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Author(s): P. J. Gehring

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Name P. J. GEHRING Department H&ER Phone 6-1089

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PERSPECTIVES ON CARCINOGENS

by

P. J. Gehring
Health and Environmental Research
Dow Chemical U.S.A.
Midland, MI 48640 U.S.A.

Presented at the Florida Audubon Society, "You and the Environment and Cancer" A Symposium on Environmental Carcinogenesis. October 26-28, 1978, Orlando, Florida.

As evidenced by the holding of this conference, there is great anxiety that cancer is increasing in epidemic proportions. Some have associated this alleged increase with modern technology and in particular man-made chemicals. This often made, but seldom documented association, has resulted from the often quoted estimate that 80 to 90 percent of cancer is attributable to environmental agents. Unfortunately, the qualifier for this statement seldom accompanies it. "Environmental agents" include sunshine, smoking, poor or overnutrition, etc.

Wynder and Gori (1977) estimate that radiation from the sun contributes 8 to 10 percent of our cancer load; tobacco 9 percent for females and 29 percent for males; and diet 59 and 41 percent for females and males, respectively. Their estimate for occupation was less than 5 percent. Dr. John Higginson of the International Agency for Research on Cancer (IARC) (1976) has estimated that less than 1 to 3 percent of cancer is attributable to occupation and attributes most environmentally induced cancer to our personal habits.

Thus, a reasonable question to address, at least briefly, is whether cancer is increasing and if it is why? Figure 1 depicts the age-adjusted cancer death rate in the U.S. for men between 1930 and 1974 Cox, (1976) and Devesa and Silverman (1978). There has been a dramatic decrease in cancer of the stomach perhaps associated with improvements of food preservation; refrigeration instead of smoking and curing agents as well as lesser contamination with bacteria and fungi. Lung cancer has increased dramatically and this has been attributed primarily to

smoking. Not only has lung cancer been associated with smoking, but 30 to 50 percent of the mortality in males from cancer of the esophagus, kidney, bladder, and pancreas has been related to tobacco usages, Wynder and Gori, (1977).

Increases in cancer of the prostate and leukemia should be noted. These may be occupationally related to exposure to chemicals because the increases began in about 1945 and plateaued about 1960. The latency period for cancer development from exposure to cancer causing chemicals, that is the time between first exposure and development of cancer, is about 20 years on the average varying from 3 or 4 to 30 or more years. In the U.S., the chemical industry was initiated in the early 30's and really boomed in the 40's and early 50's. Another observation of importance in assessing the liklihood of this relationship is that among chemists excess cancer deaths attributed to malignant lymphoma and carcinoma of the pancreas have been reported, Li, et al (1969). It is notable that mortality from these types of cancer appear to be decreasing in recent years; perhaps improved control of chemical exposure in the work environment has contributed to this apparent decrease.

Figure 2 depicts the age-adjusted total cancer death rates for men including and excluding those caused by cancer of the respiratory system which have been attributed primarily to smoking.

Figure 3 shows similar information for U.S. females. As in U.S. males, deaths from stomach cancer have decreased. Deaths from cancer of the uterus have decreased also, which some have associated with better diagnostic procedures and treatment while others suggest it is due to

better hygienic practices. Note the recent rise in lung cancer. It appears that with the benefits of more social freedom, including smoking, some risks are also reaped. Figure 4 shows the age-adjusted total cancer death rates in the U.S. for women. There has been a decrease, not an increase; however, it is my opinion that this trend will change unless women are more successful than men in kicking the smoking habit.

Is cancer increasing, if so why? The data presented here indicate it is not increasing except for that associated with smoking. Similar analyses have led to the same conclusion by the American Cancer Society--"The overall incidence of cancer has decreased in the past 25 years" (76 Cancer Facts and Figures). A valid comment is that this encouraging conclusion results from better diagnosis and treatment and, therefore, fewer deaths. Unfortunately, this is not the case, since longevity subsequent to the diagnosis of cancer has seen little improvement for most types of cancer in the last 15 to 20 years, Levin, et al, (1974).

I must conclude that the contribution of modern technology to the genesis of human cancer in toto is not dramatic and further there is no definitive epidemic of cancer other than that which appears to be relatable to our personal habits.

Does this mean that modern technology (chemicals) is vindicated for its contribution to cancer in humans? No! There have been and undoubtedly will continue to be cases where cancer has occurred as a result of technology, developed and used indiscriminately. This is not to be condoned or accepted. Industry needs to clean up its act and when

needed appropriate regulation of industry should be instituted to assure that it does. However, hip-shooting with shotgun type regulations to satisfy vocal advocates will reap more harm than reward and should, therefore, be closely scrutinized. Further, the public must not be boondoggled into believing that returning to a premodern technology life style will benefit them much. Unless we start cleaning up our personal act, it is fictional to believe our goal will be realized by getting industry to clean up its act. Industry has and continues to institute major corrective efforts; the public must be urged to do the same.

As disturbing or even more disturbing than misdirected reaction to the alleged cancer epidemic is impetuous pursuit to identify the possible culprits which are a priori assumed to be man-made chemicals. Frequently, this has been accomplished by administration of amounts of chemicals many-fold greater than those to which people are exposed. For numerous chemicals, these doses have exceeded those needed to produce serious illnesses, even death. This methodology has been justified because in animals, as in people, the spontaneous incidence of cancer is sufficiently large to preclude identification of a carcinogen unless the dose causes in the neighborhood of a 10 percent increase above background.

Using this approach, nearly 2000 chemicals have been found to cause an increase in cancer in some species of laboratory animals, by some route of administration, under some conditions. For example, a survey of results obtained on 70 chemicals tested in the National Cancer Institute's bioassay program revealed 49 percent were positive, 28 percent were negative and 23 percent required retesting. Positive results are

reported, frequently in the news media, and an effort is launched to ban the chemical or restrict human exposure to the lowest possible level, a laudable but unworkable and likely unneeded concept.

To justify further banning or severely restricting exposure to chemicals found capable of producing cancer in animals is the often stated concept that there is no threshold below which cancer causing chemicals will not cause cancer.

