# APPENDIXES

# Appendix I

# SACCHARIN ANIMAL TEST DATA

## INTRODUCTION

Data presented here show that consumption of high levels of saccharin is associated with increased incidence of bladder cancer in rats. The analysis is presented in two parts. First, the evidence for the carcinogenicity of saccharin in laboratory animals is reviewed. Second, methods of extrapolation from animal data to human exposure are explained, and some models are applied to the data from the two-generation rat studies to arrive at some estimates of the potential effect in humans.

Current Guidelines for Animal Testing. The National Cancer Institute (NCI) has published guidelines for testing suspected carcinogens in laboratory animals (121). Salient points include:

1. Groups of 50 animals of one sex and one strain should be started on the experiment at 6 weeks after birth or at weaning. Control groups should also contain 50 animals. (In practice 100 animals [50 M, 50 F] should be used at each dose).
2. The chemical should be administered by a route that mimics human exposure.
3. At least two doses, MTD (maximum tolerated dose) and MTD/2 or MTD/4 should be administered.
4. Treatment should be continued long enough (in practice generally 24 months) to produce a maximum response.
5. Animals should be sacrificed (usually at 24 months) and necropsied according to detailed pathology procedures.
6. Tests should be conducted in two species, and the results of the more sensitive one given greater consideration.

Additionally, a subcommittee of the National Academy of Sciences (115) has recommended that:

7. Exposure to the chemical for two generations should be considered. This procedure exposes the animals of the second generation to the chemical in *utero,* which may represent the most sensitive stage of the animal's life.

While none of the carcinogenicity tests of saccharin meets all of these criteria, the experiments considered positive most closely approach the current standards. Because of the test conditions or the small number of animals tested, some other experiments would not have detected the carcinogenic effects of saccharin.

## TESTING OF SACCHARIN IN RATS

Since 1949, at least 10 feeding experiments have been carried out in rats to test potential carcinogenic effects of saccharin. Only four of these experiments have been published; three in refereed journals. The others have remained in the files of the sponsoring institutions.

Rats have been used in three types of feeding experiments. First, in most experiments weanling animals were started on diets containing saccharin and fed such diets for 2 years or until death. Second, the most convincing experiments involved feeding of saccharin over two generations. This design exposed second generation animals to saccharin from the moment of conception until termination of the experiment. Third, in cocarcinogenesis experiments, saccharin was fed to rats that were also exposed to a single low dose of a known carcinogen.

Many of these studies have been reviewed by subcommittees of the Committee on Food Protection, National Academy of Sciences (NAS) (114, 116, 117), and by Reuber (141). In general, the NAS committees found the evidence for the carcinogenicity of saccharin unconvincing, but no NAS committee has reviewed the 1977 Canadian Study (67). Reuber's analysis of the data from the same experiments led him to conclude that a number of experiments have shown saccharin to be a carcinogen. '

The Office of Technology Assessment found Reuber's review (141) invaluable as a guide to literature that was hard to locate. However, the OTA analysis disagrees in detail with many of Reuber's conclusions. Wolfe and Johnson (189) cited Reuber's analysis in their testimony before the House Committee on Interstate and Foreign Commerce. At that hearing, they pointed out that high doses of a potential carcinogen are required to produce a detectable number of cancers in small numbers of experimental animals. This argument is generally accepted. Reuber, however, drew attention to a few cancers that occurred at a dose of 1/100 or 1/10 of the maximum dose administered but did not occur at any higher dose. Such results are inconsistent with the accepted argument. If the cancers were induced by saccharin ingestion, more cancers should have occurred at all higher doses. In many examples cited by Reuber, no increase in cancers occurred with increasing doses. It appears likely that the few cancers mentioned were spontaneous ones that occurred by chance in saccharin-fed animals. All experiments that Reuber cited as positive are mentioned below, and the OTA analysis is compared to his.

### A. Two-Generation Rat Feeding Experiments

#### 1. 1977 Canadian Study (67)

*(a) Experimental Design*

Two groups of 100 (50 M, 50 F) Charles River (COBS) rats were used. The control group was fed a standard laboratory ration. The experimental group received the same ration but with saccharin, purified of ortho-toluenesulfonamide (OTS), added to comprise 5.0 percent of the diet. (The shorthand designation "5-percent rats" will be used for such experimental animals.) The diet was adjusted weekly to maintain a constant saccharin dose. The experiment began when the rats were 30 days old. At

age 100 days, members of the $F_0$ (parental) generation were mated. The $F_0$ generation was continued on the saccharin diet, and the $F_1$ (offspring, progeny, or second) generation animals were fed the same diet received by their parents, At death, each animal was subjected to "gross and microscopic examination. "

*(b) Results*

Weight: $F_0$ and $F_1$ animals weighed less than controls throughout the experiment (table 4).

**Table 4.—Animal Weights in 1977 Canadian Study**
(grams)

| | Weeks on test | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 15 | 48 | 80 | 110 | 124 |
| $F_0$ Male controls | 111 | 320 | 518 | 701 | 791 | 745 | 633 |
| 5-percent rats | 112 | 298 | 462 | 614 | 682 | 658 | 614 |
| Percent difference* | 0 | 7 | 11 | 12 | 14 | 12 | 03 |
| Female controls | **98** | 206 | 282 | 364 | 448 | 488 | 467 |
| 5-percent rats | **99** | 190 | 262 | 323 | 379 | 409 | 375 |
| Percent difference | 0 | 8 | 7 | 11 | 15 | 16 | 20 |
| $F_1$ Male controls | 90 | 315 | 501 | 720 | 756 | 736 | 673 |
| 5-percent rats | **72** | **272** | 452 | 630 | 677 | 686 | 632 |
| Percent difference | 20 | **14** | 10 | 12 | 10 | 7 | 6 |
| Female controls | 81 | **192** | 272 | 372 | 447 | 504 | 499 |
| 5-percent rats | 67 | **180** | 261 | 331 | 379 | 396 | 394 |
| Percent difference | 17 | **6** | 4 | 11 | 15 | 21 | 21 |

'Calculated as: $\frac{\text{weight of controls-weight of 5-percent rats}}{\text{weight of controls}} \times 100$

Life Span: No significant differences between controls and experimental (table **5).**

**Table 5.—Mean Time to Death in 1977 Canadian Study**
(days)

| Diet | $F_0$ | | $F_1$ | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Controls | 686±22 | 695±25 | 665±22 | 699±19 |
| 5 percent | 679±22 | 731±25 | 623±22 | 706±19 |

Bladder Cancer Incidence: Increases in males of both generations and in $F_1$ females (table 6).

Other Cancer Incidence: The pathologist for the experiment is still examining other organs, but so far, cancer of no other organ has been associated with saccharin ingestion. The pathology had not been completed by October 1977.

Fertility, gestation, live delivery, and lactation index: No significant differences between controls and experimental.

**Table 6.—incidence of Bladder Tumors in 1977 Canadian Study**

(Rats with tumors/Rats examined (Percent))

| | $F_0$ | | | $F_1$ | | |
|---|---|---|---|---|---|---|
| | Benign | Malignant | Total | Benign | Malignant | Total |
| **Males** | | | | | | |
| Controls | 1/36(3%) | 0/36(0%) | 1/36(3%) | 0/42(0%) | 0/42(0%) | 0/42 (0%) |
| 5-percent rats | 4/38(11%) | 3/38(8%) | 7/38(19%) | 4/45(9%) | 8/45(18%) | 2/45 (27%) |
| **Females** | | | | | | |
| **Controls** | 0/38(0%) | 0/38(0%) | **0/38(0%)** | 0/47(0%) | 0/47(0%) | 0/47 (0%) |
| **5-percent rats** | 0/49(%) | 0/40(0%) | 0/40(0%) | 0/49(0%) | 2/49(4%) | 2/49 (4%) |

*(c) Discussion*

The author's discussion of this experiment was not available in October 1977,

*(d) Comments by Others*

These data led to the Canadian Government's decision to ban saccharin (66). The FDA's proposed ban is based on this study (50). Reuber accepts the data and conclusions as evidence for the carcinogenicity of saccharin (141).

(e) OTA Comments

OTA has not been able to evaluate this entire study because it is not yet completed. In particular no information is available about bladder stones, urothelial changes, or tumors at other sites. Only one dose level of saccharin was tested. This experiment was primarily directed toward assessing the carcinogenicity of orthotoluenesulfonamide (OTS), a contaminant previously found in commercial saccharin. A committee of the National Academy of Sciences (NAS) had suggested that OTS might be responsible for the carcinogenicity associated with saccharin in earlier experiments (116). The 1977 Canadian experiment clearly showed that OTS is not a carcinogen (data not presented in this report).

Unlike some carcinogenicity experiments, no animals were sacrificed for necropsy at scheduled intervals during this experiment. Animals were examined daily for clinical signs of tumors, and those diagnosed as probably having tumors were isolated and examined twice daily. Moribund animals were sacrificed for necropsy. Less than 1 percent of animals died unobserved and were lost to the experiment because of autolysis.

The mean time to death for animals in the experiment is shown in table 5. It ranged from about 21 to 25 months. In this experiment, large numbers of animals survived long enough to develop tumors.

Throughout the experiment, animals on the 5-percent saccharin diet had lower weights than controls. The results from the experiment show that male rats were more sensitive to saccharin than females and that saccharin caused bladder tumors.

These tumors showed only low invasiveness and no metastasis (62). Both $F_0$ and $F_1$ were examined. Tumors were found in both generations but only in males in $F_0$. The higher frequency in $F_1$ may be related to that generation's in utero exposure, but the difference in cancer incidence between $F_1$ and $F_0$ is not statistically significant.

### **2. 1973** FDA Study (49)

#### *(a) Experimental Design*

The design was similar to the 1977 Canadian Study. Six groups of 96 (48 M, 48 F) Charles River rats (Sprague-Dawley) were fed diets supplemented with different amounts of sodium saccharin: 0, 0.01, 0.1, 1.0, 5.0, or 7.5 percent of diet. Histological examinations were conducted on only the $F_1$ (second) generation rats.

#### *(b) Results*

Weight: Rats fed 5- and 7.5-percent saccharin were about 15-percent lighter than controls and those eating lower levels of saccharin.

Life Span: No significant differences between the controls and experimental rats.

Stones and Parasites: Analysis of data presented in this paper showed that there was no association between bladder stones and bladder cancer. Neither was there any correlation between parasitic worms, or their eggs, and bladder cancer.

High Doses of Sodium: One group of animals was given a diet containing a sodium salt at the same level as that ingested by the 7.5-percent sodium saccharin-fed rats. There was no increase in tumors.

Tumor Incidence: At death or sacrifice each animal was examined for macroscopic tumors (table 7), and organs were excised, fixed, and stained for subsequent microscopic pathology. Some results from the microscopic examinations are shown (tables 8-1 1).

