UPDATE: PROPER PERSPECTIVES IN EXTRAPOLATION OF EXPERIMENTAL CARCINOGENESIS DATA TO HUMANS

by
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I. Introduction

Currently many have expressed the view that the regulation of carcinogens allows minimal discretion for decision-making. To those concerned with food additives and food processing, the Delaney Clause of the Food Drug and Cosmetic Act represents the legislation which can be invoked to ban the addition of characterized carcinogenic food additives to the food supply where such chemicals or processes have demonstrated a carcinogenic response in experimental animals. Obviously, the legislation does not provide for weighing benefits against risks. Indeed, to many scientific groups the “amendment” does not provide much flexibility in evaluation of risk. However, one phrase in the clause, namely, “by appropriate methods,” in this writer’s opinion, should be more judiciously utilized. Therefore, this report will dwell mostly on how appropriate and/or relevant are the experimental procedures for defining carcinogenicity and the ultimate assessment of risk.

It has been suggested by some that the “regulators” first calculate the human risk of cancer by use of laboratory animal and mathematical models for extrapolation of data to human risk and then balance these estimated risks against certain benefits. This procedure for extrapolation of risk is commonly referred to as quantitative risk assessment, and, has had some appeal, in that one can provide discrete numbers, i.e., numbers of cancer deaths per one million or one hundred million of the population. These ratios or numbers can be compared to other numbers of cancer risk from expected rates to ascertain any differential comparative rates of increases or decreases. These procedures have some flexibility, depending on how you look at this process, in that this characteristic of flexibility may be increased or decreased. The increased flexibility is the ability to compare quantified risks with computed benefits. The decreased flexibility is the inability to decide on a material or chemical on a list of apparently more hazardous chemicals, and, this obviates a prioritization scheme.

The prevailing view is that qualitative interpretation of animal data is usually a scientifically sound procedure because animals may respond similarly to man to carcinogenic chemicals and, therefore, substances that cause cancer in “properly designed” experiments using “properly interpreted data” should predict accurately as to hazard and safety in man. Some reservations are held about whether many experiments are properly designed to meet biochemical and pharmacological criteria. Furthermore, serious reservations are held as to whether one can use appropriately the quantitative extrapolation from laboratory animal data to human events or experiences. The reason for this concern is that we have not advanced our scientific knowledge to that point to make regulatory decisions strictly on the basis of quantitative risk assessment.

In a communication, dealing with quantitative risk assessment, from the Director of the National Cancer Institute to the Commissioner of the Food and Drug Administration, he cites six cogent reasons for concerns as to quantitative risk assessment (1). These concerns are delineated in the following section on quantitative assessments followed by a section on qualitative assessment.

II. SPECIFIC PROBLEMS IN QUANTITATIVE RISK ASSESSMENT

A. MATHEMATICAL MODELS

Various mathematical models have been used. Indeed, the National Academy of Sciences (2), in 1978, cited ten different statistical models and the Food Safety Council has similarly referenced some models available for low dose risk assessment (3). All these models cannot be described in detail but the significant point is that they predict different answers. For example, a multistage model provides an estimated risk five million times higher than the lowest estimate using another model. Obviously, the best model would be that one which is reflective of the biological
mechanism of cancer in man. Thus, one is left with the probability of using one's favorite model to predict risk while our current knowledge on biology of cancer is insufficient to permit one to use a uniformly accepted model for extrapolation.

B. SPECIES SENSITIVITIES

This factor will be discussed again under qualitative factors. Exposure to a given carcinogen for one species or even one strain within a species will show a different response (tumor incidence) than others. The response in one species may be negative while in another it could be high. Using data from various sources one can illustrate that an estimated risk of 4.2 cancers for USA populations (220 million) extrapolated from mouse or rat data could be as low as no cancer or as high as 4.2 x 10^5 cancers. Thus, opportunity is large for errors in estimates.

C. ADDITIVE EFFECTS

Over the years, each agency makes regulations to protect the public in terms of a chemical or agent in question (standard, tolerance, etc.). It is hypothesized that in the low dose exposure response region of a curve, especially in the linear phase, an incremental dose from another low level exposure to a carcinogen could produce a proportional increase in cancer. While this may seem generally correct, agents or chemicals can act in a neutralizing or inhibitory way (example, DES inhibition on aflatoxin). Thus, one regulatory agency may permit a different level of exposure to a carcinogen than another.