To illustrate, a typical dose-response curve is shown in Figure 5. Response is the proportion of the population that suffers a specific effect. The solid part of the curve shows, not unexpectedly, that the percentage of a population suffering an effect increases with increasing doses. This is the form of the dose-response curve when the response of individuals within a population is distributed normally, a characterstic feature of most pharmacological or toxicological reactions to chemicals. The concept of a "threshold"--a dose below which no response will occur--has been accepted, but unproven, for most pharmacological and toxicological reactions. After finding the dose that produces no adverse response in animals, it has been common practice to assume that 0.1 to 0.0002 of that dose will be a safe level of exposure for man.

For chemical carcinogens, however, the "threshold" concept is not universally accepted. Some believe that cancer can be induced after the reaction of a single chemical molecule with a single critical site

on DNA (or perhaps other macromolecules) in a single cell. Once such a reaction has occurred, this theory holds, the cell is programmed irreversibly and there is thus a finite probability of initiating a new line of cells growing in a disorganized manner, thus producing a tumor. This mechanism predicts that there will be a finite incidence of cancer in a population no matter how low the dose of a carcinogen. Indeed, the incidence of cancer will be a linear function of dose rather than a linear function of the logarithm of the dose as depicted in Figure 5.

Unfortunately, the boundaries of this concept are beyond experimental resolution. In Figure 5, the dilemma is represented by the boxed portion in the lower left corner. The question is whether the incidence of cancer quickly reaches zero as the dose is decreased, as predicted by the "threshold" theory (dot/dash curve) or whether the incidence will decrease as predicted by extrapolation of the known experimental dose-response data (short-dash curve) or, indeed, whether the incidence may be greater than predicted by such extrapolation (long-dash curve).

The resolution of this question is difficult, if not impossible. To decide which curve is correct would mean collecting data on the response to low doses of the chemical concerned. But to do this--to put data in the boxed section of the curve--would mean measuring low incidences of cancer in animal populations, say 0 to 15 percent. But the low incidences can only be distinguished with any degree of statistical certainty from the "natural" rate of cancer in animals by trials involving impossibly large numbers of both control and treated animals. Thus only relatively high doses can, in practice, yield statistically significant data.

Thus, since no absolutely safe dose of a carcinogen can be established, it follows that to assure absolute protection for everyone no exposure whatsoever can be allowed to occur.

Inability to identify an absolutely safe dose of a chemical carcinogen, or for that matter a chemical producing other manifestations of toxicity, must be conceded. However, this concession provides little contentment since society's coexistence with naturally occurring carcinogens is now, and has been always, a fact of life. To face the issue of how to deal with carcinogens we must rely on risk assessment using the best available technology together with rational regulation to reduce risk to an acceptable level rather than to erroneously and misleadingly attempt to eliminate risk.

To illustrate the necessity of regulation which allows some low level of risk, consider the commonly accepted biochemical mechanism for cancer production. The scientific rationale underlying nonacceptance of a threshold for chemical carcinogens, or more leniently supporting the use of linear extrapolation to assess the risk of low level exposure to carcinogens, is that chemical carcinogenesis is mediated by an electrophilic (electron seeking) attack on DNA, the computer code of the cell which programs its existence. Such reactions are called alkylation reactions. The resulting reactions with the DNA bases--thymine, cytosine, guanine and adenine--lead to misprogramming of the genetic code and eventually genesis of a population of cancer cells. Unfortunately, a host of chemicals possess this capability.

For example, included are all compounds which can be polymerized into plastics, since polymerization is accomplished via alkylation reactions. Indeed, to eliminate chemicals with the capacity to alkylate DNA will, in essence, eliminate the chemical industry, because production of almost all products from chemistry rely at some point upon a similar reaction.

However, it is inadequate to regulate only chemicals possessing some potential to be electrophilic alkylating agents. Thanks to the pioneering work of the Miller's at the University of Wisconsin, it is now known that many chemical carcinogens, not in themselves electrophilic alkylating agents, are transformed to such agents in the body. For example, 2-acetylaminofluorene, an extremely potent carcinogen, is itself not capable of reacting with DNA, but in an attempt to eliminate the compound from the body, metabolic reactions occur which result in the production of a potent electrophile which reacts with DNA. Considering the possibility of such reactions for other chemicals leads to the conclusion that there are, in essence, very few chemicals which do not possess the potential to react with DNA by electrophilic attack. Either the chemical itself or a biotransformation product formed from it will react in such a manner.

Even natural constituents of the body have such an electrophilic alkylating capacity. Proteins and fats, indeed DNA itself, are produced by enzymatically mediated polymerization through alkylation reactions.

It can be argued that these reactions occur on an enzyme and, consequently, reaction with DNA is precluded. However, it must also be accepted that some, albeit small amounts, of the activated intermediates get off the enzyme and are available for reaction with DNA.

There may be a finite probability that any amount of a carcinogen capable of alkylating DNA will react in just the right manner to induce cancer. However, elimination of all chemicals possessing such activity is totally lacking in logic. To illustrate the futility of utilizing such ill-founded logic, NIOSH attempted to develop a Criteria Document for seven different vinyl compounds in which the initial exposure limits recommended for vinyl chloride and vinyl acetate were 0.04 ppm and 5 ppm, respectively; a 125-fold difference. In development of the limit for vinyl chloride, molecular mechanisms leading to the formation of the epoxide chloroethylene oxide, which could ultimately react with DNA, were cited as evidence that a safe level of exposure was unattainable.

To be consistent, the same logic should be applied to vinyl acetate. In animals tests, vinyl acetate has not been demonstrated to cause cancer, primarily because the compound is hydrolyzed quickly by esterases. However, vinyl acetate will undoubtedly be epoxidized to some degree in the body and in this form will react with DNA. Therefore, vinyl acetate is a potential carcinogen regardless of whether or not such an effect can be demonstrated in an animal system. Furthermore, a few people in the population have a deficiency of esterases and undoubtedly their capacity to hydrolyze vinyl acetate is deficient as well, an occurrence

which will render them more susceptible. For consistency, anyone advocating the "lowest feasible" or zero exposure approach for handling potential carcinogens must do so for vinyl acetate and for that matter all comppounds having a double bond between two carbon atoms.

In science, setting forth a hypothesis is not only valid but the very essence of scientific advancement. Equally important, however, is defining the boundaries of the preconceptions encompassed in the hypothesis. Such an assessment is even more critical when the hypothesis is to be used for regulation. The "one hit" rationale for advocating a no-acceptable level of exposure to carcinogens is based on the probability that one molecule of the agent may react with DNA and miscode it to produce aberrant cancer cells. Although deceptively convenient, its simplicity, if applied equitably, leads to condemnation of essentially all chemicals, man-made and naturally occurring. Obviously, the preconceived boundaries of this hypothesis have been assessed inadequately.