**Table 7.—incidence of Macroscopic Tumors in Rats Surviving 18 Months or More in 1973 FDA Study**

| Dose (Percent) | Rats with tumors/Rats examined (Percent) | |
|---|---|---|
| | Male | Female |
| 0 | 2/29 ( 7%) | 1 /27( 4°/0) |
| 0.01 | 2/28 ( 7%) | 3/30(1 0°/0) |
| 0.1 | 5/29 (14%) | 3/32( 9°/0) |
| 1 | 3/28 (11%) | 5/32(1 6°/0) |
| 5 | 4/24 (17%) | 7/29(240/o) |
| 7.5 | 8/26 (36%) | 9/32(280/o) |

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Other Pathologies: A particular type of kidney hyperplasia "colyceal polyposis" occurred more frequently in the 7.5-percent rats than controls ($p < 0.05$). This hyperplasia is not considered to be precancerous,

**Table 8.—incidence of Neoplasms in 1973 FDA Study**

| Dose (Percent) | Rats with neoplasms/Rats examined (Percent) | | | |
|---|---|---|---|---|
| | 18 Months | | 24 Months | |
| | Males | Females | Males | Females |
| 0 | **4/7(57%)** | **5/6(83 %)** | 19/29(65%) | 21/27 (78%) |
| 0.01 | — | — | 10/28(36%) | 32/30 (107%) |
| 0.1 | — | — | 14/29(48%) | 31 /32 ( 97%) |
| 1.0 | — | — | **12/28(43%)** | 20/32 (62%) |
| 5.0 | — | — | **9/24(38%)** | 34/29 (117%) |
| 7.5 | 0/7( 0%) | 3/5(60%) | **20/26(77%)** | 38/32 (119%) |

**Table 9.—incidence of Bladder Tumors in Rats Surviving 18 Months or More in 1973 FDA Study**

| Dose (Percent) | Rats with tumors/Rats examined (Percent) | | |
|---|---|---|---|
| | Papillomas of the Urinary Bladder | Carcinomas of the Urinary Bladder | Total Tumors of the Urinary Bladder |
| **Male Rats** | | | |
| 0 | **0/25(0%)** | 1/25( *4%)* | 1/25( *4%)* |
| 0.01 | **0/16(0%)** | *0/16( O%)* | *0/16( O%)* |
| 0.1 | **0/27(0%)** | *0/27( O%)* | *0/27( O%)* |
| 1.0 | 0/22(0%) | *0/22( o%)* | *0/22( o%)* |
| **5** | 0/21(0%) | *1/21( 5%)* | *1/21( 5%)* |
| **7.5** | 1/23(4%) | *6/23(23%)* | *7/23(30%)* |
| **Female Rats** | | | |
| 0 | **0/24(0%)** | **0/24( 0%)** | **0/24( 0%)** |
| 0.01 | **0/23(0%)** | **0/23( 0%)** | **0/23( 0%)** |
| 0.1 | **0/24(0%)** | **0/24( 0%)** | **0/24( 0%)** |
| 1.0 | 0/30(0%) | 0/30( 0%) | 0/30( 0%) |
| **5** | 0/28(0%) | 0/28( 0%) | 0/28( 0%) |
| **7.5** | 0/31(0%) | 2/31( 6%) | 2/31( 6%) |

**Table 10.—Incidence of Mammary Gland Tumors in Rats Surviving More Than 18 Months in 1973 FDA Study**

| Dose (Percent) | Rats with mammary gland tumors/Rats examined (Percent) | | |
|---|---|---|---|
| | One/Rat | Two or More/Rat | Total |
| **Male Rats** | | | |
| 0 | 5/29(17%) | 1/29( 3%) | **6/29(20%)** |
| 0.01 | 8/25(32%) | 6/25(24%) | **14/25(56%)** |
| 0.1 | 9/27(33%) | 0/27( O%) | **9/27(33%)** |
| 1.0 | 8/27(30%) | 0/27( O%) | **8/27(30%)** |
| **5** | 7/25(28%) | 0/25( O%) | **7/25(28%)** |
| **7.5** | 7/24(29%) | 0/24( O%) | **7/24(29%)** |
| **Female Rats** | | | |
| 0 | 5/26(19%) | 1/26( 4%) | **6/26(23%)** |
| 0.01 | 8/30(27%) | 6/30(20%) | **14/30(47%)** |
| 0.1 | 9/34(26%) | 4/34(12%) | **13/34(38%)** |
| 1.0 | 8/30(27%) | 4/30(13%) | **12/30(40%)** |
| **5** | 7/27(26%) | 5/27(18%) | **12/27(44%)** |
| **7.5** | 7/32(22%) | 2/32( 6%) | **9/32(28%)** |

**Table11 .—Incidence of Urinary Bladder Hyperplasia in 1973 FDA Study**

| Dose (Percent) | Rats with hyperplasia/Rats examined | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Months on Saccharin | | | | | | | | Total | |
| | 6 | | 12 | | 18 | | 24 | | | |
| | M | F | M | F | M | F | M | F | M | F |
| 0 | 1/29 | 1/48 | 1/12 | 0/7 | 0/7 | 0/6 | 8/25 | 2/24 | 10/73 | 3/85 |
| 0.01 | 1/31 | 0/49 | 1/18 | 0/5 | 1/6 | 0/4 | 3/16 | 0/23 | 6/71 | 0/81 |
| 0.1 | 0/35 | 0/48 | 0/15 | 0/3 | 0/4 | 0/5 | 4/27 | 0/24 | 4/81 | 0/81 |
| 1.0 | 0/32 | 0/50 | 1/15 | 0/4 | 0/7 | 0/6 | 3/22 | 3/30 | 4/76 | 3/90 |
| 5 | 3/20 | 1/37 | 0/16 | 1/16 | 0/7 | 0/7 | 3/21 | 3/28 | 6/64 | 5/88 |
| 7.5 | 4/18 | 3/35 | 4/15 | 0/5 | 4/6 | 0/5 | 7/23 | 7/31 | 19/62 | 10/76 |

*(c) Discussion*

The authors of this study concluded that bladder tumors were associated with ingestion of the maximum amount of saccharin. Other experts agreed with the classification of the urinary bladder tumors reported by the authors of the study (116,181).

*(d) Comments by Others*

The NAS committee (1 16) agreed with the conclusions of the authors, but considered it unfortunate that histological examinations were not carried out on the $F_0$ generation. It also suggested that the OTS impurity in the saccharin might be the active carcinogen. The 1977 Canadian Study (55) eliminated the basis for that objection because saccharin free of OTS was associated with a higher incidence of bladder cancer than controls, and OTS was not,

The committee speculated that ingestion of large amounts of sodium saccharin might lead to bladder stones and that the stones might be the causative agent for bladder cancer. Reuber (141) analyzed the evidence from this experiment and concluded that although 67 percent of the treated male rats at 12 months had stones, no rat with stones developed either hyperplasia or tumors.

The NAS committee also suggested that another agent, parasites, might have been the causative agent, but there is little evidence to support the suggestion. No bladder parasites were observed, and indeed, there appears to be little reason for assigning a carcinogenic role to the parasites (147).

Reuber agreed that the high levels of dietary saccharin are correlated with bladder cancer. He further argued that urinary bladder hyperplasia is a precursor of cancer and that those data, as well as the total number of tumors and number of mammary tumors, further strengthen the causal relationship between saccharin and cancer.

Dr. M.A. Weinberger, Director, Division of Pathology, Food and Drug Administration (FDA), wrote a memo in 1974 (181) agreeing with the pathology of the FDA Study. He stated that: (1) the incidence of tumors in the 7.5-percent rats was significantly different from that in the controls, (2) there was no evidence for stones or parasites playing a role in the genesis of the tumors, (3) tumors of no organ other than the urinary bladder were associated with saccharin, and (4) the bladder tumors were of low invasiveness and resembled those found in the WARF Study (165, 177).

(e) OTA *Comments*

Table 7 is derived from data presented in a preliminary report of this experiment (51), and from data that were cited by Reuber (141). Table 8 is based on the final report (49) and includes data from microscopic pathological examinations. Reuber concluded that total tumor incidence increased in parallel with increasing dose, but the more complete data do not support that conclusion, In males the incidence in controls was higher than at all doses except 7.5 percent. In females there were two peaks, one at 0.1 percent, the other at 5 and 7.5 percent.

Only the 7.5-percent dose in $F_1$ males and females was associated with an incidence of bladder cancer greater than controls. Similarly, the incidence of bladder hyperplasia increased only at the highest dose. In fact, low doses of saccharin were associated with a lower incidence of hyperplasia than in controls.

A large increase in number of mammary tumors was noted at the lowest dose (0.01) percent), but the incidence did not increase with higher doses. By this measure, there is again no clear-cut relationship between dosage and effect. These data are examples of cases that Reuber considers positive, and OTA does not.

None of the other experiments described here reported high rates of bladder hyperplasia or mammary gland tumors. The data in the WARF study are complete enough to suggest that such pathologies would have been noticed and reported if they had occurred. Furthermore, no such pathologies have been associated with saccharin ingestion in the 1977 Canadian Study (67).

3. Wisconsin Alumni Research Foundation (WARF) Study (165, 177)

*(a) Experimental Design*

This experiment was similar in design to the 1973 FDA Study, but the number of rats in each group was smaller. Five groups of 40 Sprague-Dawley rats (20 M, 20 F) were used, and sodium saccharin was added to the rations at O-, 0.05-, 0.5- or 5.0-percent levels. $F_1$ rats born to mothers ingesting saccharin were maintained on the identical diet for 100 weeks. "Most" tissues of $F_1$ rats (not $F_0$) were sectioned histologically. No rats were sacrificed at scheduled intervals. Rats that became moribund or died during the test were necropsied, and survivors were necropsied at 100 weeks.

*(b) Results*

Weight: ($F_1$ generation): 5-percent rats had reduced weights at weaning and gained weight more slowly, but reached the same levels as controls.

Life Span: No significant differences between controls and experimental.

Hematology: No significant differences between controls and experimental.

Reproduction: No significant differences between controls and experimentals.

Tumor Incidence: Results of histological examination of tissues from the rats are shown in tables 12-14.

**Table 12.—Total Number of Tumors in 1974 WARF Study**

| Dose (Percent) | Rats with tumors/Rats examined | |
|---|---|---|
| | Males | Females |
| o | 3/20 | 1 2/20 |
| 0.05 " | 2/20 | 6/20 |
| 0.5 | 2/20 | 9/20 |
| 5.0 | 14/20 | 18/20 |

*(c) Discussion*

The authors concluded that overall tumor incidence was increased in the 5-percent males compared to controls. Furthermore, they pointed out that five squamous cell carcinomas of the uterus and seven transitional cell carcinomas of the bladder were seen only in saccharin-fed groups.

The authors underline the sporadic appearance of some tumors by mentioning that of the tumors seen in three or more animals, one (subcutaneous adenofibroma) occurred most frequently in the control population.

**Table 13.—Incidence of Urinary Bladder Tumors in 1974 WARF Study in Rats Surviving 18 Months or Longer**

| Dose (Percent) | Rats with tumors/Rats examined | | | |
|---|---|---|---|---|
| | Males | | Females | |
| | Benign | Malignant | Benign | Malignant |
| 0 | 0/12 | 0/12 | 0/1 6 | 0/1 6 |
| 0.05 " : : : : : : : : : : : | 0/10 | 0/10 | 0/1 4 | 1/1 4 |
| 0.5 . . . . . | 1/12 | 0/12 | 0/1 5 | 0/1 5 |
| 5.0 ....., . . . . . | 1/1 5 | 7/15 | 0/20 | 0/20 |

**Table 14.—Incidence of Ovarian and Uterine Tumors in 1974 WARF Study**

| Dose (Percent) | Rats with tumors/Rats examined | |
|---|---|---|
| | Benign | Malignant |
| o | 0/20 | 1 /20 |
| 0.05" : : : : : : : : : : : : : : : : : : : : : : : | 0/20 | 1 /20 |
| 0.5 . . . . . | 1 /20 | 2/20 |
| 5.0 . . . . . | 2/20 | 4/20 |

*(d) Comments by Others*

The NAS committee (116) leveled the same criticisms at this experiment that it did at the 1973 FDA Study (49). They suggested that OTS, bladder stones, or parasites might be the active carcinogen.

Reuber (141) accepted the conclusions of the authors and also drew attention to the increased number of female reproductive system tumors at high doses.

(e) *Comments by OTA*

No symptoms of acute toxicity were noted except slower weight gain in the 5-percent rats.

This experiment suffers from the small number of animals, but its results are consistent with the other two-generation experiments. Ingestion of saccharin at the highest dose resulted in an increase in male bladder cancer. The increase in female reproductive cancers was not seen in other two-generation experiments, and it is not considered to be an important finding.

