

3.

SACCHARIN RISKS

The experiment that determined cancer incidence in both generations found more tumors in the second. The numbers of tumors between first and second generations were different, but the probability that the difference would occur by chance alone was about 50 percent. This probability gives little evidence against equality. However, the apparent difference in incidence between the two generations raises a suspicion that *in utero* and breast-feeding exposure may be important factors in saccharin's causing bladder cancer. Although the direction of the difference is consistent with the hypothesis of greater risk to the second generation, the differences are not statistically significant at the 5-percent level.

In one of the two-generation experiments (165,177), an increased number of uterine cancers was associated with ingestion of 5-percent saccharin. This correlation has not been found in the other experiments, and the increase may have been a random event.

To conclude, the two-generation experiments showed that saccharin caused an increase in bladder cancer in second generation animals, especially among males. In the one experiment in which the first generation was also examined, the increase fell just short of the standard test of significance. No cancer of any other site has been convincingly associated with saccharin.

Other Animal Experiments. Publications from one laboratory report that saccharin *promotes* the growth of bladder tumors that were *initiated* by previous exposure to another chemical (69,70). This "cocarcinogenic" activity of saccharin is of potential importance because humans are exposed to many chemicals in addition to saccharin.

Implantation experiments showed that saccharin also causes cancer in mice (5,25). In those experiments, pellets of cholesterol containing saccharin were implanted in the bladders of mice. About 50 percent of animals exposed to saccharin in this way developed bladder cancers, compared to 13 percent in animals exposed to cholesterol only.

The particular combination of chemicals in the cocarcinogenesis experiment and the route of administration in the implantation experiments do not mimic human exposure to saccharin. Taken by themselves, results from these experiments would be considered warnings that saccharin may be a carcinogen. Taken in conjunction with the two-generation experiments, they support the conclusion that saccharin is a carcinogen in rats, and they show that it causes cancer in mice.

No association between saccharin ingestion and cancer was found in experiments using hamsters and monkeys. The hamster experiments were one-generation tests, and just as in the one-generation rat experiments, no association was found (4). Experiments showed that ingestion of saccharin at up to 500 mg/kg body weight for 7 years did not cause illness in monkeys, did not alter their metabolism, and did not cause cancer in the few animals that have died and been necropsied (35). But the monkey experiments, unlike the positive rat experiments, did not involve lifetime exposure to saccharin, used forced feeding procedures, and examined a very small number of animals.

A general problem occurs when discussing experiments on dangerous substances. What conclusions are to be drawn when some experiments show the substance caused cancer in animals and other experiments do not? In the particular case of saccharin, all two-generation experiments have been positive. A number of other

experiments have led some to conclude that saccharin is not a carcinogen. The Office of Technology Assessment reviewed those experiments and found none comparable in design to the three positive experiments. Furthermore, some others were too insensitive to have detected the carcinogenic effect of saccharin. This statement is no indictment of those experiments; cancer testing is rapidly evolving, and many older experiments are not now considered to be satisfactory. The positive two-generation studies come the closest of all that have been conducted to meeting the current testing standards.

The information gathered in this review of animal studies, especially the uniformly positive two-generation experiments, leads to the conclusion that saccharin should be considered a carcinogen for animals.

