
		Males	females	Males	Females
High-dose Sprague-Dawley Rat Study	0	29/50	44/99	10/50	11/49
	250	33/49	46/50	12/49	6/50
	500	35/50	48/50	1 5/50	12/50
	1,000	40/49	45/50	1 3/49	13/50
Fischer 344 Rat Study	0	48/49	39/49	1 5/49	14/49
	250	49/50	46/50	2/50	10/50
	500	45/50	50/50	1 5/50	11/50
	1,000	44/49	45/50	13/49	16/50
Low-dose Sprague-Dawley Rat Study	0	21/34	26/34	3/34	7/34
	176	1 3/34	24/35	5/34	11/35
	879	17/35	29/33	9/35	9/33
	2644	23/32	33/35	6/32	12/35
Mice Study	0	25/49	35/50	21/49	32/50
	75	30148	35/50	26/48	28/50
	150	36/50	40/47	32/50	37/47
	300	46/51	42/48	43/51	40/48

SOURCE Crump 1978

Table 37.–Levels of Significance of Various Goodness-of-Fit Tests Performed on Data in Table 36

Test 1 Chi-square goodness-of-fit test (3 d, f.) of no dose-related effect.
Test 2 Test of the hypothesis no dose-related effect versus the alternative hypothesis of a one-stage model (Crump, Guess, and Deal, 1977)
Test 3 Chi-square goodness-of-fit test (2 d.f) of a one-stage model

		Test 1	Test 2	Test 3
		High-dose Sprague -Dawley rats		
All neoplasms	Males	31	< 01	92
	Females	64	49	47
Malignant neoplasms	Males	73	.21	64
	Females	38	.18	25
		Fischer rats		
All neoplasms	Males	13	.50	06
	Females.	< 01	.05	01
Malignant neoplasms	Males	<01	28	<,01
	Females	48	.26	37
		Low-dose Sprague -Dawley rats		
All neoplasms	Males ,	*03*	*.03*	*07*
	Females.	03	< 01	47
Malignant neoplasms	Males	29	16	23
	Females	61	17	61
		Swiss MBR1IBR mice		
All neoplasms	Males	< 01	< 01	*72*
	Females	*05*	< 01	47
Malignant neoplasms	Males	< 01	< 01	76
	Females	.01	<01	14

SOURCE Crump 1978

Before using these data to estimate extra risks for furazolidone residues, it is first necessary to assess the level of furazolidone residue likely to occur in food products from animals exposed to furazolidone. In 1971 it was announced by FDA that a method for measuring residues of furazolidone would be required that would reliably measure residues as low as 2 ppb. The FDA concluded in 1976 that there was at that time no method available for reliably measuring residues of 2 ppb.[15] Thus there currently is no way to know if food products from animals treated with furazolidone do not have at least 2 ppb in them.

Table 38 presents estimates of extra risk at a dose of 2 ppb based on the rodent data in table 36. Since these estimates are all very

[15] 41 F.R. 19919, May 13, 1976.

Table 38. –Estimates of Extra Risk From a Dose of Two Parts Per Billion of Furazolidone Using the Data in Table 36

		Most likely estimate of extra risk	*Upper 97 5% confidence limits for extra risk*
		High-dose Sprague -Dawley rats	
All neoplasms	Males	1/1 **500000**	1 /760 000
	Females	1 /375 000,000	1/4 800000
Malignant neoplasms	Males	1/6 900,000	1/1 **900,000**
	Females	1/6 900000	1/2 100,000
		Fischer rats	
All neoplasms	Males	0	1/5 500,000
Malignant neoplasms	Females	1/9,400 *000*	1**/2,200,000**
		Low-dose Sprague -Dawley rats	
All neoplasms	Males	1**/490,000**	1/21 **0000**
	Females	1 /260,000	1/130,000
Malignant neoplasms	Males	1/1 **300000**	1**/430,000**
	Females	1/1300 000	1/390 000
		Swiss MBR1IBR mice	
All neoplasms	Males	1/**200,000**	1**/** 120,000
	Females	1 /470 000	1 1230,000
Malignant neoplasms	Males	1 /220,000	1/130000
	Females	1 /420,000	1 /21 0000

SOURCE: Crump, 1978

nearly linear with dose at risks below 1 percent, risk estimates for other doses can be determined from the table by simply multiplying by the appropriate factor. For example, to compute risks at 20 ppb, multiply the results in table 38 by 10.

The risk estimates in table 38 are based on the statistical procedures of Crump et al. (1977) associated with a multistage model. To obtain the "most likely estimates, " the particular multistage model was selected (Guess and Crump, 1976) that maximized the likelihood of the data. Risk estimates are not given for the Fischer rats for "females, all neoplasm," nor for "males, malignant neoplasm," because these data are not consistent with the multistage model. For the most sensitive result—namely, in the mice—the extra risk at a dose of 2 ppb furazolidone exceeds the proposed regulatory "no residue" risk level of $1/10^6$ by two to five times.