The high number of spontaneous tumors in the female rats contrasts to other experiments in which the spontaneous incidence was nearly equal between the sexes or higher in males. Even so, bladder cancers were associated with the highest dose of saccharin in males,

The following tumors were noted to occur only in control animals in this experiment: adrenal adenoma, islet cell adenocarcinoma, papilloma with squamous metaplasia of the uterus. Such findings underline the difficulty of interpreting small numbers. Furthermore, the following tumors occurred in 1/40 controls and in 1/160 experimental: pituitary carcinoma, thyroid adenocarcinoma, subcutaneous fibroma, subcutaneous fibroadenoma, subcutaneous sarcoma, and subcutaneous adenocarcinema.

### 4. Summary: OTA Discussion of the Two-Generation Feeding Experiments

There is general agreement among the authors of the experiments, the NAS committees, and Reuber that:

(1) Exposure of rats to 5-or 7.5-percent saccharin from the moment of conception to death was associated with an increased frequency of urinary bladder cancers. These tumors were of low invasiveness and had no reported metastasis.

(2) These same conditions resulted in slower weight gain in all experiments and lower adult weights in two of the three experiments.

Reuber's and OTA's analyses of these data further argue that:

(3) Bladder tumors were not associated with stones or parasites in at least two of these experiments.

(4) Increased frequencies of bladder hyperplasia were associated with 7.5-percent saccharin in the 1973 FDA Study.

Additionally, OTA notes that:

(5) In all experiments more bladder cancers were found in males.

These experiments cannot be interpreted as showing a threshold for saccharin induction of bladder cancers at about 5- or 7.5-percent dietary saccharin. The frequency of tumors at the highest doses is so low that larger numbers of animals would have been needed to detect cancers at lower levels.

If these data fit a no-threshold model, an almost linear relationship between dose and tumor number might have been seen in the 1973 FDA Study in which both 5- and 7.5-percent doses were used. While no such relationship was seen, the data do not eliminate there being one. The design of the other two experiments precludes any conclusions being drawn about dose response.

The NCI Guidelines have suggested that carcinogenicity testing is best conducted at doses that produce no apparent toxicity (121). In all cases in which increased numbers of tumors were detected (5 and 7.5 percent), weight gain was not normal, and in two of the three, final weights were less than those of the controls. Reduced final weights and slower weight gain are symptoms of toxicity. The difference in weights

in the 1977 Canadian experiments exceeds the 10-percent difference acceptable to the NCI, but the weight difference is greater among females, who had fewer tumors than the males.

## B. One-Generation Rat Feeding Experiments

### 1. 1948-49 FDA Study (46)

#### (a) *Experimental Design*

Groups of 3-week-old Osborne-Mendel Rats, 20 per group (10 F, 10 M) were fed saccharin at 0,01-, 0.1-, 0.5-, 1- or 5-percent levels for 2 years. The control group included 54 rats. Animals were to be carried on the experiment for 2 years, but some died earlier. At death or sacrifice, most rats were examined both grossly and microscopically, but bladders were not included in the list of examined organs.

The microscopic slides as well as some preserved organs from this experiment were held at FDA, and in 1969, Long and Habermann (93) examined those samples. All bladders were described as "grossly normal." The one sectioned for histology was normal. Paraffin blocks of kidneys were sectioned and examined.

#### *(b) Results*

Weight: 5-percent rats were somewhat lighter than controls; other saccharin-fed rats did not differ from controls.

Life Span: No difference was observed between saccharin-fed and control animal groups.

Tumors: In the 5-percent rats, seven rats were found to have thoracic lymphosarcomas, which is near the incidence seen in control rats. However, four of those seven rats had abdominal lymphosarcomas, a much higher frequency than the usual 1:15-20 for abdominal: thoracic lymphosarcomas.

Long and Habermann's (93) reexamination of the 1948-49 FDA materials produced a more detailed exposition of the incidence of lymphosarcomas. Table 15 is taken from Long and Habermann's pathology reports.

**Table 15.—Incidence of Lymphosarcomas in 1948-49 FDA Study**

| Dose (Percent) | Rats with tumors/Rats examined (Percent) | | |
|---|---|---|---|
| | Lymphosarcomas of the Thorax | Lymphosarcomas of the Abdomen | Total Lymphosarcomas |
| o | 9/54 (17%) | 0/54( 0%) | 9/54 (1 7%) |
| 0.01" | 8/14 (57%) | 0/1 4( 0%) | 8/14 (57%) |
| 0.1 | 5/1 6 (31 %) | 0/1 6( 0%) | 5/16 (31%) |
| 0.5 | 2/1 5 (1 3%) | 0/1 5( 0%) | 2/15 (13%) |
| 1 | 1/1 8 ( 6%) | 0/1 8( 0%) | 1/18 ( 6%) |
| 5 | 7/1 7 (41%) | 3/1 7(1 8%) | 10/17 (58%) |

Other Pathologies: Long and Habermann presented the data concerning kidney lesions in table 16.

**Table 16.—Incidence of Kidney Lesions in 1948-49 FDA Study**

| Dose (Percent) | Rats with lesions/Rats examined (Percent) | | |
|---|---|---|---|
| | Epithelial Hyperplasia | Calcification | Venous Thrombosis |
| 0 | **1 /33 ( 3%)** | 1/53 ( 2%) | 0/53 ( 0%) |
| 0.01 | **0/13 ( 0%)** | 0/13 ( 0%) | 0/3 ( 0%) |
| 0.1 | **0/15 ( 0%)** | 0/15 ( 0%) | 0/15 ( 0%) |
| 0.5 | 1/15 ( 7%) | 2/15 (13%) | 0/15 ( 0%) |
| 1 | 3/18 (17%) | 1/18 ( 6%) | 0/18 ( 0%) |
| 5 | 13/17 (76%) | 13/17 (76%) | 4/17 (24%) |

*(c) Discussion*

The authors concluded that saccharin produced no adverse effects at doses less than 5 percent, that the 5-percent dose caused only slight toxic effects, and that only the ratio of abdominal to thoracic lymphosarcomas was remarkable. Long and Habermann (93) drew attention to the increased number of thoracic lymphosarcomas at 0.01 percent and noted that the incidence was significantly greater than in the controls. They tempered that conclusion with comments about the decreasing incidence at all higher doses except 5 percent. They attached less importance to the number of abdominal lymphosarcomas than did the original report of this study (46).

*(d) Comments by Others*

The NAS committee (116) quoted the original observation that saccharin-fed rats had increased incidence of abdominal lymphosarcomas (46) but made no other comments. Reuber (141) also cited the increased incidence of abdominal lymphosarcomas, and he treated the renal pathologies as evidence for a precancerous condition.

*(e) Comments* by *OTA*

The lymphosarcoma data present some difficulties in interpretation. Four groups of 20 control animals were used, and the incidence in the controls ranged from 0/20 to 4/20. Fitzhugh et al. (35) did not present these data in tabular form; in fact, they did not mention lymphosarcomas at doses less than 5 percent, but did mention that the ratio of abdominal to thoracic lymphosarcomas was remarkably high. They found the ratio to be 4:7, rather than the 1:15 to 1:20 they expected. Long and Habermann (93) did not consider this difference to be significant and pointed out that the abdominal tumors occurred only in animals with thoracic tumors. The distribution of lymphosarcomas across the dose range has two peaks, one at 0.01 percent, the other at 5.0 percent. This distribution may reflect a difference in frequency of spontaneous tumors among the groups,

The renal pathologies may be considered to be examples of acute toxicity. Indeed, their frequencies might fit a threshold model. If the kidney lesions are precancerous, such cancers must develop quite slowly because no excess of kidney

tumors has been noted in any experiments, An excess of kidney calyceal polyposis was noted in 7.5-percent animals in the 1973 FDA Study, but that condition is not precancerous. Furthermore, a careful examination of 600 saccharin-fed rats did not find an excess of kidney lesions (112).

2. Lessel Study, 1948-49 (91)

*(a) Experimental Design*

Groups of 40 Boots-Wistar rats (20M, 20F) were fed rations of O-, 0,005-, 0.05-, or 5. O-percent saccharin for 2 years. This experiment was intended to complement the FDA 1948-49 Study.

*(b) Results*

Body Weight: The 5-percent rats weighed less than other groups.

Life Span: The 5-percent rats had decreased lifespans.

Food Consumption: The 5-percent rats ate more even though they gained less weight and died earlier.

Tumors: Animals were examined for tumors at death or sacrifice (table 17), and the urinary bladder was examined for pathology (table 18).

**Table 17.—Number of Survivors and Number of Tumors in 1948-49 Lessel Study**

| Dose (Percent) | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | Survivors at 2 Years | Tumors | | Survivors at 2 Years | Tumors | |
| | | Benign | Malignant | | – Benign | Malignant |
| 0 | 6 | 1 | 1 | 9 | 5 | 4 |
| 0.005 : : : : : : | 12 | 2 | 1 | 13 | 6 | 3 |
| 0.05 . . . . . | 8 | 4 | 2 | 10 | 4 | 0 |
| 0.5. . . . . | 8 | 2 | 2 | 9 | 0 | 1 |
| 5.0. . . . . | 3 | 1 | 1 | 2 | 2 | 0 |

**Table 18.—Male and Female Rats Ingesting Saccharin With "Gross Abnormalities" of the Urinary Bladder[a] in 1948-49 Lessel Study**

| Dose (Percent) | Rats with abnormalities/Rats examined (Percent) | |
|---|---|---|
| | Males | Females |
| 0 . . . . . | 2/20 (1 0°/0) | 0/20 ( 0°/0) |
| 0.005 . . . . . | 1 /20 ( 5%) | 0/20 ( 0%) |
| 0.05 . . . . . | 4/20 (20%) | 0/20 ( 0%) |
| 0.5. . . . . | 1 /20 (10%) | 0/20 ( 0%) |
| 5 . . . . . | 5/19 (26%) | 3/18 (17%) |

[a]Rats surviving 6 months or longer.

*(c) Discussion*

The author concluded that tumor incidence was unaltered at all dose levels.

*(d) Comments by Others*

The NAS committee (116) quoted from Lessel's reports extensively and summarized by saying that his detailed description of examination of bladders (table 18) ". . . underscore some of the problems encountered in long-term testing. " It then commented that the study was incomplete because not all bladders were examined microscopically.

Reuber (141) drew attention to the observations that one 5-percent rat had bladder mucosa hyperplasia, and two early bladder tumors were seen in 5-percent females. One tumor was associated with a stone; the other was not.

*(e) Comments by OTA*

All but one of the tumors reported in table 17 were detected at 22 to 24 months. Because of the small number of animals alive at 24 months, it is difficult to assess the significance of the numbers of tumors.

The highest incidence of bladder "gross abnormalities" (table 18) was associated with the highest dose of saccharin. These abnormalities occurred at a higher dose than did the majority of the tumors.

The conclusion reached by Reuber (141) that saccharin increased tumors in males has to be qualified because of the low incidence of tumors in the control males. In all studies except this one and WARF, spontaneous tumors occurred more frequently in males or at about the same frequency in both sexes. The incidence seen in males here may be lower than the average incidence if larger numbers of controls had been studied. Alternatively, of course, it might be argued that the incidence in control females was abnormally high, and that the female data might have been positive if a better determination of spontaneous incidence had been made, These opposing arguments again point up the problems of trying to analyze data from too few animals.

3. National Institute of Hygienic Sciences (Tokyo) (123)

*(a) Experimental Design*

A control group of 54 rats was fed a standard laboratory diet. The experimental group of 54 rats was fed a diet increasingly rich in saccharin according to the schedule given in table 19. Rats were killed and inspected for cancers at various times from 6 to 24 months.