EXTRAPOLATION TO HUMANS

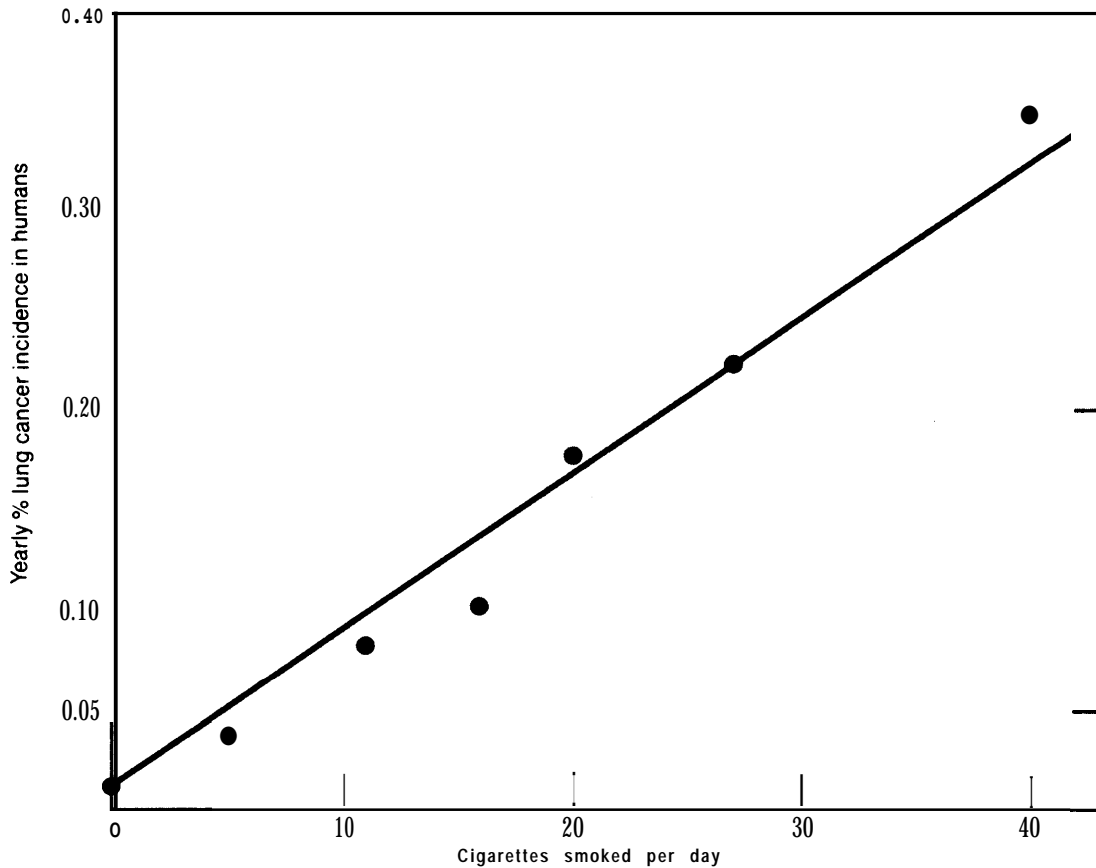
As explained above, standard procedure in animal experiments is to feed substances at the "maximum tolerated dose," which for saccharin is 5 percent of the diet. According to normal dose-response relationships, if cancer is produced in animals at high dose levels, it will also be produced at low dose levels, but in fewer animals.

Substantial evidence for this dose-response relationship exists for animals, but some of the most convincing evidence is derived from human experience. An example of such a dose-response in humans is incidence of cancer resulting from cigarette smoking. As shown in figure 1, the incidence of lung cancer in humans is greater for people who smoke a lot than for those who smoke only a little. At the lowest exposure levels for which there are data, about five cigarettes a day, only a very small fraction of people get lung cancer. But because very large groups of people were examined, these few cases could be detected.

To test these low doses of cigarettes in rats, one would have to design an experiment with thousands of rats in order to be able to detect the same incidence of cancer that occurred in people, a study that would be neither economically nor experimentally feasible. So that a smaller number of animals can be used, only higher doses are tested, a procedure resulting in a higher percentage of animals that develop cancer. In fact, because it is practical to use only about 100 or so animals in a cancer test, very high doses are chosen in an effort to cause cancer in at least 10 percent of the animals. (Cancer in 10 percent of people would clearly be a disaster.) All the evidence that has been accumulated so far suggests that this procedure is reasonable. Therefore, an amount of saccharin equivalent to 800 diet drinks a day was not an unreasonable dose to give to rats; if saccharin causes cancer in rats at such high doses, it is also very likely to cause it at lower doses.

Saccharin was found to be among the weakest carcinogens ever detected in rats, as illustrated in figure 2. The doses of a number of different carcinogens which cause cancer in half of the animals (rats or mice) treated are compared in this figure. There is over a million fold range of doses. In other words, chemical carcinogens are very different in their carcinogenic potencies. For example, aflatoxin (AF-B1), a substance produced by certain fungi and found in moldy peanuts and certain grains, causes cancer in 50 percent of rats at a dose of more than one million times less than the dose of another carcinogen, trichloroethylene (TCE), a chemical that, until recently, was used to extract caffeine in the manufacture of instant coffee. It has been classified as a