Because elimination of potential carcinogens, man-made and naturally occurring, is illogical and moreover impossible, it is necessary to return to the basic elements of chemical toxicity to develop parameters to assess risk as reliably as feasible. These are absorption, distribution, biotransformation, excretion, and reaction with the receptor (the critical site in the cell). Since doses of chemicals used in bioassays for carcinogenic activity are frequently many-fold greater than those to which people are exposed, it is of particular importance to consider how the dose of a carcinogen may influence these parameters.

To illustrate how the fate of a chemical may change with dose refer to Figure 6 which shows the typical chemical processes that determine the fate of a chemical (C) in the body. Each arrow represents a first-order rate process. The dominant process governing the fate of the chemical may be different for different species, different chemicals, and frequently, for different doses of the same chemical. Interfering with excretion or detoxification will enhance covalent binding of a reactive, electrophilic ("electron-seeking") metabolite (RM) to macromolecules (RM-M).

For example, furosemide, a diuretic, is excreted predominantly intact in the urine when low doses are given patients. When high doses are administered, the ability of the kidney to excrete the compound is overwhelmed causing a disproportionate increase in the formation of toxic metabolites which react with macromolecules. As another example, bromobenzene is transformed in the liver to the chemically reactive bromobenzene-3,4-epoxide. This molecule is detoxified enzymatically by conjugation with glutathione. However, if large doses are administered, glutathione is depleted and the reactive metabolite reacts instead with macromolecules.

The barrels in Figure 7 illustrate how dose may alter the fate of a chemical. As more and more fluid (putative carcinogen) flows into the barrel, elimination via the lower slit (excretion and "normal" metabolism) is overwhelmed, resulting in disproportionate increases in the amount of fluid in the barrel and/or elimination through the upper slit simulating excretion via other routes, metabolism via other pathways and, in some cases, reactions with macromolecules.

A more complete and more rational model depicting the fate of a chemical in the body is shown in Figure 8. This model encompasses the realism that many processes, including transformation of a noncarcinogenic material to a carcinogenic one and subsequently its detoxification, can be overwhelmed if the processes are subjected to amounts beyond their capacity to handle. Rather than being portrayed best by first-order rate processes, these reactions are described more accurately by dose-dependent or what we call Michaelis-Menten kinetics in accordance with the equation:

$$\frac{\partial C}{\partial t} = \frac{V_m C}{K_m + C} \quad .$$

In this equation $\partial C/\partial t$ is the rate of change in the concentration of the chemical at time t, C is the concentration of chemical at time t, V_m is the maximum rate of the process, and K_m, the Michaelis constant, is equal to the concentration of the chemical at which the rate of the process is equal to one-half V_m.

Reactions of reactive metabolites with macromolecules in the body (both nongenetic and genetic) are, however, first-order rate processes. Replication of genetic material, with the reactive metabolite bound covalently to it, is assumed naively to be a first-order rate process. This replicated genetic material gives a finite probability, albeit very small, of programming the cell to become cancerous. Genetic material with reactive metabolite bound to it can, in reality, be repaired and there is evidence that, like detoxification and excretion, the repair process can be overwhelmed. Hence, this process is assumed to be governed by Michaelis-Menten kinetics.

This model can be quantified as a series of differential equations, Gehring and Blau (1977). Integrating these equations allows the following to be determined as a function of the dose administered: (a) the amount of nongenetic material bound covalently with reactive metabolite, (b) the amount of genetic material bound covalently with reactive metabolite and then repaired, and (c) the amount of genetic material bound covalently with reactive metabolite but not repaired. Figure 9 shows the results. In this graphical representation the foregoing three parameters were divided by the administered dose (C_o). The respective parameters are labeled as CBN/C_o, CBG/C_o, and $RCBG/C_o$. If these parameters did not change disproportionately with increasing doses, each of the respective lines in Figure 9 would be parallel to the abscissa.

From these curves, it is clear that as dose increases there is a disproportionate increase in the amount of reactive metabolite bound to genetic material escaping repair (curve designated $RCBG/C_o$) and, hence, a disproportionate increase in the likelihood of cancer. This highly conservative model demonstrates why excessive doses of some chemical cause discernible increases in cancer, while smaller doses may have no effect. It illustrates clearly that data generated from studies of animals given large doses cannot be extrapolated directly to predict the incidence of cancer caused by exposure to smaller amounts of the same chemical. Such extrapolation assumes that the fate of the chemical does not change with dose--an assumption which requires the curve of unrepaired "damaged" genetic material to be a straight line parallel to the abscissa. Clearly it is not.

Validation of Model: Repair Mechanisms - Data developed for two potent carcinogens; N-ethyl-N-nitrosourea (ENU) and dimethylnitrosamine (DMN) demonstrate the appropriateness of certain aspects of the model, specifically the existence of a "swampable" repair mechanism. Administration of rather high doses of ENU to newborn rats result in a high incidence of malignant tumors of the central and peripheral nervous system. At such dose levels, liver tumors do not develop. Data generated by Goth and Rajewsky (1974) correlate these events with ethylation of the O-6 position of guanine in DNA, Figure 10 (i.e. alkylation of DNA bases, as discussed earlier). Excision repair by enzymatic removal of the ethylated guanine from DNA occurs readily in liver but only poorly in nervous tissue. It has been shown by Gerchman and Ludlum (1973) that O-6 alkylation of guanine results in misincorporation of bases in a bacterial nucleic acid polymerase system. Thus, in nervous tissue the O-6-ethylguanine in DNA has a greater opportunity to miscode DNA replicated for daughter cells. The ethylated guanine may pair with thymine rather than cytosine. As a consequence, cancer occurs in nervous tissue but not in liver.

DMN given as a large single dose produces kidney tumors in rats, but does not produce liver cancer. Recently, Pegg, et al, (1976, 1977) have demonstrated that excision repair of DNA having guanine methylated in the O-6 position occurs readily in the liver of rats given both a high and low dose of DMN. In kidney, however, repair of DNA having O-6 methylated guanine occurs readily only after administration of low non-carcinogenic doses, Figure 11.