**Table 19.—Saccharin Feeding Schedule for Rats in Japanese Study (undated)**

| Days | Dose (Percent) |
|---|---|
| 0 - 20 . . . . . | 2 |
| 21 - 60 . . . . . | 3 |
| 61 -150, . . . . . | 4 |
| Greater than 150 . . . . . | 5 |

*(b) Results*

Life Span: There was no apparent effect of saccharin on life span.

Tumors: The number of tumors found was reported; some of those results are shown in table 20.

**Table 20.—Incidence of Tumors in Rats Necropsied at 24 and 28 Months in Japanese Study (undated)**

| Months | Rats with tumors/Rats examined | |
|---|---|---|
| | Control Rats | Saccharin Rats |
| 24 months | 5/1 1 | 5/1 1 |
| 28 months | 0/3 | **5/6** |

*(c) Discussion*

The author's discussion of these experiments is not available.

*(d) Comments by Others*

Reuber (141), based on data not quoted here, concluded that the saccharin-fed rats had twice as many tumors as controls.

This experiment was not mentioned by any NAS committees (114, 116, 117).

*(e) Comments by OTA*

Table 20 is an excellent (although extreme) illustration of the difficulties of interpreting some experiments. The numbers are very small and do not lend themselves to interpretation. For example, given *5/11* positive control rats at 24 months and 0/3 positive controls at 28 months, it is difficult to come to any conclusion about the frequency of spontaneous tumors.

4. Litton Bionetics Study (119)

*(a) Experimental Design*

Two studies (Saccharin I and II) were run in parallel. In each experiment, 52 Charles River Rats (26 M; 26 F) were fed either 1- or 5-percent saccharin for 104 weeks. The control group for each experiment was 40 rats (20 M, 20 F).

Complete necropsies were performed.

*(b) Results*

Life Span: Feeding of saccharin did not affect longevity (see table 21), but an epidemic of chronic murine pneumonia killed many l-percent male rats in Saccharin II.

Tumors: Animals were necropsied and examined for cancers (table 21).

Other Pathologies: One 5-percent male had bladder hyperplasia in Saccharin I. There were 17 cases of glomerulonephritis among the 26 l-percent males in Saccharin I; none in Saccharin II.

**Table 21.—Survivors, Tumors, and Pneumonia in 1973 Litton Bionetics Study**

| Dose (Percent) | Survivors at 18 Months | | Incidence of Pneumonia | | Tumors in Males | | Tumors in Females | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | Benign | Malignant | Benign | Malignant |
| **Saccharin I** | | | | | | | | |
| 0 | 20/20 | 18/20 | | | 10 | 0 | 27 | 1 |
| 1 | 24/26 | 21/26 | | | 12 | 1 | 27 | 1 |
| 5 | 21/26 | 24/26 | | | 17 | 2 | 26 | 0 |
| **Saccharin II** | | | | | | | | |
| 0 | 18/20 | 19/20 | | | 14 | 3 | 13 | 0 |
| 1 | 18/26 | 26/26 | 18/26 | | 11 | 1 | 23 | 1 |
| 5 | 23/26 | 23/26 | 11/26 | 4/26 | 3 | 1 | 25 | 2 |

One 5-percent female rat had a urinary bladder papilloma (Saccharin II).

*(c) Discussion*

The authors concluded that saccharin was not associated with carcinogenesis.

*(d) Comments by Others*

Reuber (141) reports that these data were analyzed by statisticians at the NCI, who concluded that tumor incidence was higher in the female rats in the Saccharin 11 experiment. The males in Saccharin II were stricken with pneumonia, and the number of early deaths may have caused the reduced incidence of tumors seen in that group. It was also concluded (113) that there were no increases in malignant tumors. The NAS committee (116) was apparently interested only in bladder tumors. Its discussion of these experiments noted only that a single bladder papilloma was found in a 5-percent female.

Reuber (141) presented figures somewhat different from those in table 21 which supported the conclusion that the total number of tumors was higher in saccharin-fed female rats than in female rats in Saccharin II.

*(e) Comments by OTA*

This experiment is unique in that females appeared to be more sensitive than males to saccharin. The males in Saccharin II had a high incidence of pneumonia, and some animals died. While this factor may account for that group's having fewer tumors, it could have had no effect on Saccharin I in which females were also more sensitive than males.

The frequency of spontaneous tumors in female controls between the two experiments is quite different. The significant difference between experimental and control females in Saccharin II depends on the much lower spontaneous rate in that experiment. There was no increase in tumor incidence as the saccharin dose was increased fivefold from 1 to 5 percent.

Toxic effects, bladder hyperplasia and chronic glomerulonephritis were noted in some saccharin-fed rats, but not in both experiments. The incidence of glomerulonephritis was higher in the l-percent than in the 5-percent male rats in Saccharin 1.

5. Bio-Research Consultants (122)

*(a) Experimental Design*

Twenty-five male Charles River Rats (derived from Sprague-Dawley) were fed O-, 1-, or 5-percent saccharin diets from the age of 8 weeks through 104 weeks or longer. All animals that died after the first 6 months were necropsied except those badly autolyzed. Two experiments were run, using saccharin from different sources.

*(b) Results*

Body Weight: Saccharin had ". . . no significant effect upon body weight. '

Life Span: The l-percent rats in one experiment ". . . showed increased mortality after the first year of study, " but the difference was not considered significant.

Tumors: Results are presented in table 22.

**Table 22.—Number of Tumors in Male Rats in 1973 Bio-Research Consultants Study**

| | Rats with tumors | | | | |
|---|---|---|---|---|---|
| | Study 1 | | Study 2 | | Control |
| **Dose** (Percent) | 1 | 5 | 1 | 5 | o |
| Animals in Group | 13 | 12 | 15 | 14 | 16 |
| Tumors | | | | | |
| Pituitary Adenoma | **9** | **5** | 7 | 6 | 7 |
| Parathyroid Adenoma | **0** | **0** | 0 | 0 | 1 |
| S.C. Fibroma or Fibrosarcoma | **2** | **0** | 1 | 1 | 3 |
| Adrenal—Medullary | 1 | 1 | 3 | 1 | 2 |
| —Lymphangioma | 1 | **0** | 0 | 0 | 0 |
| Breast—Adenocarcinoma | 1 | **0** | 0 | 0 | 0 |
| Bladder—Noninvasive CA | **0** | 1 | 1 | 0 | 1 |
| —Papilloma | 1 | **0** | 0 | 1 | 0 |
| Stomach—Epidermoid CA | **0** | **0** | 1 | 0 | 0 |
| —Papilloma | **0** | 1 | 1 | 1 | 0 |
| Kidney—Tubular Adenoma | 1 | 0 | 0 | 0 | 0 |
| Spleen—Hemangioma | 0 | 1 | 1 | 0 | 0 |
| Skin—Epidermoid CA | 0 | 1 | 0 | 0 | 0 |
| Lymphoma | 0 | 0 | 1 | 0 | 0 |
| Lymph Node Hemangioma | 0 | 0 | 0 | 1 | 0 |

*(c) Discussion*

The authors concluded that their data did not show a correlation between saccharin and cancer.

*(d) Comments by Others*

The NAS committee (116) concurred with the authors' conclusion. Reuber (141) eliminated pituitary adenomas from his considerate ion because of its high incidence in all groups, and he concluded that saccharin was associated with higher incidences of total tumors and malignant tumors.

*(e) Comments by OTA*

Using the data in table 22 and eliminating the pituitary tumors from all groups and the parathyroid adenoma from the controls (because ‘‘neck organs were not included in the tissues to be examined under the contract’ ‘), OTA obtained the numbers shown in table 23. There is little difference in occurrences among the groups and no increase between the l-percent rats and the 5-percent rats.

**Table 23.—Tumors Other Than Pituitary in Male Rats in 1973 Bio-Research Consultants Study**

| Dose (Percent) | Rats with tumors/Rats examined | | |
|---|---|---|---|
| | Group 1 | Group 2 | Control |
| o ..... | | | 6/1 6 |
| 1 ..... | 7/1 3 | 9/1 5 | |
| 5 ..... | 5/1 2 | 5/1 4 | |

The data in table 22 show that the number of bladder lesions was not increased by saccharin ingestion. Only in the l-percent rats of Group 2 was there an increase in malignant tumors. No such increase appeared in either the 5-percent group or in the other l-percent group. The significance of the l-percent increase is therefore not clear.

6. Schmahl Study (152)

*(a) Experimental Design*

Groups of 104 Sprague-Dawley Rats (52 M, 52 F) were fed diets containing saccharin beginning at age 70 to 90 days until the animals died. After death, each animal was necropsied, and every bladder was examined histologically. These groups ingested either O-, 0.2-, or O. S-percent saccharin.

*(b) Results*

Weight Gain: No significant differences between experimentals and controls.

Blood Chemistry and Pressure: No differences between experimental and controls.

Life Span: No significant differences between experimental and controls.

Tumors: No significant differences between experimentals and controls.

*(c) Discussion*

The author concluded that there was no detectable carcinogenic effect of saccharin.

*(d)* **Comments** *by Others*

The NAS committee (116) listed this experiment in a table but made no comment about it.

Reuber (141) accepted the conclusion of the authors that no bladder cancers

were caused by saccharin but faulted the study for a number of technical reasons. He concluded, from inspection of the data, that the incidence of lymphomas and leukemias was higher in the 0.5-percent rats.

*(e) Comments by OTA*

This study has numerous shortcomings, but it is not immediately apparent why Reuber (141) singled this one out for criticism. As in other experiments: (1) this one was not published in a refereed journal (but see next paragraph); (2) histological examinations were carried out only when "during the dissection, conspicuous conditions were found, (but histology was performed on all bladders); and (3) the rats had a high spontaneous frequency of tumors. Additionally, the results were not separated by sex. Results are stated as number of tumors, and the number of animals examined is not given. The higher dose of saccharin was tenfold less than those used in the positive two-generation experiments.

Schmahl has published a paper in German. OTA did not review that paper, and the amount of overlap between data reviewed here and those presented in the German publication (151) is unknown. The translation available to OTA reports one lymphosarcoma in the controls, four in the 0.2-percent group, and two in the 0.5-percent group. The total is higher among the experimentals, but it does not increase with dose. In the case of leukemia, there was one in the controls, zero in the 0.2-percent group, and one in the 0.5-percent group.

**7.** Munro et al. (112)

*(a) Experimental Design*

Groups of 120 Charles River CD (COBS) rats (60 M, 60 F) were fed diets containing saccharin from weaning until 120 weeks. Constant dose levels were obtained by adjusting saccharin amounts at weekly intervals. The levels fed were O, 90, 270, 810, or 2430 mgm saccharin/kgm body weight/day. (The maximum level is approximately 5 percent, and these levels have been converted to percent of saccharin in the diet in the data presented here.)

Animals were visually examined each day for clinical signs of toxicity. Blood chemistry, urine chemistry, body weights, and food intake were determined. Animals dead or moribund during the experiment and all survivors at 120 weeks were subjected to detailed gross necropsy.

*(b) Results*

Body Weight: The 5-percent rats, both M and F, had reduced body weight and slight diarrhea at all times after 10 weeks.

Life Span: "A dose-related increase in mortality was observed in treated male rats. . but not in female rats. . ."

Hematology Examinations: No difference between controls and experimental.

Urine Chemistry Composition: No difference between controls and experimental.

Examination for Bladder Parasites: No ova of T. *crassicauda* were observed in urine, and no parasites were observed in any bladders.

Pathology: Detailed tables of neoplasms and other histological findings are presented, The incidence of lymphomas and leukemias found in this study are given in table 24.