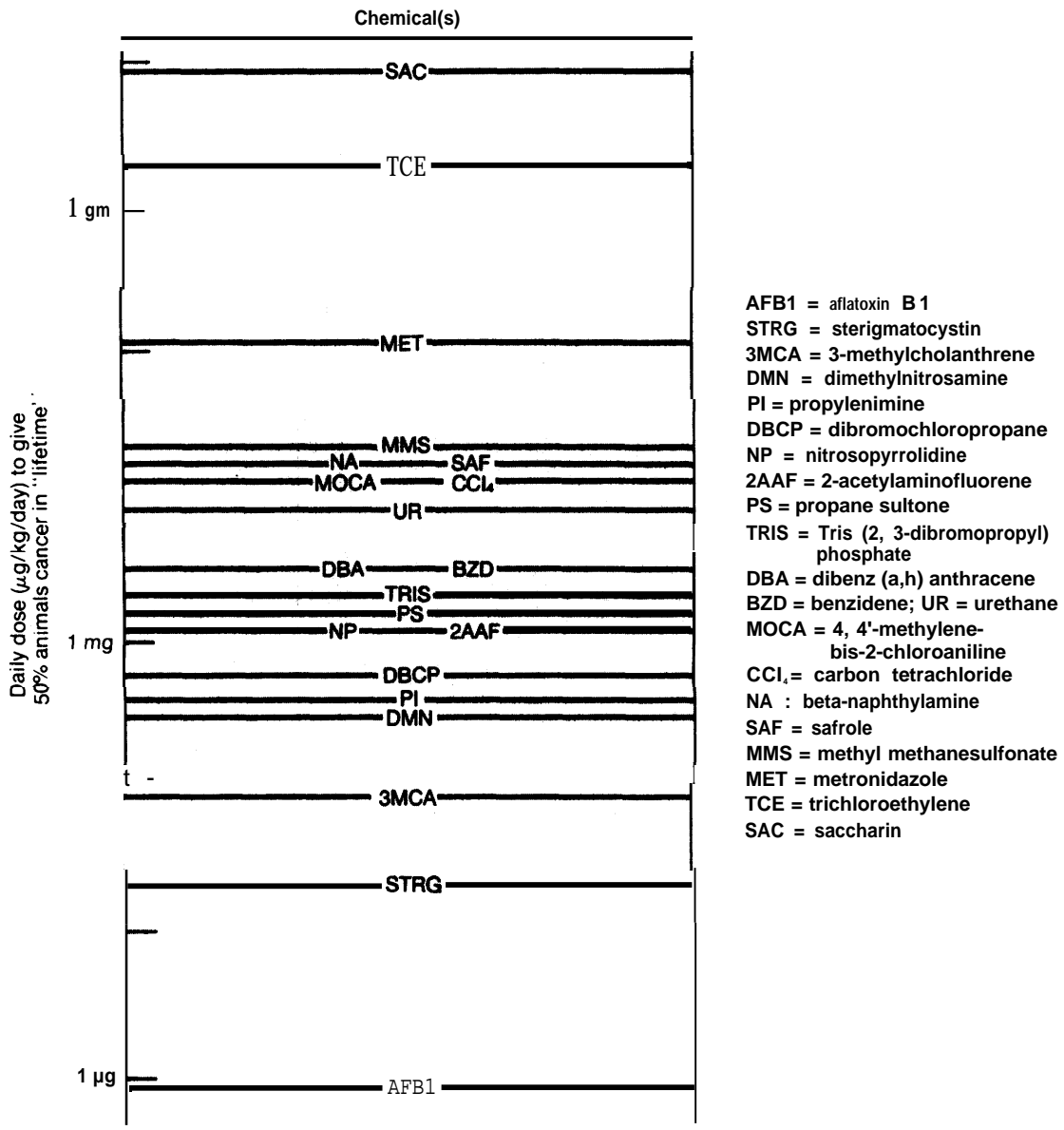
Figure 1—Incidence of Lung Cancer in Humans



food additive because small amounts are left as residue in the coffee, and it was recently banned by the FDA under the “Delaney clause.” If this millionfold range of carcinogenic potency in rats has any correspondence in people, it is clear that a tremendously different degree of human risk results from eating a peanut butter sandwich with a trace of aflatoxin in it as compared to drinking a cup of decaffeinated coffee containing the same amount of TCE. Where does saccharin fall on this millionfold scale? It actually extends the scale in the weak direction—it is slightly weaker than TCE.