D. SYNERGISTIC EFFECTS

Current bioassays are oriented around exposures to a single carcinogen. Human populations never receive single exposures and, as such, some agents can synergize other agents to augment carcinogenic effects (examples, cigarette smoking and asbestos exposure). Additionally, exposures to carcinogens in air could be augmented by exposures to carcinogens in water, diet, drugs and the workplace. This is reflecting the concept of integrated carcinogenic stress. As mentioned earlier, one cannot generalize because in some cases antagonistic effects could yield lower than expected cancer rates (examples, protective effects of retinoids or vitamin A in lung cancer and vitamin C in blocking nitrite — secondary amine interaction to form carcinogenic nitrosamines).

E. LOW DOSE EXTRAPOLATION

Consideration of biological effects in the low dose-exposure region of a dose-response curve comes under consideration in qualitative assessment or risk. Specifically, one usually extrapolates from high dose exposure of a small number of rodents to a low dosage exposure to predict for a large number of people. All of this is predicted on the assumption that the incidence of tumors is proportional to the dose. This assumes linearity. But, if linearity does not hold, and reasons to believe in certain cases that it does not, then an overstatement of risk is present. One could certainly use more estimates of average exposure to estimate risks as well as data on distribution of exposure. Such data and information is rarely available.

F. SPECIFICITY OF TUMOR SITES

Most agents may have a specific or major target site for tumor induction. The animal reflects a tumor specific site but this may not hold for man. Thus, risk estimates made from animal data on one type of tumor would lead to assessment for one form of cancer whereas another form of cancer for man may prevail which is not estimated. All of the above factors are enumerated in Table 1.

III. LIMITING FACTORS IN QUALITATIVE RISK ASSESSMENT BASED ON ANIMAL MODELS

Previous remarks have been made concerning the adequacy of experimental designs and the problems encountered in interpretation of data and findings from such investigations. In Table 2 some of the limiting factors which should be considered in this regard are enumerated. If such penetrating analyses were always performed in the evaluation of carcinogenic studies, it is conceivable that the scientific community at large would be less tempted to engage in debates on the safety and risk of environmental chemicals.

| TABLE 1 |
| LIMITING FACTORS IN QUANTITATIVE RISK ASSESSMENT |
| • PREDICTIVE VARIANCE IN USE OF MATHEMATICAL MODELS |
| • VARIANCE IN ESTIMATES FROM SPECIES AND STRAIN SENSITIVITIES |
| • SIGNIFICANCE OF ADDITIVE EFFECTS |
| • SIGNIFICANCE OF SYNERGISTIC EFFECTS |
| • SIGNIFICANCE OF ANTAGONISTIC EFFECTS |
| • ERRORS IN PREDICTABILITY FROM HIGH DOSE TO LOW DOSE EXPOSURE |
| • ERRORS IN ASSESSMENT FROM EXTRAPOLATION FROM ANIMAL SITE SPECIFICITY TO CANCER TYPES IN MAN |

Reference: Upton, A. C. (1979)
TABLE 2
LIMITING FACTORS IN QUALITATIVE RISK ASSESSMENT BASED ON ANIMAL MODELS (CARCINOGENICITY STUDIES)

- INAPPROPRIATE ROUTE OF ADMINISTRATION
- IMPROPER TEST SPECIES AND STRAINS (SUSCEPTIBLE SPECIES AND STRAINS)
- ROLE OF DIET, NUTRITION, DIET AND WATER CONTAMINANTS
- CONTAMINANTS IN TEST CHEMICAL
- EFFECTS OF IMMUNOSUPPRESSION - HORMONAL ACTION
- EXCESSIVE BODY BURDEN - LIPOPHILIC SUBSTANCES
- TIME-TO-TUMOR FORMATION - POTENCY
- EXCESSIVE DOSING - INTOXICATION - METABOLIC OVERLOAD
- CHEMICAL FUNCTIONING AS NUTRIENT AT LOW DOSE


A. INAPPROPRIATE ROUTE OF ADMINISTRATION

Food additives, for example, should be tested through oral administration and not by subcutaneous injection. Bladder implantation, while a curious and interesting technique for showing some stimulatory effect, may not have relevance for interpretation of results for agents normally ingested.

B. INAPPROPRIATE TEST SPECIES AND STRAINS

Frequently sub-human primates are considered closest to man in comparison of biological response. However, it is not always practical to use monkeys. The rodent which is frequently used as the test species, should be of known genetic history and spontaneous tumor incidence. Purebred or inbred strains are frequently used where a heightened effect or accelerated tumor incidence is looked for by observing an additive response of the test chemical to a given agent that induces tumors of the same type and organ site. Some consider this procedure tantamount to recommending to an analytical chemist that he use a dirty test tube.