**Table 24.—Number of Leukemias and Lymphomas in Examined Animals in 1974 Munro Study**

| Dose (Percent) | Rats with leukemias and lymphomas/Rats examined | |
|---|---|---|
| | Males | Females |
| o .. | 2/57 | 5/56 |
| 0.2 | 2/51 | 3/56 |
| 0.6 | 5/54 | 2/52 |
| 2 | 2/52 | 1 /56 |
| 5 | 7/54 | 1 /54 |

*(c) Discussion*

Authors state "saccharin administration was not accompanied by an increase in tumor incidence. "

*(d) Comments by Others*

The NAS committee (11) discussed only the incidence of urinary bladder tumors. They concurred with the authors' conclusion that there was no relationship between dosage and incidence of tumors as shown in table 25.

**Table 25.—Number of Urinary Bladder Cancers in 1974 Munro Study**

(Rats with tumors)

| Tumors | Dose (Percent) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | o | | 0.2 | | 0.6 | | 1.7 | | 5 | |
| | M | F | M | F | M | F | M | F | M | F |
| Angiosarcoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Transitional Papilloma | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 |

Reuber (141), discussing the same data, cited the NAS report (116), which reported that two of the four bladder papillomas were reclassified as carcinomas. He concluded that the small number of bladder tumors suggest ". . that saccharin may well be carcinogenic for the urinary bladder. " He drew attention to the incidence of leukemias and lymphomas in males, concluding that saccharin-fed males displayed slightly increased incidence of tumors compared to control males.

Reuber (141) also pointed out that certain "unusual or rare tumors" were observed in treated, but not in control rats as shown in table 26.

**Table 26.—Tumors in Saccharin-Fed and Not in Control Rats in 1974 Munro Study**

| | Tumor | Seen in |
|---|---|---|
| 1. | Hepatocellular Carcinoma (liver) | 0.6 percent males |
| 2. | Adenocarcinoma (prostate) | 0.6 percent males |
| 3. | Endometrial Adenocarcinoma | 1.7 percent females |
| 4. | Endometrial Adenocarcinoma | 5.0 percent females |
| 5. | Malignant Mesenchymal Tumor (uterus) | 5.0 percent females |
| 6. | Cholangiocarcinomas X 2 (liver) | 5.0 percent females |

*(e) Comments by OTA*

Bladder cancers do not appear to be related to saccharin dosage. There was evidence of some toxicity (diarrhea, slower weight gain) in both males and females as well as earlier death in males on the 5-percent diet compared to controls.

The conclusion that the small number of bladder cancers is higher in the experimental animals than in the controls is difficult to accept. If the angiosarcoma in the control group is accepted as a bladder cancer, then there was one tumor in 113 control animals; in the larger number of experimental animals, there were four tumors in 429 animals. Even zero tumors in 113 animals would not be significantly different from four tumors in 429.

The leukemia and lymphoma data for males suggest an increase due to saccharin ingestion. However, the female data are quite different. The incidence in female controls is higher than in any experimental group. These differences may reflect sex differences or, alternatively, some sampling artifact. The authors attached no significance to these findings.

The argument that the experimentals had tumors not seen in control animals must be weighed against the occurrence of some tumors in the control population that were not seen in the experimentals (table 27).

**Table 27.—Tumors in Control and Not in Saccharin-Fed Rats in 1974 Munro Study**

| | Tumors | Rats with tumors/Rats examined | |
|---|---|---|---|
| | | Controls | Experimental |
| 1. | Adenocarcinoma (large intestine) | 1/1 13 | 0/429 |
| 2. | Fibrosarcoma (urethra) | 1/1 13 | 0/429 |
| 3. | Hemangioendothelioma (skin) | 1/1 13 | 0/429 |
| 4. | Fibrosarcoma (uterus) | 1 /56 | 0/21 8 |
| 5. | Osteogenic sarcoma (rib) | 1/1 13 | 0/429 |

This experiment has a number of features to recommend it: (1) it was published in a refereed journal; (2) the causal role of stones or bladder parasites was eliminated; and (3) reasonable numbers of animals were used.

The 1977 Canadian Study pathology records show that the mean time for appearance of bladder tumors is about 24 months. In Munro et al. (112), only about 10 animals remained alive in each group at 24 months (62). If more animals had lived that long, the incidence of bladder tumors might have been higher.

### 8. Summary: OTA Discussion of One-Generation Feeding Experiments

Certainly the best documented study (and one of only three published) is Munro et al. (112). The authors of that study concluded, "saccharin administration was not accompanied by an increase in tumor incidence" (in that experiment). The NAS committee and OTA agree with that conclusion. As mentioned in our discussion of this experiment, the small number of animals alive at 24 months may be considered a flaw in the experimental design. Had there been more animals, more tumors might have been detected. This same general criticism can be made for other experiments: too few animals were used, or too few survived 24 months.

The remainder of the experiments inspire less confidence. Different investigators concentrated on different pathologies. This fact may account for the relative absence of similar lesions in the reports of different studies. Although each of these experiments can be faulted, the investigators in all of them drew the conclusion that saccharin was not carcinogenic.

## C. Cocarcinogeiss Experiments With Rats

Hicks and colleagues (69, 70) devised a model system for studying induction of bladder cancer in Wistar rats. A single dose of N-methyl-N-nitrosourea (MNU) was instilled through a urethral catheter into the bladder. 'Such a single dose of 2.0 mg MNU acts as an initiator, but it does not produce a carcinoma unless additional doses of MNU are administered. Three additional instillations at biweekly intervals resulted in all animals developing either transitional cell carcinomas or transitional plus squamous cell carcinomas of the bladder epitheliums.

Hicks et al. (69) added saccharin to the drinking water of animals that received a single dose of MNU. Appropriate controls were run (see table 28). MNU alone caused hyperplasia but no tumors. Saccharin produced two mildly hyperplastic responses. The combination of saccharin and MNU produced pathology in 11 of the 12 bladders examined, and tumors in 5. The dose of saccharin (2g/kg body weight/day) is equivalent to about 4-percent dietary saccharin.

Bladder stones were found in about 20 percent of the MNU animals, none of the saccharin animals, and 6/12 MNU + saccharin animals. Each of the five tumor-bearing MNU + saccharin rats had bladder stones.

At the time this experiment was published, about one-fourth of the rats had been examined. No followup paper detailing the results of the study of the remaining animals has been found.

The second paper by Hicks et al. (70) provides additional data about saccharin as a cocarcinogen, but it does not specify what data included in reference 57 were published in reference 56, nor does it say whether results in reference 57 were obtained on animals mentioned in reference 56.

These data show that spontaneous tumors occur rarely in Wistar Rats, and Hicks (68) states that none has been seen in 10-years observation of about 600 control rats. MNU alone produced no tumors. Saccharin was associated with a small number of late-appearing tumors. In contrast, the number and time of appearance of tumors were markedly changed when both MNU and saccharin were administered.

**Table 28.—Cocarcinogenicity of Saccharin in 1973 Hicks Study**

| Group | Treatment | Total No. of Animals in Group | No. Killed so far for Examination | Time Killed | Condition of Bladder Epitheliums |
|---|---|---|---|---|---|
| A | None | 50 | 12 | Between 9 and 56 weeks | Normal |
| B | 2.0 mg MNU by intra-vesicular instillation | 50 | 12 | Between 3 and 50 weeks | 3/1 2 hyperplastic. All killed 12 wks. after dosing<br><br>9/1 2 normal. Killed between 12 and 50 wks after dosing |
| c | 2.0 g kg$^{-1}$ body weight d$^{-1}$ saccharin in drinking water | 50 | 12 | Between 9 and 56 weeks | 1 0/12 normal. 2/1 2 mildly hyperplastic. Killed after 36 wks. |
| D | 2.0 mg MNU at week 6 plus 2.0 g kg$^{-1}$ body weight d$^{-1}$ saccharin in drinking water from week O | 50 | 12 | Between 9 and 56 weeks | 1/1 2 normal.* 6/1 2 hyperplastic* 5/1 2 epithelial tumors.* |

• **See Table 29.**

For a control, MNU-treated rats were also treated with cyclophosphamide, which causes urothelial necrosis followed by hyperplasia in both ‘animals and man. No tumors resulted from treatment with both of these agents.

The authors’ conclusion from these experiments was that the model will detect weak bladder carcinogens, and that saccharin is such a weak carcinogen. Furthermore, these experiments show that stones were associated with some but not all tumors, that some bladders had stones but no tumors, and that tumors occurred in animals free of the worm Trichosomoides *crassicauda.*

Mohr (108) is in the process of repeating Hicks’ experiments. Early results from his experiments have failed to demonstrate the earlier appearance of cancers in rats

treated with MNU + saccharin. Therefore, at the present time the published experiments show that saccharin is a cocarcinogen, and unpublished work, still in progress, show that it may not be a cocarcinogen.

**Table 29.—Bladder Histology of Rats Treated With MNU and Saccharin in 1973 Hicks Study**

| Condition of Epitheliums | **No.** of Animals | Time Killed | **No.** of Animals with Calculi in Bladder |
|---|---|---|---|
| "Normal" (3 cell thick) | 1 | 20 weeks | o |
| Mildly hyperplastic (up to 6 cell layers) | 2 | 3 and 10 weeks | 1 |
| Grossly hyperplastic plus invasion of epitheliums by blood capillaries | 4 | 12,13,16 and 30 weeks | 1 |
| Papillary outgrowths and/or polyploid nodular hyperplasia with solid downgrowths | 2 | 8 and 24 weeks | 2 |
| Invasive carcinoma with both transitional and squamous cell elements | 3 | 21, and 50 weeks | 3 |

**Table 30.—Incidence of Bladder Tumors in Cocarcinogenicity Experiments in 1975 Hicks Study**

| Treatment | **No.** of Rats | No. with Bladder Tumors | Percent Incidence of Tumors | No. of Weeks First Tumor Seen |
|---|---|---|---|---|
| None . . . . . | 98 | o | o | — |
| MNU . . . . . | 124 | 0 | 0 | — |
| Saccharin . . . . . | 253 | 4[b] | 1.6 | **95** |
| MNU + Saccharin[a] . . . . . | **79** | 46 | 58.0 | 8 |

[a]**Given at 4-8 percent dietary levels (2-4 g/kg body weight/day),**
[b]**Tumros observed macroscopically** in males; **histology underway.**

Reuber (141) reviewed these studies and concurred that saccharin was a cocarcinogen in these studies. He commented that the data in table 29 show that at 12 weeks following MNU + saccharin, there were severe hyperplastic lesions; at 24 weeks, there were nodules; and at 50 weeks, there were carcinomas.

The hyperplasia at 12 weeks may be a precursor to cancer. Hicks (70) pointed out, however, that while the combination of MNU + cyclophosphamide produced hyperplasia and hyperpolyploid cells in the epitheliums, no cancers resulted.

These studies provide an interesting model for possible exploration. They also provide data consistent with saccharin's being a carcinogenic agent.

## TESTING OF SACCHARIN IN MICE

### A. One-Generation Feeding Experiments

At least four one-generation saccharin feeding experiments have been carried out in mice. All of them were considered negative by their authors. Reuber (141) has selected some data from these experiments and presented them as positive. OTA concludes that some data from two studies may show saccharin's carcinogenicity, and that a third study is inconclusive or negative; the fourth study was not reviewed,

1. National Institute of Hygienic Sciences, Tokyo (124)

*(a) Experimental Design*

Genetically homogeneous mice of the dde strain were fed saccharin for 21 months. Each group contained 100 mice (50 M, 50 F), and saccharin was fed at O, 0.2, 1,0, or 5.0 percent of the diet.

*(b) Results*

Mortality: Ingestion of saccharin had no effect on mortality.

Body Weight: There were no significant effects of saccharin on body weight.

Tumor Incidence: Results are in table 31.

*(c) Discussion*

The authors' discussion was not available.