Some evidence suggests that the potency of carcinogens in rodents may be a rough indicator of their potency in people. The evidence is admittedly fragmentary and subject to considerable uncertainty. However, to compare the strength of carcinogens in animals and people requires data on people. Because controlled experiments cannot be conducted on people, available information is limited to the few studies in which epidemiologists have been able to determine that a substance caused human cancer. This information is very difficult to obtain; estimates of dose levels of substances that have caused human cancer have been possible for only six substances. In most of these cases, a rough correlation exists between potency in rodents and in people (107). Given the enormous range of biological potencies of carcinogens possible, this rough correlation is quite important. If this same correlation holds for saccharin, then it seems reasonable to predict from the rat studies not just that saccharin

Figure 2-Carcinogenic Potency of Chemicals in Rats and Mice



Data calculated from experiments in the literature by Sawyer, Hooper, Friedman and Ames (unpublished). (Though it is clear that there is a millionfold range in carcinogenic potency, the exact location of individual points may change slightly as the calculations are refined.)

is likely to cause cancer in people, but also that it is likely to be a relatively weak carcinogen in people. It must be kept in mind that while some extrapolations have been validated, others are more complex. In the case of diethylstilbestrol (DES), for example, the chemical caused cancer in the liver in animals, but in the female reproductive organs in humans. Nevertheless, although the animal experiments did not predict the organ site, they did show that the chemical was a risk.

Is saccharin likely to be so weak a carcinogen in people that it should cause no concern? Probably not, for several reasons. *First*, in assessing potential human risk, one must take into account not only the potency of the carcinogen, but also the number of people who are likely to be exposed to it and the amount of the carcinogen to which they are likely to be exposed. For example, if one group of people is exposed to a weak carcinogen and another group to a more potent carcinogen, the actual number of cases of cancer can be larger in the group exposed to the weak carcinogen if either (a) the number of people in the group exposed to the weak carcinogen is much greater than in the group exposed to the more potent carcinogen; or (b) the number of people exposed in the two groups is the same, but the group exposed to the weak carcinogen is exposed to much higher doses. In the case of saccharin, because so many people are consuming saccharin in substantial amounts, risk estimates range up to several thousand expected new cases of cancer each year. This number of cases is substantial and, in fact, constitutes a large fraction of the current total incidence of bladder cancer. *Second*, the degree of uncertainty in these extrapolations is sizable and maybe wrong by a factor of 10 or possibly even 100, a factor that would raise the potential risk to clearly unacceptable levels. *Third*, saccharin is not the only carcinogen to which people are exposed, and the total body burden of carcinogens is of greatest concern. Any increment, even a relatively small one, to an already substantial burden of carcinogens must be taken very seriously.

SHORT-TERM TESTS

A number of sensitive short-term tests have been developed for use in predicting whether substances are likely to cause cancer. It seemed possible that conducting a rapid and coordinated battery of short-term tests on saccharin might clarify some of the uncertainties of the animal cancer tests. Government and industry officials are discussing how short-term test results should affect regulatory decisions concerning substances to which humans are exposed. The saccharin test battery, which took about 3 months to complete, illustrates one way short-term tests can be applied to a particular regulatory problem.

Twelve short-term tests on saccharin were commissioned by the OTA; 10 have been completed. The battery of tests was designed to determine, as definitively as possible within the time limits of this study, whether highly purified saccharin* is mutagenic or interacts with DNA. The test battery included many of the most sensitive short-term tests available. Criteria for including a test in the battery were: (1) sensitivity and validity for detecting carcinogens; (2) complementarily with the other tests and with test literature on saccharin; and (3) ability to be completed within the time constraints. Saccharin had been tested previously in only two** of these twelve short-term tests. The experiments reported here were conducted by the developers of the tests or recognized experts, who generously donated their time to this study. The

*All tests were conducted using the same sample of saccharin that was used in the most recent Canadian carcinogenicity tests in rats. This material, even though highly purified, still contains very small amounts (about 20 ppm) of impurities and is referred to as "impure saccharin." For this reason, all participating laboratories also received a sample of saccharin that had been specially purified to remove essentially all traces of impurities and is referred to as "pure saccharin."

**Several sex-linked recessive lethal tests in *Drosophila* have been published, with somewhat conflicting and uncertain results. Results obtained by Stolz, et al. (162) using the *Salmonella*/Ames test were independently confirmed for the OTA test battery.

results of these tests are discussed in appendix II, and a list of principal investigators is presented in table 38 of appendix II.