C. ROLE OF DIET, NUTRITION, DIET AND WATER CONTAMINANTS

Whether a chow or semi-synthetic ration is used influences the observed tumor response and incidence. The levels of protein, fat and carbohydrate also affect the final results. Overlooked too often is the possibility of diet contamination and the fact that the contaminants can synergize or potentiate the response of the test chemical. Not considered also is the potential effect of carcinogenic and mutagenic biorefractories present in the drinking water. Currently identified are 23 carcinogens, 30 mutagens, and 11 promoters (5).

D. CONTAMINANTS IN TEST CHEMICALS

Quite frequently a minor contaminant in a test chemical may be responsible for the observed response. This would suggest in many instances that the purified chemical and the technical grade be tested to ascertain the true result not conditioned by contamination.

E. EFFECTS OF IMMUNOSUPPRESSION AND HORMONAL ACTION

Studies in laboratory animals (6,7) and man (8) have shown evidence that immunosuppression, especially cell mediated immunity using thymectomy and antilymphocyte serum, can enhance the chances of developing spontaneous neoplasms and, accordingly, increase the risk of development of cancers in response to exposure of known carcinogens. Tumors thus induced may arise from transformed cells which are free to multiply under such suppressed cell mediated immunity. Similarly, under artificial laboratory conditions, an alteration in hormonal status could also reveal a positive response as an artifact in exploring the carcinogenic activity of a chemical. Indeed, illustrations of this interaction under laboratory conditions, and, one may assume that more will follow, as, for example, the challenge of the chemical in terms of response enhanced by an estrogen in the milieu.

F. EXCESSIVE BODY BURDEN—LIPOPHILIC SUBSTANCES

Failure to consider the lipophilic properties of certain chemicals, such as the organochlorine compounds, which are retained in the adipose tissue, and, ultimately, released either through overload (sink phenomena) or starvation, has led to disastrous results in a bioassay. The embarrassing feature was that high incidence of mortalities resulted from the overload phenomena. Also, it should be emphasized that those chemicals which have binding properties may be assumed to pose a greater hazard than those rapidly metabolized or excreted. It is also interesting that lipophilic chemicals such as DDT or the metabolite DDE are retained for years after the initial exposure and appear not only in organs and lipid tissue but also in the circulating blood supply and spill over into the milk during lactation. If any of these chemicals are carcinogens they may thus provide a constant carcinogenic insult.
G. TIME-TO-TUMOR FORMATION

Working on the original hypotheses of Druckrey (9), Jones (10), and later Jones and Grendon (11), developed a concept on latency period where cancer incidence could occur beyond the lifespan. In terms of time this, in effect, is envisioning a threshold effect in that tumors or cancer could be induced beyond the average lifespan of animals and/or man. Some investigators (12) challenge a time-to-tumor formation concept in terms of a practical threshold because, they claim, that human data in the USA shows dose median age relationship, and, thus argue, that the relationship between dose and latent period may be an artifact of high doses producing many tumors per animal, the first of which could kill the host before the others can become clinically noticeable. The arguments are curious in that such considerations are not resorted to in defense of propositions relevant to the weaknesses of metabolic overload in experimental design. If the Jones-Grendon concept holds then one has another criteria in definition of potency and the ultimate quantitation of risk.

H. INTOXICATION AND METABOLIC OVERLOAD

This may be one of the most significant weaknesses in experimental design. Ancillary to this is the ultimate publication of results that must be interpreted from the standpoint as to whether they are scientifically or toxicologically invalid. This is an area that has been the subject of many reviews. For example, the selection of a high dose of maximum tolerated dose is justified on the basis that this increases the "power of the test" when small groups of animals are used. Obviously, such alternatives have appeal. They are fully understood, but, what is often neglected, is the influence of that dose on enzyme systems and metabolic pathways. Biochemically and pharmacologically, such an admonition should be appreciated. Some will say that they know of no cases where experiments have exhibited this phenomena of overdosing. They have but to examine some of the studies reported in the literature. Chemical carcinogens exhibit large differences in the dose required for a given level of tumor induction but many share common biological properties including metabolizing to electrophilic intermediates capable of interacting with DNA. The potential for intoxication is always present and, if tumors are produced in this dose-response region, one cannot be sure that this is not an artifact or non-representative response due to an abnormal overload. This is not to say that high doses necessarily produce cancer. Indeed, many times the chemical is lethal at the MTD. As stated above, if the animal survives, one needs to be sure that the test is toxicologically relevant.