**Table 31.—Incidence of Tumors in Necropsied Male Mice in Japanese Study (undated)**

| Dose (Percent) | Rats with tumors/Rats examined | | | | |
|---|---|---|---|---|---|
| | Time of Necropsy | | | | |
| | Unspecified | 12 mo. | 18 mo. | 21 mo. | Total |
| o ..... | 1 /27 | 0/5 | 6/5 | 1 2/13 | 1 9/50 |
| 0.2. ..... | 1 /23 | 1/5 | 2/5 | 0/17 | 4/50 |
| 1.0. ..... | 3/22 | “ 0/5 | 2/5 | 8/1 8 | 1 3/50 |
| 5.0. ..... | 1/1 9 | 1/5 | 4/5 | 20/21 | 26/50 |

*(d) Comments by Others*

Reuber (119) concluded that there was a significant increase in ovarian tumors in mice ingesting saccharin (see table 32).

**Table 32.—incidence of Uterine Cancers in Necropsied Mice in Japanese Study (undated)**

| Dose (Percent) | Rats with tumors/Rats examined | | | | |
|---|---|---|---|---|---|
| | Time of Necropsy | | | | |
| | Unspecified | 12 mo. | 18 mo. | 21 mo. | Total |
| 0 | **0/26** | **0/5** | 1/5 | **0/14** | **1 /50** |
| 0.2 | **0/22** | **0/5** | **0/5** | **3/18** | **3/50** |
| 1.0 | **0/29** | **0/5** | **0/5** | **7/11** | **7/50** |
| 5.0 | **0/28** | **0/5** | **0/5** | **6/12** | **6/50** |

*(e) Comments by OTA*

Interpretation of this experiment is difficult. If the data for uterine cancers at 21 months are considered alone, cancer incidence appears to increase with saccharin dose (table 32). However, the data for females throughout the experiment do not support that conclusion. The data do not convincingly show that saccharin caused or did not cause cancer,

Data from animals examined at 21 months support Reuber's conclusion. While there is no clear-cut relationship between dose and incidence, the incidence in the treated animals is higher. Nevertheless, this effect may be an artifact of this experiment; the following experiment did not report an increase in uterine cancers in mice fed 5-percent saccharin.

**2.** Bio-Research Consultants (122)

*(a) Experimental Design*

Groups of 26 male and 26 female randomly bred mice were fed saccharin at 10,000 ppm (about 1 percent) or 50,000 ppm (about 5 percent) of their diet. All animals dying after 6 months were necropsied, and survivors at 2 years were sacrificed and necropsied. Two experiments were run in parallel with saccharin from different sources as the only variable between them.

*(b) Results*

Data from determining tumor incidence are shown in table 33.

*(c) Discussion*

The incidence of bladder cancer did not differ in the experimental and controls. None of the three bladder tumors was malignant. One group of treated male mice displayed an increase in incidence of vascular tumors, but the increase was neither statistically significant nor confirmed in the duplicate experiment.

**Table 33.—incidence of Tumors, Bladder Tumors, and Vascular Tumors Found in Necropsied Mice in 1973 Bio-Research Consultants Study**

| Dose (Percent) | Mice with tumors/Mice examined | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| | Total | Bladder | Vascular | Total | Bladder | Vascular |
| Controls | | | | | | |
| 0 | 7/1 9 | **1/1 9** | **1/1 9** | 14/17 | 0/17 | 1/17 |
| Experiment 1 | | | | | | |
| 1 | 16/1 4 | 0/14 | 1/14 | 7/14 | 0/14 | 1/14 |
| 5 | 1 3/15 | 0/15 | 2/15 | 17/18 | 0/18 | 4/18 |
| Experiment 2 | | | | | | |
| 1 | 1 3/15 | 0/15 | 1/15 | 15/14 | 0/14 | 4/14 |
| 5 | 1 7/19 | 2/19 | 8/19 | 16/18 | 0/18 | 3/18 |

*(d) Comments by Others*

Reuber (141) noted an increase in lung tumors in l-percent male mice (data not shown) and increased numbers of total and vascular tumors in male mice.

*(e) Comments by OTA*

These data support Reuber's contention concerning an association between saccharin and an increase in total and vascular tumors in males; furthermore, the number of vascular tumors was increased in saccharin-fed female mice. Because of the absence of an additional increase in lung tumors at 5 percent, however, OTA does not accept the conclusion from these data that lung cancer was caused by saccharin.

The other mice-feeding experiments have produced no results that support these findings. OTA interprets this experiment as being more positive than its authors proposed, but in need of verification.

3. Roe **et** al. **1970 (144)**

*(a) Experimental Design*

The general design of these experiments resembles the cocarcinogenesis experiments of Hicks (69,70). Female Swiss albino mice were treated with an oral dose of 50 mg benzo [a] pyrene (BP) in 0.2 ml polyethylene glycol (PEG). Seven days after this exposure, one group of 50 treated animals was started on a diet which contained 5-percent saccharin. Suitable controls, PEG alone, BP + PEG, PEG + saccharin were included and two other sweeteners, cyclamate and sucrose, were tested in parallel.

Animals were examined for "obvious tumor development" at weekly intervals and daily for general health, and sick animals were killed. All killed animals, those that died unobserved, and all those terminally sacrificed at 18 months were examined. All organs including the bladder were examined microscopically, but microscopic examinations were made only of identified or suspected neoplasms.

*(b) Results*

Treatment with BP or BP and saccharin did not affect longevity, although BP-treated saccharin-fed mice weighed less throughout the experiment than did non-BP-treated saccharin-fed mice. The authors had no explanation for this finding and considered it possibly spurious.

Microscopic examination for tumors produced the results shown in table 34.

**Table 34.-Tumor Incidence (in Necropsied Animals) and Survival in 1970 Roe Study**

| Treatment | Dead Before 18 Mos.[a] | Tumors of Forestomach | Other Tumors |
|---|---|---|---|
| PEG | 26/1 00 | 0 | 10 |
| PEG & BP | 30/1 00 | 0 | 14 |
| PEG & Saccharin | 6/50 | o | 0 |
| PEG & BP & Saccharin | 1 0/50 | o | 4 |
| | **Sacrificed at 18 Mos.[a]** | **Tumors of Forestomach** | **Other Tumors** |
| PEG | 65/1 00 | 2 | 24 |
| PEG & BP | 61/1 00 | 21 | 29 |
| PEG & Saccharin | 36/50 | o | 13 |
| PEG & BP & Saccharin | 32/50 | 11 | 12 |

[a]**Mice with** tumers/Mice examined.

*(c) Discussion*

Administration of BP resulted in an increase in forestomach tumors, but feeding of saccharin did not cause a further increase. No macroscopic bladder tumors were observed.

*(d) Comments by Others.*

None.

*(e) Comments by OTA*

The absence of microscopic pathological examinations from this experiment makes it impossible to compare these results to experiments that included such examinations.

Sacrifice of animals at 18 months, of course, eliminated any possibility of detecting tumors that would have developed later. Because of this fact, the results of this experiment cannot be compared to those of the other two experiments (122,124) in which mice were sacrificed at 21 or 24 months.

4. Verschuuren, et al. (143)

OTA was unable to obtain this report.

## B. Implantation Experiments in Mice

Pellets of cholesterol containing saccharin were implanted into the urinary bladders of mice. Control animals received cholesterol pellets only. In both experiments (5,25) the incidence of tumors was significantly higher in the experimental.

**Table 35.—Survival of Mice Living More Than 175 Days After Bladder Implantation and incidence of Changes in Mouse Bladders With Implants of Sodium Saccharin Suspended in Cholesterol in 1971 Bryan Study**

| Experiment no. | Mice Examined | Average Survival (days) | Squamous Metaplasia | Carcinomas | | |
|---|---|---|---|---|---|---|
| | | | | Total | Percentage | P Value |
| | | Cholesterol alone | | | | |
| 1 | 63 | 378 | 1 | 8 | 13 | |
| 2 | 43 | 394 | 3 | 5 | 12 | |
| | | Saccharin & Cholesterol | | | | |
| 1 | 66 | 375 | 3 | 31 | 47 | <.001 |
| 2 | 64 | 396 | 6 | 33 | 52 | <.001 |

Positive results from implantation experiments were judged as warning signals of the possible carcinogenicity of saccharin (117). However, doubts were raised about how closely implantation mimics normal ingestion of saccharin, and the significance of tumor induction by implantation was questioned. The special concern about saccharin being a carcinogen of the bladder may have grown (at least partially) out of these studies. No regulatory action has been based on these studies.

## TESTING OF SACCHARIN IN OTHER ANIMALS

## A. Hamster Experiments (6)

### 1. Experimental Design

Groups of randomly bred Syrian golden hamsters were given 0.0-, 0.156-, 0.312-, 0.625-, or 1.25-percent saccharin in their drinking water for life. A group of 30 males and 30 females was fed at each level. Gross and histological examinations were performed on all hamsters.

### 2. Results

No tabular data were presented. The incidence of tumors was 10.1 percent in 169 controls and 14.9 percent in the 299 animals ingesting saccharin.

### 3. Discussion

The authors reported that the organ distribution and histological types of

neoplasms were within the range found for spontaneous tumors. They concluded that saccharin was not carcinogenic.

#### 4. Comments by Others

None.

#### 5. Comments by OTA

This experiment appears to have been well-executed. It produced no evidence for carcinogenicity. The levels of dietary saccharin given to the hamsters were less than those given to rats, and the experiment was of one generation duration. Therefore, while it is not positive, neither does it contradict the findings of the two-generation rat experiments.

### B. Monkey Experiments (35)

#### 1. Experimental Design

Aqueous sodium saccharin was administered by stomach tube to rhesus monkeys for 6.7 years. Doses of 20, 100, or 500 mg saccharin/kg/day (6 days a week) were given respectively, to 2, 2, and 3 monkeys. Three animals of each sex were used as controls. Routine hematological examinations and assays of serum components were carried out at 6-month intervals. During the sixth year of the test, urine was examined to determine whether long-term administration had caused any detectable metabolic adaptation.

#### 2. Results

Three saccharin-fed monkeys died during the 6.7 years of testing. There were no tumors in their bladders. No metabolic adaptation was detected in the saccharin-fed animals, and no pathologies were associated with the sweetener.

#### 3. Discussion

The authors concluded, “Our experience to date tends to reinforce the mounting evidence of noncarcinogenicity” (35).

#### 4. Comments by Others

None.

#### 5. Comments by OTA

The results of this experiment support the thesis that ingestion of saccharin by monkeys for 6.7 years does not induce new patterns of metabolism. This experiment may not have been suitable to test carcinogenicity because of the small numbers of animals involved and because the route of ingestion does not mimic human exposure. But it does show that ingestion of saccharin for 7 years produced no ill effects in monkeys.

## SUMMARY STATEMENT ABOUT ANIMAL TESTING

In summary, the three most sensitive tests (49,67,165) have produced data showing saccharin is a carcinogen. While none of the three studies considered alone might have allowed a conclusion about the effect of saccharin on the bladder, the three taken together allow conclusions to be made.

The data from all three experiments show that: (1) saccharin-fed second generation rats had more bladder cancers than did control animals, (2) the bladder cancers were only weakly invasive and did not metastasize, (3) bladder cancers occurred in animals ingesting saccharin at 5 or 7,5 percent of their diet, and (4) male rats are more sensitive than females.

A statistical analysis of the results of the two-generation rat feeding experiments is shown in table 36. Results are presented as a fraction; the numerator is the number of bladder cancers, and the denominator is the number of animals (at least 18 months old) examined. In each experiment, the number of cancers found in the saccharin-fed animals exceeded the number found in the controls.