It should be noted that the estimates of risk are higher for the mice and low-dose rats than for the high-dose rats because the high-dose rats had lower or approximately equivalent tumor rates as rats and mice in the other experiments. High-dose rats were fed 250 ppm, 500 ppm, or 1,000 ppm furazolidone. Low-dose rats were fed approximately 17.6 ppm, 87.9 ppm, or 264.4 ppm furazolidone; and the mice were fed 75 ppm, 150 ppm, or 300 ppm furazolidone. When extrapolated to extra risks from a dose of 2 ppb furazolidone (table 38), the low-dose experiments result in higher incidence of tumors than the high-dose experiments.

Two ppb may be the residue level in meat, but it is not the dose to which humans are exposed. These risks can be translated at low doses for mice into comparable risks for humans in the following way: Furazolidone is used extensively in chickens and turkeys and for limited periods in swine, In the estimates of effects from banning selected antibacterial, banning nitrofurans (of which furazolidone is one) was estimated to have an effect on chickens and turkeys but not on pork. (See table 23.) Thus, human exposure from meat consumption comes from chickens and turkeys.

For DES, Americans were assumed to consume an average of 2,3 lbs of beef a week and 24 lbs of food a week. Thus beef was assumed to average about 0.2 ppb DES, for an average dose of 0.02 ppb. Taking a population of approximately 200 million and a total beef supply in 1976 of 25,969 million lbs (see table 22), the average amount of beef consumed by Americans was approximately 2.3 lbs a week (25,969 million lbs of beef - 200 million people - 52 weeks). This correlates with the 2.3 lbs used in calculating the DES risk, where average dose from DES residues was 0.02 ppb.

A comparable calculation for furazolidone is as follows: Total chicken and turkey production in 1976 was 10,930 million lbs (table 22). The average weekly consumption per person was therefore:

$$\frac{10{,}930 \text{ million lbs}}{200 \text{ million people}} - 52 \text{ weeks} = \frac{1.05 \text{ lbs}}{\text{a week}}$$

Using the same caluclation as used for DES, the average dose of furazolidone per person from residues in chicken and turkey meat would be:

$$\frac{1.05 \text{ lbs a week}}{24 \text{ lbs a week}} \times (2 \text{ ppb}) = 0.09 \text{ ppb}$$

The estimates of extra risk in table 38 for a dose of 2 ppb can be used to estimate the risks for other doses by multiplying by the appropriate factor, 0.09/2, or approximately 1/20. Taking the most sensitive animal data, that for Swiss MBR1IBR mice, and multiplying by I/20, these risks are approximately 1 - (4 × 10"); 1 - (9,4 x 10^{6}); 1 - (4.4 x 10^{D}); and 1 - (8.4 x 10^{6}). Thus the risk to humans from furazolidone in poultry is 4 to 10 times less than the target "no residue" risk of 1/10'. In contrast to expected extra cancers of 200 for the "no residue" risk of l/l 0^{6}, these exposures to furazolidone are estimated to produce 20 to 50 extra cases of cancer.

These estimates also can be illustrated by contrasting the calculated exposure of humans to furazolidone with estimates of the dose of furazolidone required to produce an extra risk of $1/10^{6}$ (table 39). The most sensitive mice data result in doses of 0.41 to 0.95 ppb, as contrasted to the 0.09 ppb dose calculated for human exposure.

The mice and rat strains used in these experiments all had rather high spontaneous rates of both tumors and malignancies, Mantel (1977) has not recommended using the Mantel-Bryan procedure for data with high spontaneous rates, In his analysis of the experiments described here,[16] Mantel selected a "cut off" time and only considered tumors detected prior to this time. This modification of the data used by Mantel was applied to the mice data and was found to have relatively little effect, the maximum change in the upper confidence bounds on risk being less than a factor or two (Crump, 1978). *Therefore,* the risk estimates in table 38 would not have been significantly different if the test animals had had a lower spontaneous rate of tumor production or if the data had been modified so as to discount tumors that occur late in life.