Thus, we must conclude that alkyiation of DNA may lead to cancer induction. Further, we must conclude that administration of high doses inhibits or exceeds the capacity of the repair mechanism. Indeed, the data I have presented on ENU and DMN indicate that a discernible increase in cancer occurs only if the target tissue has a poor capacity for repair of DNA of if the doses are of sufficient magnitude to inhibit repair or exceed the capacity of the repair process.

The model does not establish that low exposures to chemicals found to be carcinogenic to animals at high doses are absolutely safe. "Absolute safety" is both unrealistic and ludicrous.

Example, Vinyl Chloride (VC) - Some chemicals require activation to a toxic form to elicit their toxic effect. Activation to the toxic form as well as deactivation of the toxic form may occur via a saturable metabolic process. For instance vinyl chloride metabolism does not increase proportionately with increasing concentrations of exposure, Table 1, Watanabe, et al, (1976a and 1976b). Rather, the amount of VC metabolized during 6 hours of exposure to various concentrations appeared in accordance with Michaelis-Menten kinetics as described by the equation:

$$v = \frac{V_m S}{K_m + S}.$$

In this equation, v and V_m are the velocity and maximum velocity, respectively, for the biotransformation of VC (expressed as µg equivalents of VC metabolized per 6 hours). S and K_m are, respectively, the concentration of VC being inhaled and the Michaelis constant (expressed as µg VC/liter air).

The amount of VC biotransformed by rats exposed to any concentration may be calculated by determining certain quantifiable values. The value v/S was determined for each exposure concentration such that the data could be analyzed in accordance with the linear Woolf-Augustinson-Hofstee transformation of the Michaelis-Menten equation, Segel, (1976),

$$v = K_m \frac{V}{S} + V_m.$$

A plot of V versus v/S is shown in Figure 12 and the values V_m and K_m determined to be, respectively 8558±1147 (SD) µg VC metabolized per 6 hours and 860±159 (SD) µg VC/liter air by fitting the model directly. The amount of VC biotransformed can now be related to the incidence of hepatic angiosarcoma.

Maltoni and Lefemine (1975) reported the incidence of hepatic angiosarcoma in rats exposed to different concentrations of VC, 4 hours/day, 5 days/week for 12 months and subsequently held for observation until death (Table 2). A plot of these data as a function of exposure concentration reveals an unacceptable plateau at high level exposures, Figure 13b. Before attempting to relate these data to the amount of VC biotransformed in accordance with the Michaelis-Menten equation using the previously determined values of V_m and K_m, the value for V_m must be adjusted for the shorter exposure duration used by Maltoni and Lefemine, 4 hours versus 6 hours. This adjustment is accomplished by multiplying V_m by 4/6. Thus, the amount of VC biotransformed daily by rats exposed to the various concentrations used in the experiment of Maltoni and Lefemine can be calculated from the equation:

$$v = \frac{5706\left(\frac{\mu g\ VC}{4\ hr}\right) \cdot S\left(\frac{\mu g}{\ell}\right)}{860\left(\frac{\mu g}{\ell}\right) + S\left(\frac{\mu g}{\ell}\right)}$$

The resulting values for v are given in Table 2. In Figure 13a, the incidence of angiosarcoma in rats is plotted as a function of the amount of vinyl chloride biotransformed, v. Obviously, the results have been rendered more interpretable when the response is related to the amount of vinyl chloride biotransformed rather than the amount inhaled. Using the foregoing equation, we can extend a projection below the levels of exposure producing an experimentally discernible response (dashed line). The probit equation for this dose-response line is probit response = -1.65+1.543 log V.

Assuming no threshold for the induction of angiosarcoma in rats exposed to VC, the exposure concentration expected to produce one angiosarcoma in 10,000 rats can be calculated. The probit percent representing an incidence of 0.01% is 1.28. Substitution of this value into the probit equation yields:

Log v = 1.8827

Antilog v = 76.33 μg VC metabolized/4 hours.

Substitution of this value for v in the equation relating v to the exposure concentration, S, indicates that the exposure concentration needed to give this value of v is 11.66 μg/l or 4.6 ppm. Hence, exposure of rats to 4.6 ppm VC for 4 hours daily, 5 days/week for 1 year can be expected to produce one angiosarcoma per 10,000 rats, assuming the dose-response curve remains valid at exposures less than those producing a discernible experimental response.

In the foregoing analysis of the dose-response data for the induction of angiosarcoma in the rat, no threshold for the response was assumed. Extrapolation below the range of doses causing an observable response may overestimate the response in rats. There is evidence that detoxification of reactive metabolites may occur more efficiently in rats exposed to concentrations of VC below 50 ppm, Watanabe, (1976c).

Indeed, further analysis of the data reported by Maltoni and Lefemine (1975) provides a suggestion of a practical threshold. In a subsequent presentation of these data, it was revealed that the latency for the development of hepatic angiosarcoma was, respectively, 64, 70, 78, 81, 78, and 135 weeks for rats exposed to 10,000, 6000, 2400, 500, 250, and 50 ppm VC (Maltoni, 1975). These results indicate that at the low levels of exposure the time required for induction exceeds considerably the mean life expectancy for rats (approximately 104 weeks). This is consistent with the work of others suggesting that multiples of a lifetime may be required for expression of cancer in response to low doses of a carcinogen (Druckrey, 1967 and Albert and Altschuler, 1973). Thus, extrapolation of the data obtained for rats below the range of exposures causing a discernible response may be expected to overestimate the projected incidence.

The foregoing discussion provides added proof that the development of angiosarcoma in rats exposed to vinyl chloride is not a function of the vinyl chloride concentration per se, but a function of the amount of

vinyl chloride biotransformed in the body. Only after this transformation is calculated do the dose-response data become meaningful. This concept is a clear indictment of the current practice in carcinogenic bioassays of using only a maximum tolerated dose and one-half the maximum tolerated dose to assess the hazard of potentially carcinogenic chemicals.

Knowing that induction of angiosarcoma is caused by exposure to the biotransformation products rather than vinyl chloride per se provides for more rational extrapolation of rat data to man. Unfortunately, data addressing the biotransformation of vinyl chloride by man are unavailable. To attempt; therefore, an extrapolation requires approximation of the V_m and K_m for man. Although possible, it is unlikely that the value for K_m will differ substantially for rats and man; thus, this constant is assumed equivalent to that for rats.