**Table 36.—Results and a Statistical Analysis of the Two-Generation Rat Feeding Experiments**

| Study | Generation | Dose[a] (Percent) | Males[b] | Females[b] | Significance |
|---|---|---|---|---|---|
| Canada, 1977 (55) | Parental | 0<br>5 | **1 /36**<br>**7/38** | 0/38<br>0/40 | p=0.075 |
| Canada, 1 977(55) | Offspring | 0<br>5 | **0/42**<br>**12/45** | 0/47<br>2/49 | p=0.003 |
| FDA, 1973 (37) | Offspring | 0<br>7.5 | **1 /25**<br>**7/23** | 0/24<br>2/31 | p=0.0l7 |
| WARF, 1973 (135) | Offspring | 0<br>5 | **0/10**<br>**8/15** | 0/16<br>0/20 | p=0.021 |

[a]Saccharin as a percent of the total diet. Five (5) percent is equal to about 2.5 g saccharin/kg body weight/day.
[b]Rats with bladder cancer/Rats examined.

To conclude, the two-generation experiments showed that saccharin caused an increase in bladder cancer and especially among males.

Two experiments (69,70) concerning the cocarcinogenic potential of saccharin are discussed in published reports. Instillation of single small doses of a chemical (methylnitrosourea, MNU) into the bladders of rats did not cause cancer; however, repeated instillations of the same chemical at 2-week intervals did cause cancer. The first dose is called an “initiator” dose, and the subsequent doses are “promotor” doses. Saccharin was shown to be a potent promotor. Ingestion of 2-percent or 4-percent saccharin before and after receiving one dose of MNU caused a significant increase in bladder cancers compared to animals receiving only MNU. Specific objections can be directed at efforts to apply these results to human conditions. It is

unlikely that humans encounter MNU, but it is equally clear that we are exposed to many other chemicals. The possibility exists that saccharin might be a cocarcinogen with other chemicals in our environment.

The results of implanting pellets of saccharin in mice bladders show that saccharin caused cancer in that animal (5,25). A number of reservations have been attached to extending the finding from implantation experiments to the human population. However, about 50 percent of animals exposed to saccharin in this way developed bladder cancers as compared to 13 percent in animals exposed to cholesterol only. Regardless of the interpretation placed on these experiments, it is important to note that saccharin has been shown to cause cancer in an animal other than the rat.

Data from one-generation rat feeding experiments have sometimes been judged to be positive and sometimes negative. Some of the studies do not support any conclusions. None of the negative experiments followed the protocols used in the two-generation experiments, and therefore no data contradict those positive results.

A committee of the NAS reported in 1970 that the two long-term rat feeding experiments available to them at that time were inadequate when judged against the current standards for animal testing (1 17). In 1974, seven additional studies were evaluated by a NAS committee (116). According to NAS, none of the studies provided positive evidence for saccharin's carcinogenicity. The design and execution of only one of those experiments, however, approaches the current guidelines for animal testing. It is more prudent to conclude that these studies were not conducted adequately than that they were negative.

Because of the deficiencies in the one-generation experiments, their data are viewed with skepticism. The data from the two-generation experiments lead to the conclusion that saccharin ingestion is associated with cancer. The lack of association in the one-generation experiments does not force any qualification of this conclusion.

At least four one-generation feeding experiments have been carried out in mice. Although none of them produced convincing evidence that saccharin causes cancer, neither are they convincingly negative. Experiments carried out with hamsters and monkeys showed that saccharin was not a carcinogen in those animals under the conditions of those experiments.

Biochemistry performed during the course of the monkey experiments showed that ingestion of saccharin for several years did not induce changes in metabolism. These results agree with others that show ingested saccharin is excreted unchanged, that is, without being metabolized (17).

This brief recapitulation of the history of saccharin illustrates how rapidly the nature of testing for carcinogenicity has evolved. Recently performed, more sensitive experiments have demonstrated the carcinogenicity of saccharin. Earlier, less sensitive experiments did not.

An important caution is attached to this part of the report. "Saccharin" is a mixture that contains the named substance and other chemicals. Although the saccharin used in the most recent experiment contains only about 20 parts per million impurities, it cannot be eliminated that an impurity is the carcinogen in saccharin. This possibility does not alter the conclusion that the mixture sold as "saccharin" is a carcinogen. Instead it may be that the chemical saccharin is safe, but the product consumed as "saccharin" may have a carcinogenic substance in it.

In summary, the three most sensitive tests have produced data showing saccharin is a carcinogen. Other experiments have not produced convincing data to support that conclusion. OTA has presented its reasons for considering some of the negative experiments to be inconclusive rather than convincing,

It was mentioned above that none of the negative experiments followed protocols comparable to the positive experiments. Therefore, there are no data from similar experiments that contradict the positive experiments. To date, none of the experiments (except implantation experiments) in species other than the rat have produced unequivocal evidence that saccharin is a carcinogen. These results may mean either that the experiments were negative or that they were not sufficiently sensitive. Whichever, it is prudent to take the results of experiments in the most sensitive species to determine carcinogenicity (121).

## EXTRAPOLATIONS FROM LABORATORY DATA TO HUMANS

### A. General Considerations

Several types of extrapolations can be applied to data from animal tests. Three types of extrapolations are listed below.

1. **From detected cancer incidence at high doses to projected incidence at low doses.** Usually, high doses of carcinogens are administered to produce a detectable number of cancers in small groups of animals; in the case of saccharin, effective doses are about 5 percent or more of the diet. A number of methods have been employed to extrapolate from incidence at high doses to predicted incidence at much lower doses.
2. From carcinogenicity **in animals to carcinogenicity in humans.** It is generally accepted that an animal carcinogen is also a human carcinogen. Extrapolation between cancer incidence in animals and expected incidence in humans is necessary to quantify the risk for human populations from exposure to a chemical.
3. **From mutagenesis frequency to carcinogenesis frequency.** Many carcinogens have been found to be mutagenic in short-term tests. This correlation serves as the basis for saying that agents shown to be mutagenic are probably carcinogenic.. Methods for quantitatively extrapolating from the mutagenic potency of a chemical to its expected carcinogenicity in laboratory animals have been developed.

The validity of conclusions reached by extrapolations depends on the accuracy and reliability of the experimental data as well as on the extrapolation procedures. Unfortunately, the saccharin data are not so good as to eliminate all misgivings about using them for quantitative estimates of human risk. The data are good enough, however, to allow the qualitative judgment that saccharin presents a potential risk to human health.

The interpretation of dose response curves depends on the model used for extrapolation. Acute toxicity testing produces dose response curves with “no-effect” or “threshold” levels. No toxicity is associated with doses below such levels. Mutagenicity testing, on the other hand, produces curves with no thresholds. Even

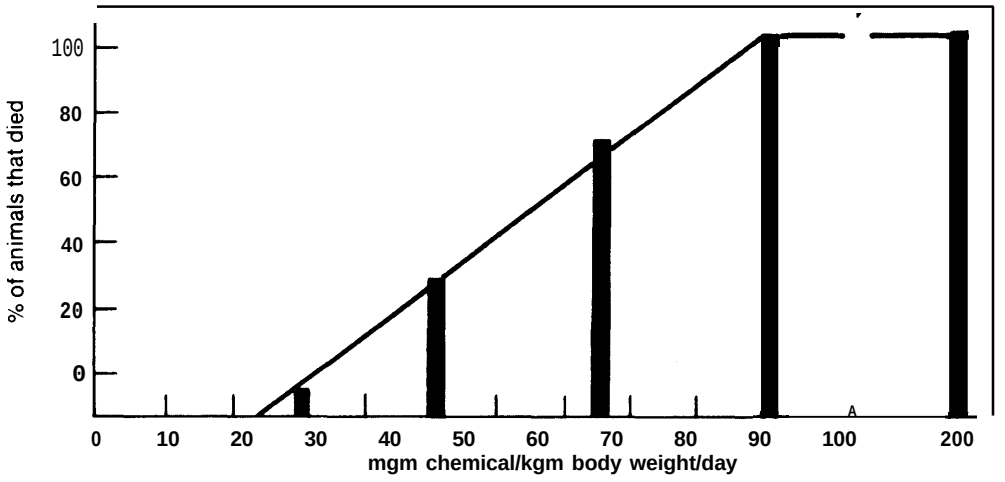
very small doses have an effect. There is a lack of agreement about which of these curves describes the incidence of cancers in a population exposed to a carcinogen, and no experiment designed to decide between the two models is accepted as definitive.

## B. Dose-Response Relationship

### 1. Acute Toxicity Testing

Toxicity is usually tested in laboratory animals by dividing a population of genetically and nutritionally similar animals into subgroups. Each group is exposed to a particular amount of the substance in question, and the animals are observed over a period of time to detect possible deleterious effects of the substance. Figure 3 is a hypothetical example of the results for such a test to determine the dose of the substance that would be necessary to cause death in a population of animals.

**Figure 3-Hypothetical Toxicity Curve**



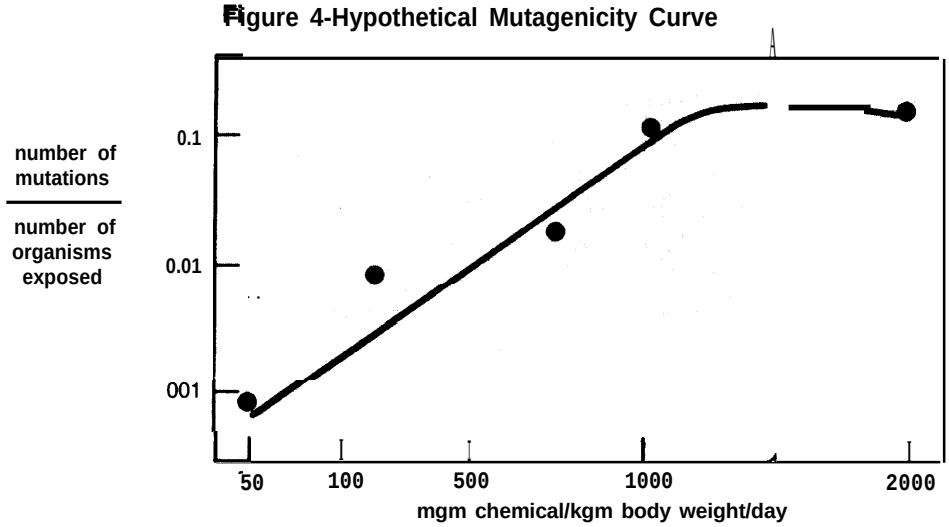

From the data, it can be concluded that doses of 100 and 200 mgm kill all animals, doses in the range from 30 to 100 mgm kill some fraction of animals, and doses of 10 to 20 mgm produce no deaths. Another conclusion to be drawn from these results is that at a dose somewhere between 20 and 30 mgm, there is a ‘‘no-ef feet level. ” Another term used to describe the no-effect level is “threshold.” Doses below the threshold level produce no effects. The line drawn in figure 3 is a “dose-response curve. ”

The outcome measured in the experiment in figure 3, death, concludes a complicated series of events. Death might result, for instance, if the substance kills cells. Increasing doses would kill more and more cells, but an organism would survive until the number of dead cells became intolerably large. Individual variations in the ability to tolerate dead cells would partially account for intermediate survival levels between doses of 30 and 100 mgm.

### *2.* Mutagenicity Testing

A different type of dose-response curve describes the induction of mutations in a population. Such a curve is shown in figure 4.

**Figure 4-Hypothetical Mutagenicity Curve**




This curve has no threshold level, As the dosage is decreased, the frequency of mutations becomes lower and lower but does not reach zero. Instead, it levels out at the frequency equal to the spontaneous mutation rate. Practically, it becomes more and more difficult to measure low level mutagenesis, i.e., at the lowest dose in figure 4, only about 1 individual in 1,000 would be expected to harbor a mutation. For technical reasons, many such mutagenic dose-response experiments have been conducted with microorganisms in which 100 million organisms can be rapidly screened.