Results from three tests—sister chromatid exchange, mouse lymphoma, and” chromosome aberration—were positive. Highly purified samples of saccharin were weakly active in these tests, and the results are clearly suggestive that saccharin itself has mutagenic properties. The results should be regarded with some caution, however. The responses were very weak in the three tests, even at the high dose levels tested. And the value of the sister chromatid exchange, mouse lymphoma, and chromosome aberration tests in predicting carcinogenicity has not yet been firmly established. However, validation of the tests has begun by testing a number of carcinogens and mutagens and a few noncarcinogens, with promising results.

The seven other completed tests represent the gamut of short-term tests. Their results were negative, a fact that complements the generally negative results already reported in the literature (85). However, their negative results neither invalidate nor cast suspicion on the positive results. Short-term tests differ in their ability to detect dangerous substances, and it is conceivable that a substance would be positive in only some of the tests.

The ten experiments described above tested highly purified saccharin, but all the saccharin used in animal cancer testing contained some levels of impurities. Some reviewers of the saccharin literature have suggested that impurities might be carcinogens. Therefore, two laboratories have used the *Salmonzella/Ames* test to test impurities in the saccharin used in the 1977 Canadian Study of rats (67).

A sample of the saccharin used in the 1977 Canadian Study was chemically fractionated and found to contain about 20 parts per million (ppm) of impurities. Approximately 12 different impurities were present, but they have not been specifically identified or separated. Mutagenic tests on the residue containing the 12 impurities have yielded positive results in two separate laboratories (162,171). Whether the mutagenic impurities account for the carcinogenic activity of the saccharin in animals is unclear. However, results of the positive short-term tests on highly purified saccharin are consistent with the conclusion that saccharin itself is a carcinogen.

EPIDEMIOLOGICAL STUDIES

Three kinds of epidemiological evidence have been examined.

1. In Great Britain, where a large increase in saccharin consumption occurred during World War II, time trends in per capita consumption of saccharin and of cigarettes have been compared with trends in death rates from cancer of the bladder (12). Increased cigarette smoking can account for the steady increase in bladder cancer mortality among males born after 1870. However, no inflection of the curve of mortality occurred following the sharp increase in saccharin consumption during World War II. These data, while revealing no association between saccharin and cancer, cover only two or three decades of increased saccharin use. Furthermore, it is not possible in such general statistical reviews to sort out specific effects of a chemical that only an unspecified proportion of the general public might use.

2. In series of patients with cancer of the bladder (so-called “cases”) and unaffected persons (so-called “controls”), some information has been obtained on their use of saccharin or beverages containing saccharin and their medical histories with respect to diabetes. In none of several studies of this type—in the United States, Canada, and Britain (11,80, 109)—were statistically significant differences found between cases and controls. Data on saccharin use were in many instances incomplete and were not available for those patients who had died of bladder cancer, making comparisons difficult.
3. Series of patients with diabetes—a group that was shown to have heavier saccharin use than the general population (11)—have been followed over many years to determine the cause of their deaths. The observed numbers of deaths from cancer have been compared with the numbers expected at the same time on the basis of cancer mortality rates in the general population of the same age and sex. This methodology permits assessment not only of bladder cancer risk, but of risk of all cancers that are likely to cause death. In the two studies conducted to date—one in the United States (79) and one in Britain (13)—no significant excess of bladder cancer mortality was observed. In both studies a small excess of cancer of the pancreas was seen. Significant deficits of certain cancers (mouth, lung and esophagus) were observed, probably related to low rates of use of tobacco and/or alcoholic beverages by the diabetics. Much of the experience of these diabetics was of too short a duration to allow full evaluation of the cancer rates. In most cases, which individuals took saccharin and which did not was not actually known.

Although the epidemiological evidence fails to document an association between saccharin use and bladder cancer in humans, this finding must be interpreted with caution. Adequate evidence is simply not available. Epidemiological studies can provide very strong evidence of the causal relationship between environment and disease—particularly when they are positive. Most of the major known carcinogens for man—cigarette smoke, ionizing radiation, asbestos, sunlight, beta-naphthylamine, and nickel—were identified by epidemiological studies in man before they were identified as carcinogens in experimental animals. However, negative epidemiological studies are more difficult to interpret. Humans are usually exposed to carcinogens in doses far smaller than are used in animal experiments. The effects are consequently less frequent, and the number of people whose experience needs to be judged to detect the cancer effect is much greater.