V_m is assumed to be proportional to the body surface area. There are considerable data in the literature showing that metabolism in general, and other physiological parameters are relatable directly to the body surface area (Pinkel, 1958 and Schmidt-Nielsen, 1970). For this reason, administration of biologically active chemicals to various species frequently gives an equivalent response when the dose is administered in proportion to the surface area of the body, i.e. dose per square meter of body surface. This body surface area relationship is gaining recognition in estimating the risk incurred by man from exposure to chemicals in the environment (Committee on Safe Drinking Water, National Research Council, 1977). In utilizing this relationship to extrapolate between species, it must be recognized that the original relationship was

Age-Adjusted Cancer Death Rates In the United States For Men

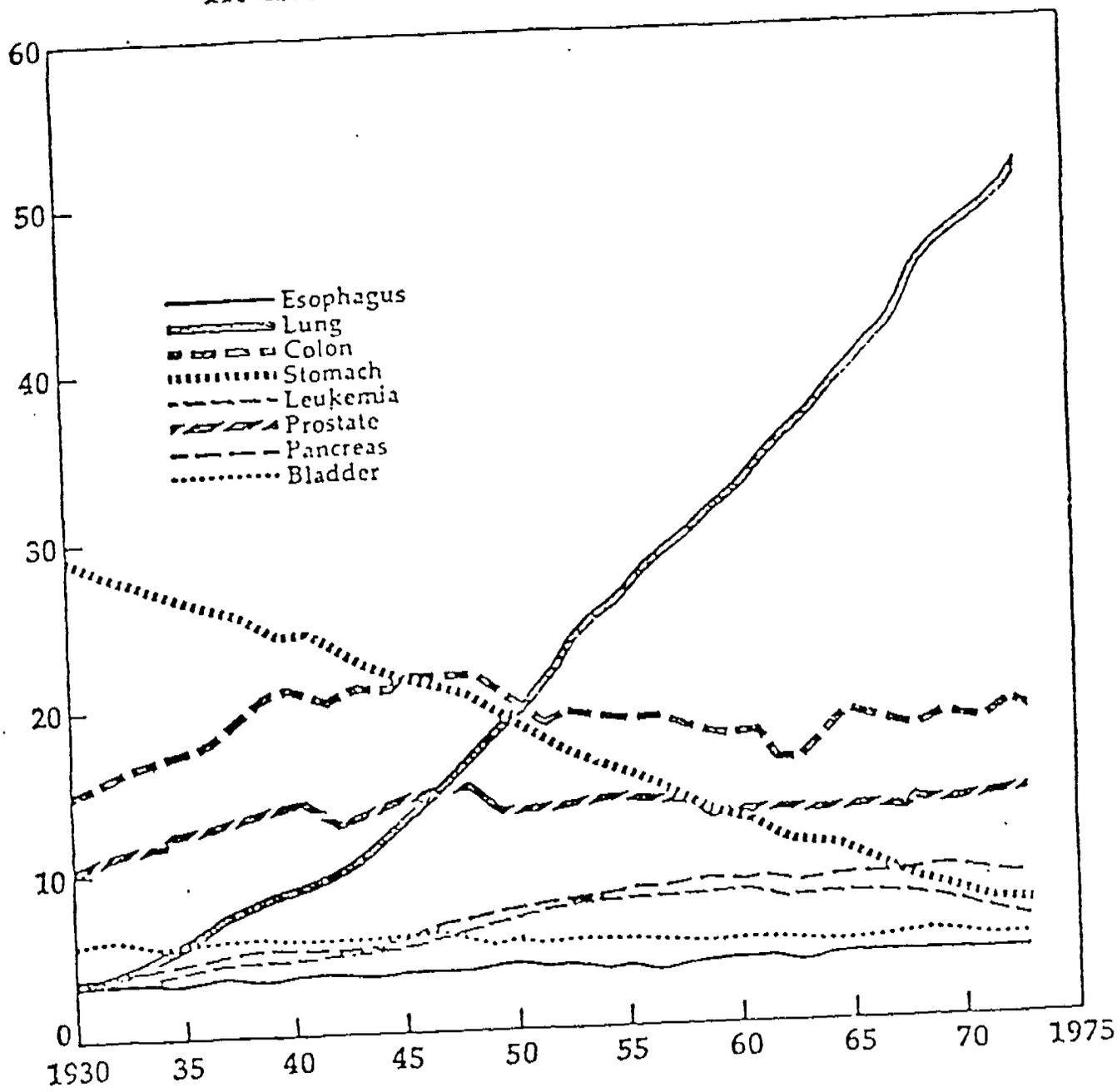

Figure 1

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Age-Adjusted Total Cancer Death Rates In the United States For Men