In contrast to toxic substances that may have to injure or destroy a large number of cells to produce death, a single interaction between an agent and DNA can produce a mutation. After the interaction, the normal cellular metabolism replicates and perpetuates the mutation, Death requires injury and subsequent pathology; mutation requires only an injury followed by normal cellular functions. These differences between the two measured events, death and mutation, may at least partially explain the differences between figure 3 and figure 4. Mutations also occur spontaneously; some number of mutations will appear even with no dose.

### 3. Carcinogenicity Testing

Examples such as figures 3 and 4 spark little controversy. Toxicity testing usually produces a curve with a threshold value, an area of increasing response to increased dose, and a plateau. Mutagenicity testing produces a curve with no threshold, an area of increasing response to increasing dose, and a plateau. In contrast, there is some controversy about whether a curve such as that in figure 3 or that in figure **4** describes the frequency of cancer as the dose of carcinogen decreases.

Some scientists suggest that an animals' immune system can protect it against cancer (27). If the immune system has a finite capacity to recognize and eliminate precancerous and cancerous cells, no carcinogen would cause a cancer until that capacity

is swamped. Such a mechanism could result in a threshold. However, there is little experimental support for this idea, and other scientists think that the immune system may promote tumor growth (138).

Many chemical carcinogens act after conversion of the administered compound to a highly reactive metabolize. This metabolize, in turn, chemically links to a vital component of the cell. In the case of some compounds that cause tissue damage, the cell contains protective agents, which inactivate the reactive metabolize. Cell damage occurs only after the supply of the protective substance has been exhausted. Additionally, other mechanisms may exist to detoxify or to excrete dangerous chemicals and metabolizes or to repair damaged DNA. If the efficiency of these protective systems is perfect until a finite limit is exceeded and if the system fails at that point, a threshold might result.

There are a number of problems involved in designing and executing an experiment to decide whether there is a threshold dose for a carcinogen. Large numbers of animals would be required, the experiment would be very expensive, and inspecting a large number of animals for cancers introduces many possibilities for human error. Even if such an experiment were adequately conducted, and a threshold obtained, this result would not necessarily mean that a threshold exists in human populations. Members of a laboratory animal population are inbred and genetically very similar. Humans, on the other hand, are outbred and genetically quite dissimilar. If all of the animals have the same capacity to detoxify small amounts of a chemical, this ability could account for the threshold. If humans have a similar capacity for detoxification, this capacity would be expected to vary widely among members of the population because of their genetic differences. Some people might be very sensitive; others less so. This wide variation makes it impossible to predict a threshold for human populations.

Carcinogenicity studies conducted with animals rely on doses many times higher than those to which humans are exposed. Such doses are necessary because of the limitations of animal experiments. Usually, groups of about 100 animals (50 male, 50 female) are exposed to a constant dose of a carcinogen. In the 1977 Canadian Study, 8.9 percent of animals exposed to 5-percent dietary saccharin from the time of weaning developed tumors. A tenfold reduction in the dose to 0.5 percent would be expected to result in a tenfold reduction in tumor frequency to about 0.9 percent. Of course, 0.9 percent of a group of 100 animals is less than one animal. Increasing the size of the exposed population would allow detection of the lower frequencies, but the cost of scaling up the experiments is very high. Those who accept the curve in figure 4 as a representation of carcinogenesis say that positive effects at high doses demonstrate a danger at any level. Those who believe that there is a threshold (figure 3) say that high dose experiments show only that high doses cause cancer, and that doses below the threshold may pose no danger. Determining a threshold level for any carcinogen has so far been impossible. Cancer, however, is a relatively common disease. If thresholds exist, they are frequently exceeded, and any additional exposure to a carcinogen might push more individuals across the threshold.

## C. Extrapolation Methods

1. Extrapolation from detected incidence of cancer at high doses to expected incidence at lowest doses.

(a) The Question Of Thresholds. As already mentioned, an argument is sometimes made that there is a dose below which a carcinogen will not cause cancer. Craig

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and Miller (36), in a review of 151 dose-response curves, found none inconsistent with a no-threshold curve. Currently, the burden of proof is on those who espouse a threshold model. The subsequent discussion assumes that if a threshold exists, it has not been demonstrated, it has not been measured, and it cannot serve as a practical tool for making extrapolations.

*(b) The Probit-Curve or the Marztel-Bryan* (103) *Extrapolation.* This paper provides an easy-to-read introduction to the problem of establishing "absolute safety. " For example, what conclusions can be drawn if 100 animals are exposed to a dose of a suspected carcinogen, and no animal develops a tumor? At the 99-percent probability level, this experiment provides assurance that the true risk is less than or equal to 4.5 percent. Increasing the number of tested animals to 1,000 (assuming none develops cancer) reduces the risk to 0.45 percent, but it does not assure absolute safety.

This method estimates the risk of cancer by assuming that the risk decreases one standard deviation (one "probit") as the dose of carcinogen is decreased by 10. In the example of 100 cancer-free animals, the risk at that dose, D, is 4.5 percent. If it is decided that a risk of 1 cancer per 100 million exposed individuals represents "virtual safety" and is thus acceptable, this method calculates (using the one probit decrease for a tenfold dose-decrease relationship) that a dose of D/8,300 is acceptable. An attractive feature of this method is that it rewards "good testing. " For example, if the dose D is shown to be safe for 100 animals, the virtually safe acceptable dose would be D/8, 300: if 1,000 animals were used, the acceptable dose would be D/1,000; and if 50 were used, the acceptable dose would be D/18,000, Testing higher doses would result in acceptance of higher permissible levels. The paper also describes the application of this procedure to estimating risks when experiments show that cancers are caused by high doses.

In another readable paper Mantel and Schneiderman (105) argue for the general application of the Mantel-By ran model. However, this extrapolation procedure has been criticized because it leads to higher doses being associated with "virtually safe" incidence than does the single-event or one-hit hypothesis.

(c.) The Single-Event or One-Hit Hypothesis. This simple model proposes that the probability of a normal cell being transformed into a cancer cell varies directly with the dose. This relationship assumes that a single cell can be transformed into a cancer cell and can develop into a tumor. Evidence supports the idea that cancers result from single transformed cells (37).

Schneiderman (153) in testimony before the Congress used a no-threshold/linear model to estimate the number of bladder cancers to be expected in the United States if people areas sensitive to saccharin as are rats. Schneiderman used this model rather than one incorporating a probit or other relationship, although he has published (105) arguments for the use of probit relationships. Schneiderman (153) estimated that continual consumption of one 12 oz. diet soda per person per day would result in 600 to 1200 new cases of bladder cancer per year.

The one-hit models are most "conservative" in that they associate the highest risk with a given dose. Application of the one-hit model is recommended by Heel et al. (72) and the National Academy of Sciences (115).

The one-hit hypothesis fits some data available about humans. The incidence of leukemia among survivors of nuclear blasts, the incidence of various tumors follow-

ing occupational and therapeutic radiation, and the incidence of lung cancers in people who smoke cigarettes all vary directly with dose (quoted in 97 and see figure 1 for cigarette data).

(d) *Multiple stage models.* This family of theories proposes that more than one independent event is necessary to cause cancer. These models project curves that are concave upwards with increasing dose. Crump et al. (37) present arguments that at low doses such multihit models approach linearity.

(e. ) Other models and discussions. Many are recommended only for the statistically sophisticated and are listed in the references of Heel et al. (72).

### 2. Extrapolations From Animal Experiments to Man

With appropriate experimental design and attention to detail, convincing results showing a relationship between cancer and exposure of an animal to a chemical can be obtained. Some discussion and extrapolation from those results allow a family of curves to be constructed that relate the observed incidence at high doses to the expected incidence at low doses.

The National Academy of Sciences has recommended that carcinogenicity testing be carried out in more than one species and that the results obtained with the most sensitive animals be applied to human populations (115). Adjustments must be made for differences in dose between animals and humans. Heel, et al. (51) recommended that doses be adjusted on the basis of relative surface areas, which are calculated as (man's weight/test animal's weight)$^{2/3}$. When chemicals are administered in the diet, doses expressed as percent dietary intake or parts per million (ppm) require no further adjustment.

Carcinogenicity testing in more than one species may be especially important because "laboratory animals are inbred. A particular strain may be very sensitive or very insensitive to a particular agent. Human populations contain individuals of widely differing sensitivities. Extrapolations from inbred animals to human populations can be better made with more data, but considerations of safety require that data from the most sensitive animal model be used for estimating human risk.

## D. Relationship Between Short-Term Tests and Animal Tests

Meselson and Russell (107) have constructed a formula that relates the potency of a chemical in one short-term test, the Salmonella/Ames test, to its carcinogenicity in animals.

Fourteen chemicals that have been adequately studied in both animal systems and in the Salmonella/Ames test were included in the calculations. When carcinogenicity was plotted against mutagenicity, 10 of the chemicals fell on or near a straight line with a slope of 1. Therefore, mutagenicity correlates with carcinogenicity. Three nitrosoamines and N-nitrosomethylurea are not so mutagenic as expected. Refinements in the Salmonella/Ames test may improve the correlation between the mutagenicity and carcinogenicity of the nitroso compounds.

This demonstrated relationship is one of the first quantitative attempts to relate short-term mutagenicity testing to carcinogenicity. Combining this procedure with extrapolations from animals to humans could enable a person (a very brave one) to predict carcinogenicity of a chemical for humans on the basis of short-term test data.

No extrapolation from short-term testing of saccharin has been made. So far, short-term tests of saccharin have shown it to be nonmutagenic in the Salmonella/Ames test. A mixture of impurities extracted from saccharin is mutagenic, but the data are not yet firm enough to base extrapolations on them.

## E. Extrapolation of Saccharin Data

The tools for extrapolation are available, and experiments have produced data with which extrapolations can be made. Even so, questions arise about whether the data are adequate for the extrapolations and whether the extrapolations make accurate predictions.

Table 37 presents a number of extrapolations that have been made to estimate human risk in a population that ingests one can of diet soft drink, containing 120 to 150 mg saccharin, per person per day. In all the extrapolations, calculations are based on data from the two-generation rat experiments (49, 67, 165). It is assumed that the population at risk is 200 million people, that life expectancy is 70 years, and that human sensitivity is the same as that of the male rat.

The data clearly show that the method chosen for dose adjustment has a sizable effect on the extrapolated figure. At present, there is no generally accepted choice among the adjustments, and this table is presented primarily to show the variety of figures possible.

**Table 37.—Estimated Risks from Saccharin Consumption**

**Estimate 1**
Dose adjusted to surface area by the expression mg/kg/day [human] = 5.6 mg/kg/day [rat]

| *Method of extrapolation* | *Cases/year* |
|---|---|
| a. linear (71) | 3,400 |
| b. quadratic (a multistage model) (71) | 15 |

**Estimate 2**
Dose adjusted to body weight by the expression mg/kg/day [human] = mg/kg/day [rat]

| *Method of extrapolation* | *Cases/year* |
|---|---|
| a. linear (1 46) | 600 |
| b. linear (1 53) | 600 to 1200 |

**Estimate 3**
Lifetime dose adjusted to body weight by the expression mg/kg/lifetime [human] = mg/kg/lifetime [rat]

| *Method of extrapolation* | *Cases/year* |
|---|---|
| a. linear (23) | 15,000 |

## CONCLUSION

The animal studies on the possible carcinogenicity of saccharin have been analyzed, and reasons have been given for ascribing more confidence to some experiments than to others. The most reliable findings have been those from two-generation rat feeding experiments.

Several competing theories on extrapolating from the results of animal tests to humans have also been discussed, followed by application of some of the mathematical models to the available data on saccharin. Different models yield different results. There is no basis for judging which, if any, of these figures is accurate.