Table 39.–Estimates of Dose in Parts Per Billion of Furazolidone Required To Produce an Extra Risk of 1 /10' Using the Data in Table 36

		Most likely estimate of extra risk	*Upper 97.5% confidence limits for extra risk*
		High dose Sprague-Dawley rats	
All neoplasms	Males	3.0	15
	Females	750	9 5
Malignant neoplasms	Males	13.7	3 9
	Females	139	4.1
		Fischer rats	
All neoplasms	Males	—	402 ppm
Malignant neoplasms	Females	189	4 3
		Low-dose Sprague Dawley rats	
All neoplasms	Males	O 988	0429
	Females	O 527	0253
Malignant neoplasms	Males	? 58	0862
	Females	2 56	0783
		Swiss MBR1IBR mice	
All neoplasms	Males	0410	0247
	Females	O 946	0454
Malignant neoplasms	Males	0431	0265
	Females	O 836	0424

I R E p o r t

There should be neither statistical nor theoretical grounds for rejecting the risk estimates in table 38 as unreasonable estimates of rodent risk at 2 ppb. Each of the 14 nitrofurans tested with the Salmonella/Ames test were mutagenic (McCann et al., 1975). Consequently, furazolidone should be considered to be a genotoxic carcinogen with the property of being linear at low dose. The upper confidence bounds on risk computed from the multistage model also have this property. (See figure 1.) Moreover, as is shown in table 37, the multistage model cannot be ruled out on the basis of a chi-square goodness-of-fit test for those data sets for which risk estimates are listed in table 38,

Since the carcinogenic effect of furazolidone in man has not been measured directly, data such as in table 36 constitute the currently available dose-response information for estimating the carcinogenic risk to man.

The assumptions underlying the kinds of risk estimates as calculated for DES and furazolidone are not unanimously accepted, and calculations based on different assumptions could lead to different estimates. The point of the foregoing quantitative exercise was to test the usefulness of a target risk approach to the definition of "no residue" and whether that approach would avoid the problem caused by using actual physical presence of residues for the definition. As discussed earlier, technical improvements in measuring

[16] Ibid.

very minute quantities of residue have led to problems in continuing to use the physical presence approach.

Present FDA regulatory authority is risk-oriented. A regulated substance must be shown to have its intended effect, but more importantly for this discussion, risks must be estimated because safety as well as effectiveness is a regulatory criterion. Thus, even if there were agreement that the 1/10'' added lifetime exposure risk of cancer was an appropriate definition of "no residue, " determining the amount of drug that corresponds to that risk level remains a problem.

Similar differences exist among researchers in quantifying the benefits of using animal drugs. The estimates summarized in the previous chapter on benefits of the use of certain antibacterial and DES produced different quantitative results, even though the USDA and Headley analyses began with the same model,

In contrast to risk assessment, FDA's regulatory decisionmaking basis does not include a quantification of benefits. Furthermore, FDA will not make an official estimate of relative effectiveness of the different drugs they approve for similar uses, FDA's position is that the Agency does not deal in relative effectiveness and that any product with the same claims may be used interchangeably as a substitute for the others (FDA, 1979). The USDA and Headley analyses on benefits lead to different results, although starting from the same model. If FDA had to quantify benefits, it most likely would have reached different quantitative results.

FDA does have to estimate risks. In contrast to the estimate of cancer risks from DES and furazolidone included in this report, FDA has indicated that, according to the estimates they have made, present DES and furazolidone uses would lead to cancer risks in excess of the proposed target risk of 1/10') (FDA, 1979). The difference in results comes primarily from two different assumptions. FDA uses the ninth decile for consumption distribution rather than average per capita consumption "to provide protection for the vast majority of the population. But use of average per capita consumption does not necessarily underestimate the risk to humans. The estimates used here assumed all beef contained at least 2 ppb of DES, when in fact DES **or** other weight-promoting chemicals are given to about 80 percent of fed cattle, and FDA itself reports that DES has dropped considerably in the dollar-volume sales list.

Second, FDA is also concerned that risks occur from both the parent drugs and their metabolizes and that for both DES and furazolidone, the parent drug represents only a small percentage of the total residue, which has not been well-characterized or shown to be safe. FDA states that: "Without identification and testing of the compounds which comprise the residue, no estimates of risk are of much value in judging the safety of the drug use. At best it may be said that the information available gives rise to the possibility that residue exposure may greatly exceed an acceptable level of risk from cancer' (FDA, 1979). The estimates used here were based on residue levels that were at the limits of detection by methods presently approved by FDA, whereas FDA's estimates are based on newer methods not yet approved. In addition, if FDA's use of the target-risk approach leads to no practical difference on how regulatory decisions are made, the FDA statement that no estimates of risk are of much value without identification and testing of the metabolites raises the question of whether a target-risk approach is any improvement over physical presence criteria. Furthermore, this would be a contradiction to the previously quoted statement by FDA's Director of the Bureau of Veterinary Medicine that the new method would "provide a mechanism whereby a reasonably safe level may be established and then, irrespective of further analytical developments, there will be that expectation that the originally set level will remain until toxicological evidence rather than analytical evidence [demonstrates that to be an incorrect tolerance" (*Food* Chemical News, Oct. 16,1978).