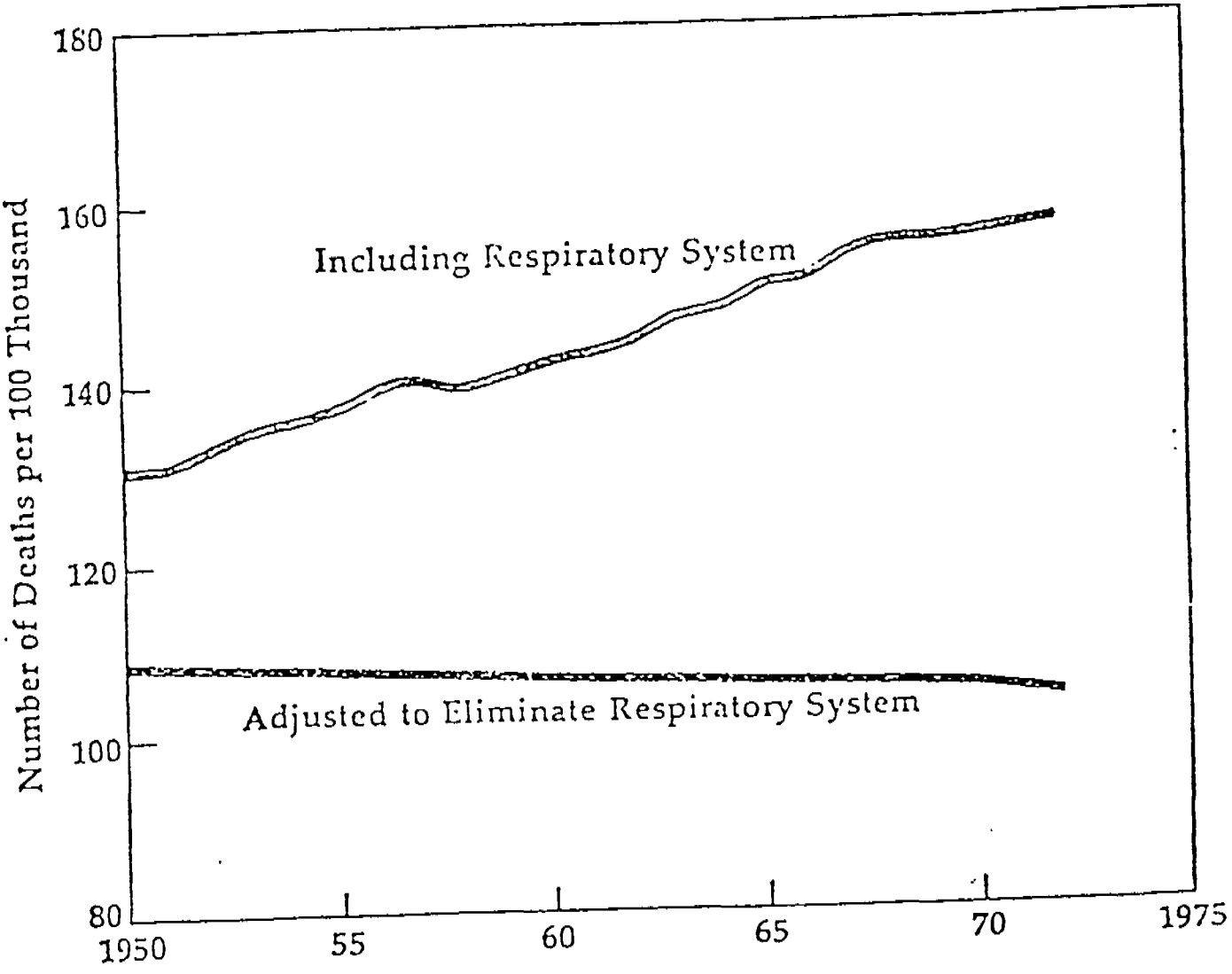

Figure 2

Age-Adjusted Cancer Death Rates In the United States For Women

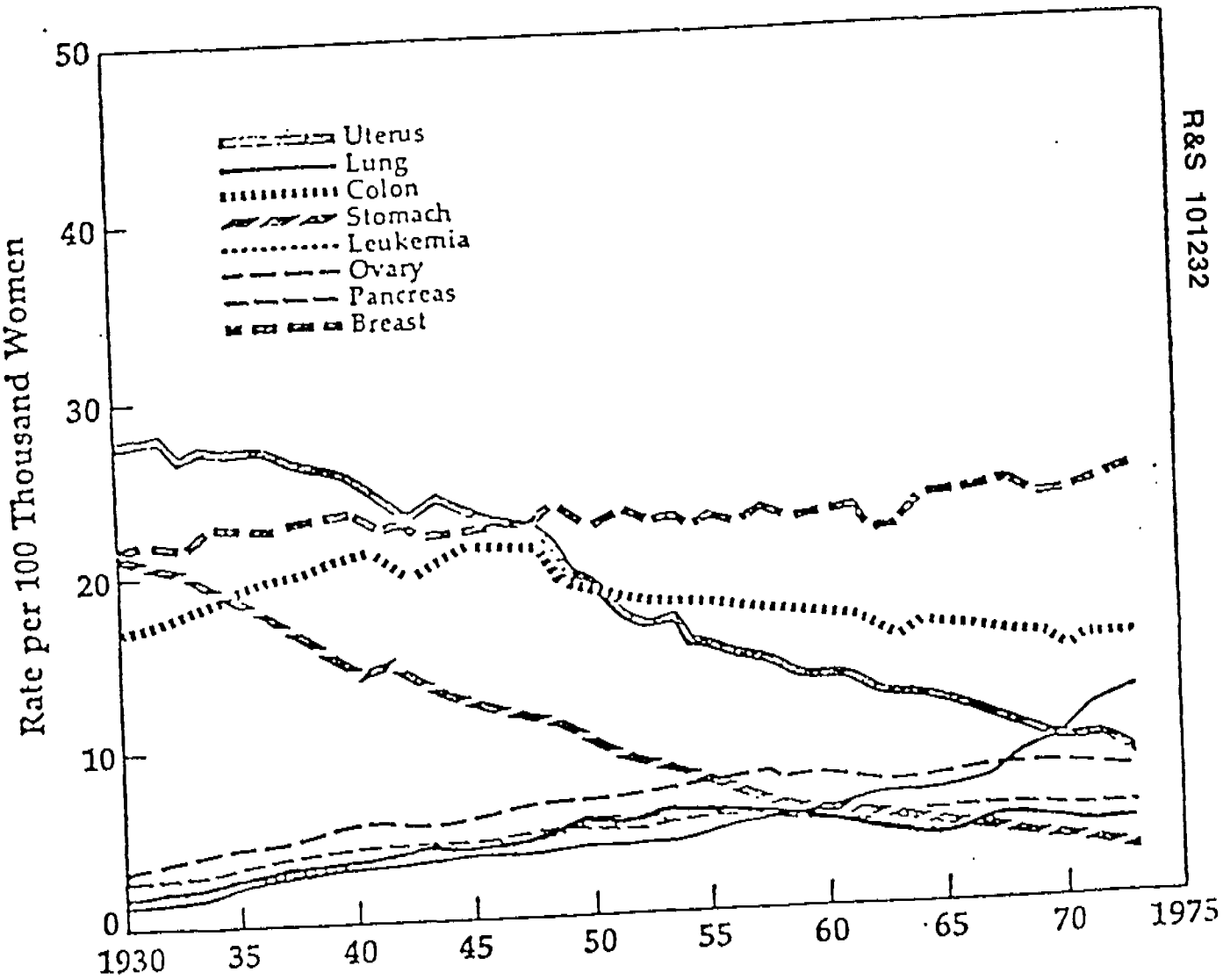

Figure 3

Age-Adjusted
Total Cancer Death Rates
In the United States For Women