I. BIOCHEMICAL INTERMEDIATES—CHEMICAL AT LOW DOSE FUNCTIONING PHYSIOLOGICALLY AS NUTRIENT

The aspect of dose dependency has been repeatedly emphasized and demonstrated in the field of nutritional sciences. Some nutrients such as selenium, calcium, xylitol and sucrose have shown some tumorigenic effects at critically high doses (13). These cases should be instructive in illustrating that a chemical at an appropriate dose level can stress certain biochemical mechanisms whereas at lower concentrations it is required for homeostasis or for proper physiological function in a mammalian system. It is hoped that a program to use biochemical intermediates (vitamins, essential minerals, amino acids and endogenous compounds or metabolites) as research probes in this area of carcinogenesis methodology will be instructive (Table 3).

IV. ASSESSMENT OF RISK WITH FOOD ADDITIVES IN MEAT PLACED IN PROPER PERSPECTIVE

Whether one is considering an additive or an adulterant, the case of nitrite in cured meat needs to be

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Physiological level required for biological function</th>
<th>Observed toxic dose for response</th>
<th>Adverse effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>0.1 - 0.5 ppm Prevents red blood cell damage</td>
<td>5 ppm in rat</td>
<td>Liver tumors</td>
</tr>
<tr>
<td>Calcium</td>
<td>Man 0.8 gms/day Bovine 15-19 gms/day</td>
<td>88 gms</td>
<td>Ultimo-branchial tumors in bulls</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Undetermined - Essential element Deficiency - effect on spleen and red blood cells</td>
<td>400-600 ug/l in water</td>
<td>Skin cancer</td>
</tr>
<tr>
<td>Sucrose</td>
<td>For energy, level ?</td>
<td>20% in diet of rodent</td>
<td>Kidney tumors</td>
</tr>
<tr>
<td>Xylitol</td>
<td>Nonessential but endogenous chemical In pentose metabolism - energy production</td>
<td>20% in diet of rodent</td>
<td>Tumors of adrenal glands - bladder</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>400 IU, - Ricetts prevention, Ca and P metabolism</td>
<td>1 ppm in diet of C3H tumor virus strain of mice</td>
<td>Mammary tumors</td>
</tr>
<tr>
<td>Orthoamino-phenols (Metabolites of tryptophan)</td>
<td>Dose ? in tryptophan metabolism</td>
<td>9-11 mg in 4 parts of cholesterol in bladder implants</td>
<td>Bladder tumors</td>
</tr>
</tbody>
</table>

Reference 13.
put into some proper perspective. The role of body burden, absorption, metabolism and blocking effects by in vivo interactions all impinge on the degree of potential risk.

A. BODY BURDEN

It is estimated that 2 percent of the exposure of humans to nitrite comes from meats cured with nitrite; the remaining 98 percent of the exposure from other sources (14). According to Tannenbaum (15), the average daily intake of nitrite from meat products is of the order of 3 mg per adult per day. Spinach on the other hand, especially when stored, may have several hundred ppm of nitrite. Rodomski et al (16) demonstrated that high vegetable diets resulted in peak urinary concentrations of 270-425 ppm. Drinking water with negligible amounts of nitrate produced a urinary output of 48 ppm of NO₃, but drinking water with added nitrite and a diet of no cured meats increased urinary levels to 87 ppm. Drinking water can be a significant contributor since recycled water using contaminated surface water (feed lot runoff of nitrogenous materials and agricultural runoff) is a source of NO₃ in potable water. An epidemiological study in England by Hill et al (17) indicated that high nitrate drinking water was presumably responsible for higher gastric cancer rates in one community over those that had lower levels of NO₃ in water.

Subjects on controlled diets excrete much more nitrate in their urine than they ingest (15). Thus, nitrate and nitrite must be formed in the body. The quantity formed this way is roughly 80-90 mg of nitrate per day according to Tannenbaum (15). Some individuals had an excess as much as 750 mg per day.