Lack of certainty as to the validity of the data on exposure in case-control studies is a further reason for reservations in interpreting negative findings. Only a portion of the population uses saccharin, and the use of statistics from the total population may dilute any association that exists among the users—perhaps to the point of making it unobservable. Such a dilution may be responsible for lack of correlation between bladder cancer and the sharp increase in British consumption of saccharin.

In the followup studies of diabetics, the same problems exist. The problem of small numbers is particularly evident in the British study (13), where there were only four deaths from bladder cancer (5.8 expected). Furthermore, in the British cohort of diabetics, information from a different sample indicated that by the end of the study period, only 23 percent of the survivors would have taken saccharin daily for 10 years, and only 10 percent for 25 years or more (11). Numbers are larger in the

American cohort, but no information exists on the use of saccharin by the American patients (80).

The period between exposure and appearance of cancer is a problem common to all these types of studies. This latent period introduces two kinds of difficulties—it again “dilutes” study groups so that they contain fewer individuals who are truly at risk than appears to be the case, and allowance cannot easily be made for the latent period because its duration is unknown. The latent period is almost certainly a matter of years, rather than months, but experience with known carcinogens in humans ranges from 2 to 40 or more years. In the context of saccharin, the latent period may be a particular problem in the studies of diabetes. A large proportion of diabetics have onset of the disease late in life and may not survive the latent period, dying of other causes before developing bladder cancer.

In all three types of epidemiological studies, making the association of primary interest is complicated by extraneous but important variables such as occupation and cigarette smoking. Such variables may not only introduce a false association, but may also hide an association that does exist.

The conditions of the two-generation animal tests are not likely to have been frequently duplicated in the epidemiological studies. The implications for humans of the apparent sex difference in susceptibility of rats are also unclear. Epidemiological data on human bladder cancer indicate that the sex difference (an excess in males compared to females) is explained by differences in exposure to known occupational carcinogens and cigarette smoke, variables that could hardly have been relevant in the animal test.

UNRESOLVED QUESTIONS

Several questions concerning the carcinogenic effect of saccharin remain unanswered:

1. What is (are) the carcinogenic agent(s) in commercial saccharin?
No conclusions can be drawn as to whether it is the chemical saccharin itself, one or more of the impurities, or combinations of both.
2. What is the significance of the increased sensitivity of the male rat bladder as compared to that of the female?
Differences in bladder cancer incidence between human males and females can be explained by exposure factors. The factors contributing to the difference in the test rats are not known.
3. Is the carcinogenic effect limited to the bladder?
The induction of tumors in a specific location in test animals does not necessarily predict that the carcinogenic effect in humans will occur in the same site. But it has been shown that a substance that causes cancer in test animals is likely to cause cancer in humans.
4. Does in utero and breast-feeding exposure lead to greater risks of cancer from saccharin?
In the one experiment which examined both first- and second-generation rats, the cancer incidence of the first (F_0) generation was just short of being statistically significant ($p = 0.075$), while the cancer incidence of the second

(F₁) generation was significant ($p = 0.003$). The probability of the difference between F₀ and F₁ cancer incidence being significant, however, is only about 50 percent. It is therefore not clear whether additional testing would show that saccharin causes cancer only in the second generation, or whether the first generation is also susceptible. If the second generation is more sensitive, two possibilities explain the differences between F₀ and F₁: 1) the additional length of exposure for the second generation in the gestational and suckling periods; or 2) the F₁ generation may be susceptible due to the special circumstances of in *utero* and/or breast-feeding exposure.

5. What are the mechanisms by which saccharin causes cancer?
Although there have been great advances in understanding the mechanisms whereby some chemicals induce cancer, nothing is known of the mechanisms by which saccharin may cause cancer. Research elucidating the mechanism could enhance future assessments of the human risk of saccharin and could conceivably shed light on the problem of bladder cancer in general. Many chemical carcinogens are converted in the body to highly reactive metabolites that bind to key components of the cell to initiate cancer. Humans may form such reactive metabolites to a greater or lesser degree than test animals, thereby influencing the relative susceptibility to the carcinogen. Although saccharin is excreted largely unchanged, a minor metabolite could become important at high doses.