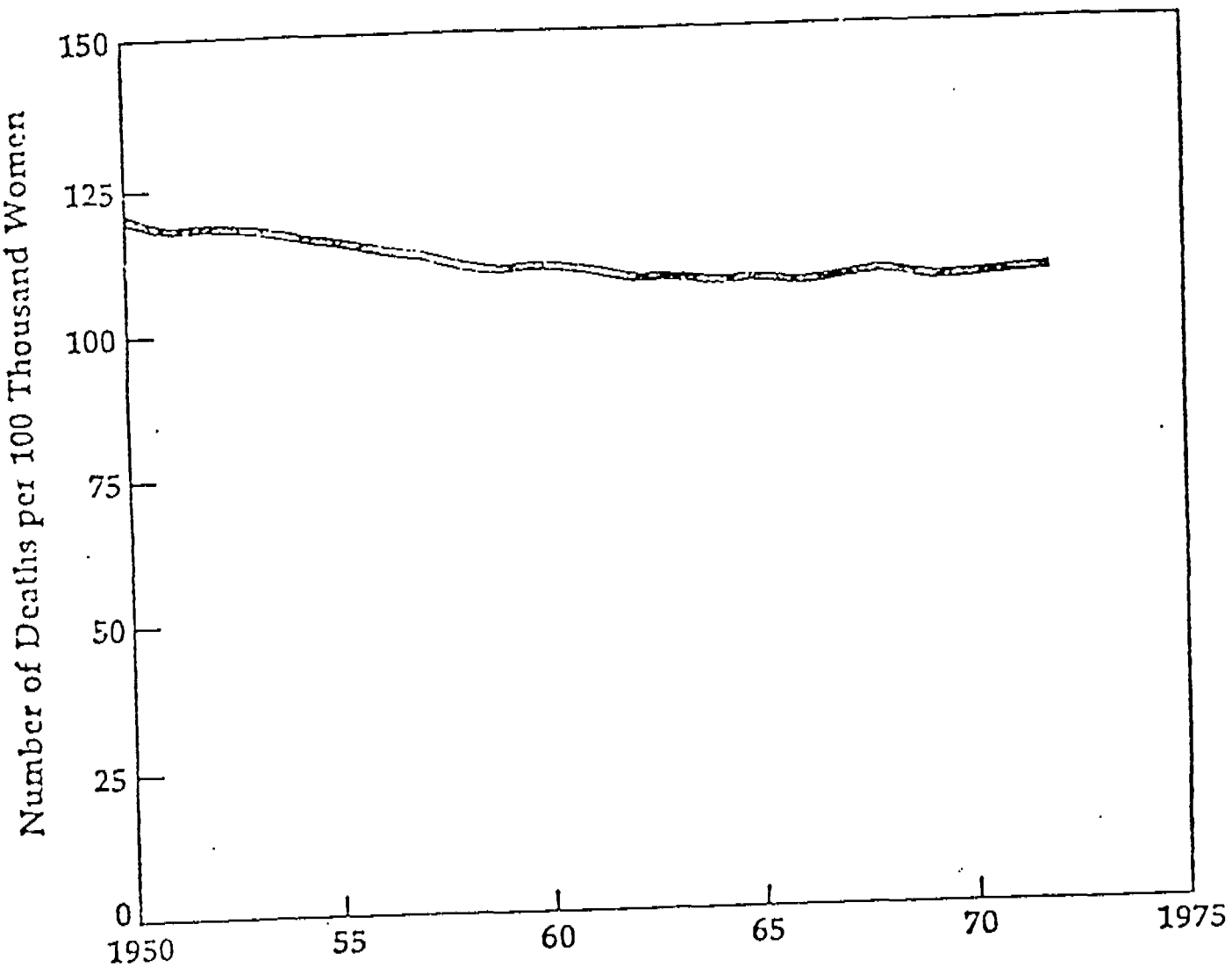

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Figure 4

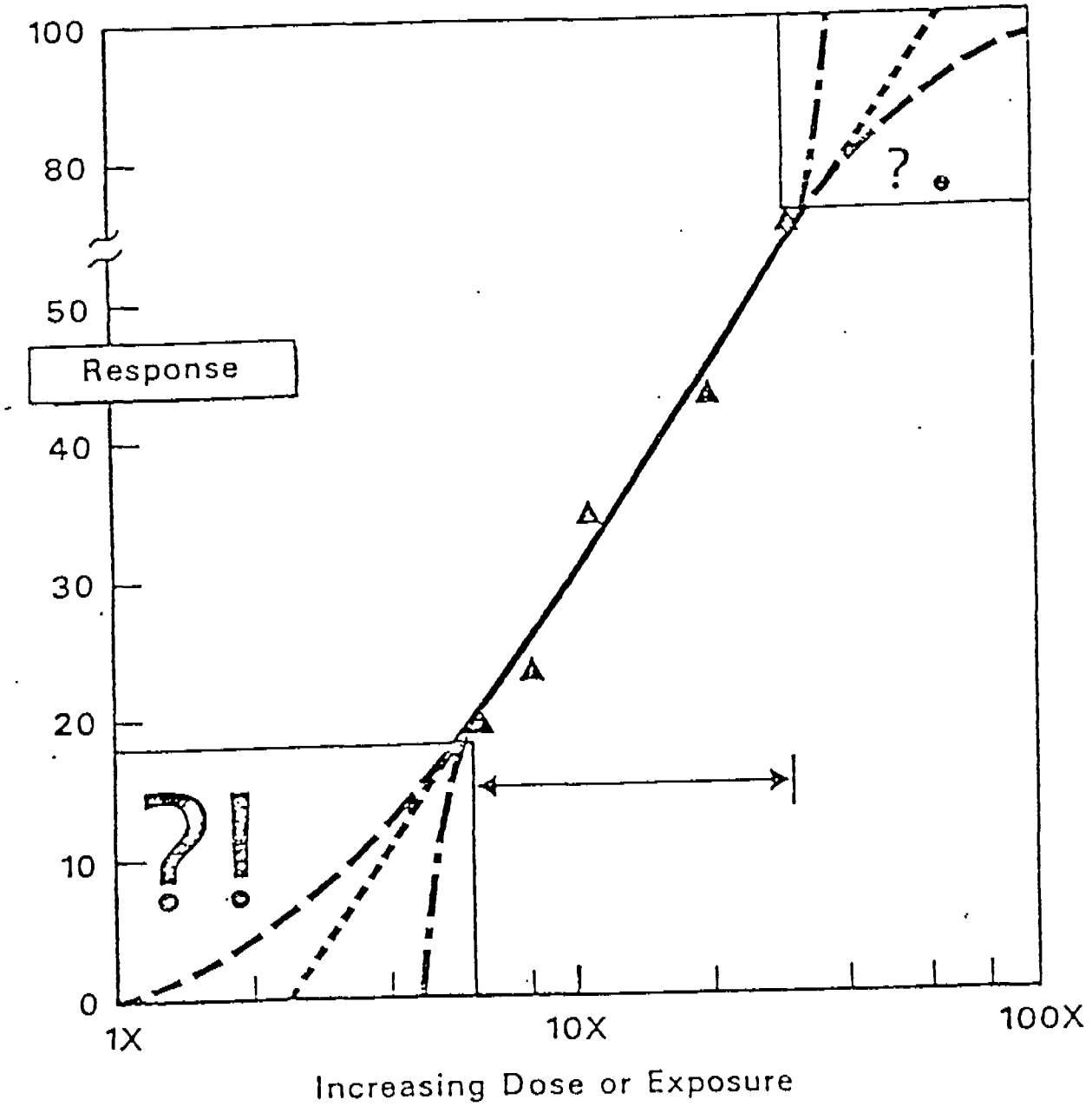
100
80
50
Response
40
30
20
10
0
?
?!
1X
10X
100X
Increasing Dose or Exposure