Saliva may contribute as low a level of nitrite as 5 to 20 mg per day or as high as 100 mg per day or 30 times the level in cured meat. The salivary nitrite from salivary glands occurs from oral nitrate which is reduced from nitrate by bacteria or endogenous synthesis which takes place in the intestine absorbed into the blood streams and recycled to tissues and the salivary glands. The occurrence in saliva could take place in a matter of hours after food is ingested. Some saliva could have several hundred or thousands of parts per million of nitrite where high nitrate vegetables are consumed (15).

The gastric nitrite is of the order of 1 ppm but can increase markedly where pH of the gastric content is such as to permit bacterial reduction of nitrate to nitrite.

Bladder infections can give rise to higher than normal nitrite levels and smoking with formation of oxides of nitrogen could in the lungs be converted to nitrite.

B. THE EXPOSURE

As indicated earlier, the various sources relate to ultimate exposure as reflected in blood levels, gastric levels, salivary levels, urinary levels and fecal levels. Food and water sources may be contributors but the series of reactions in the gut and intestine relate to the overall nitrite balance. High vegetarian diets certainly contribute marked increases in nitrite levels in the body. The lower gastrointestinal levels of nitrite, according to Tannenbaum (15), are 25 times greater than that from cured meat products.

C. INTERACTION AND BLOCKING EFFECTS

One should be cognizant of the effect of dilution and competing reactions. The dilution effect from saliva to gastric juice reduces the potential concentration of N-nitroso compounds that may be formed. Moreover, nitrite in meats entering the stomach is accompanied by amino acids and/or proteins and the blocking agents ascorbic acid (vitamin C) and alpha tocopherol (vitamin E). Mirvish et al (18) showed that sodium ascorbate inhibited adenoma induction in strain A mice where nitrite and amines were present in the diet. Gallic acid also inhibited adenoma induction. Stools of normal individuals showed a marked reduction in mutagens when four grams of vitamin C was given orally each day for two weeks. The mutagens were probably nitrosamines or nitrosamides (19).

D. EVALUATION OF RELATIVE RISK

By not adding nitrite to meat until other preventive measures are developed increases the risk of botulism since nitrite inhibits formation of botulinium toxin. This fact places regulatory agencies into quite a dilemma. The agencies have asked for a delay on any ban.

Comparing the probable risk of nitrite addition to other risks from naturally occurring carcinogens such as estrogens, calcium, selenium, ergot, vitamin D₂, xylitol (glucoronic pathway metabolite) and aflatoxin may be an interesting exercise as to relative risk. We cannot guarantee absolute safety or zero risk but since our lives are filled with relative risks perhaps it is conceivable to assess the problem in this manner. One should consider the separate risk of conversion of nitrite to carcinogenic N-nitroso compounds and the interaction of nitrite formed internally, exclusive of additions which can interact directly with various internal organs. From this viewpoint the relative risk
of gastric nitrosation from nitrite in cured meats containing blocking agents such as ascorbic acid is indeed minimal. Furthermore, from animal and human experiments, nitrite introduced orally or intragastrically can be rapidly destroyed (15). As emphasized previously, the relative risk of nitrite from cured meats to the blood is orders of magnitude less than that which could prevail through the endogenous formation of nitrite in the gastrointestinal area. One thing certain is no regulation nor legislation can control of the situation with salivary formation of nitrite and microbial formation of nitrite in the gut. Since this insult prevails, the only argument left for an adversarial position is the concept of addition of risk from orally administered nitrite.

Society will have to weigh whether that addition is permissible. In Table 4 a summarization is given of associated exposures to nitrite and the determinative factors which are requisite in an evaluation of relative risk from meat nitrite dietary insult. These variable and inadvertent exposures should be considered in setting forth a perspective on the proportionalization of total risk.

V. SUMMARY

Animal data in carcinogenesis studies are presumptive evidence which may connote some concern in terms of probable risk to man. While considerable debate ensues on extrapolation of findings from animal tests, certain criteria and predictive factors should be used in a critical evaluation of such data in terms of real relevance to man. This evaluative process should be adopted in all cases. Both understatement and overstatement of risk can occur from inappropriate design of studies. Until more sophisticated biochemical/pharmacological approaches are developed to better define the shape of the dose-response curve, one will inevitably have to accept the woefully inadequate extrapolation, and quantification of risk, from animal dose-response data using mathematical models.

The problem of orally administered or additive nitrite via cured meats is a classical problem of relative risk. Scientifically, the degree of risk would appear minimal, and, even if removed, a far larger element of risk will be in existence from endogenous formation of nitrite. Here is an issue which demands careful deliberation, application of perspective and rationalization which is indeed one for a societal decision.

REFERENCES