Figure 5

Figure 6

CHEMICAL PROCESS FOR THE FATE OF A CHEMICAL IN THE BODY

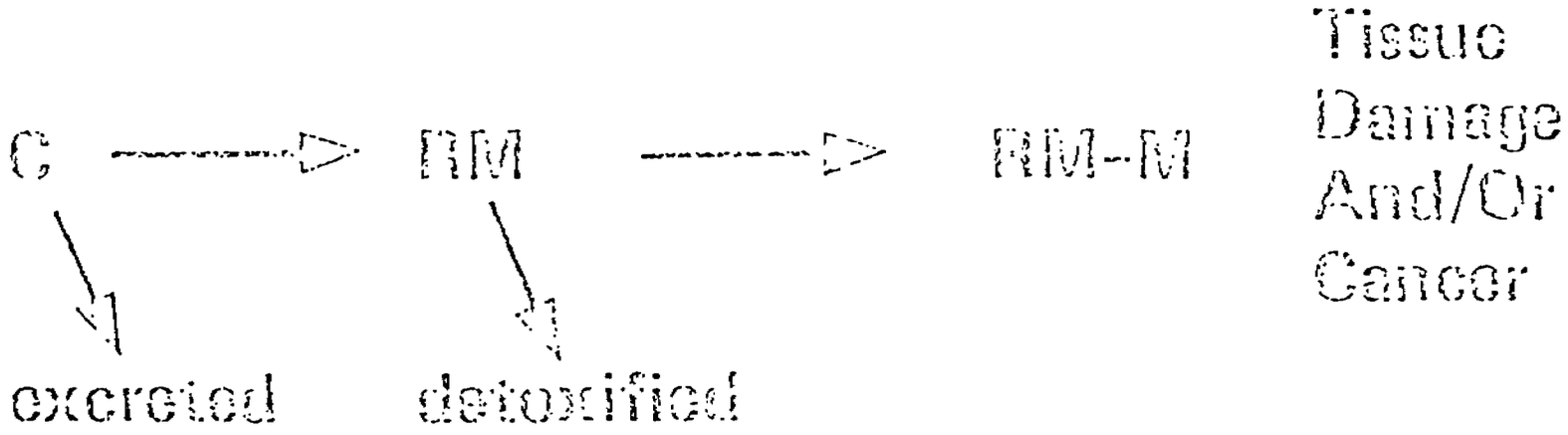

Figure 7

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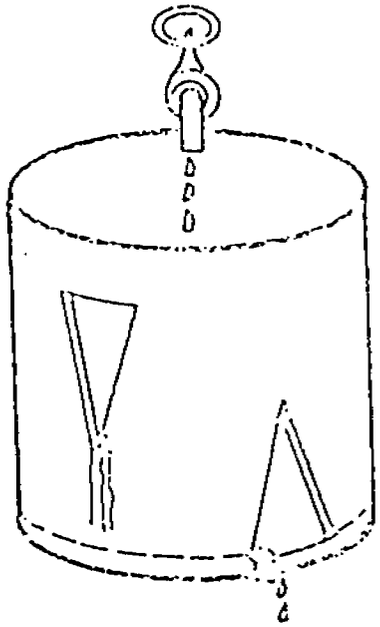

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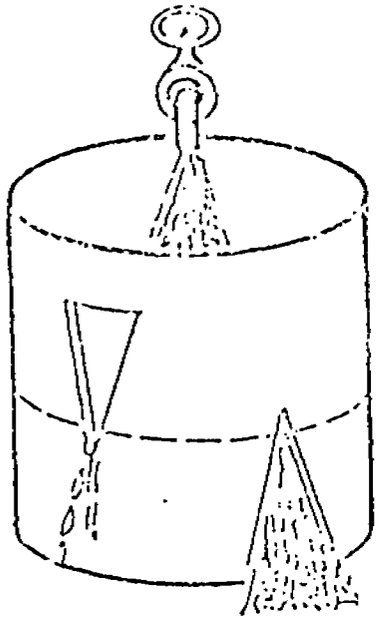
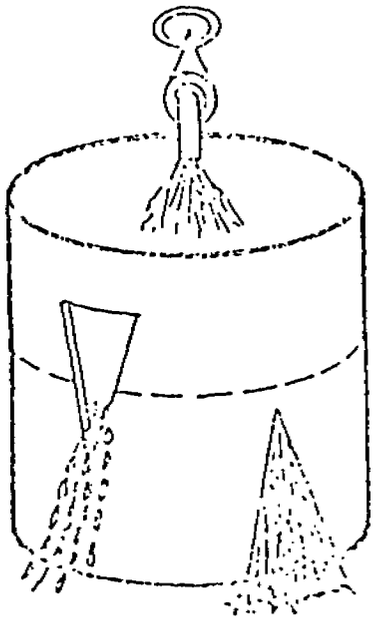


C



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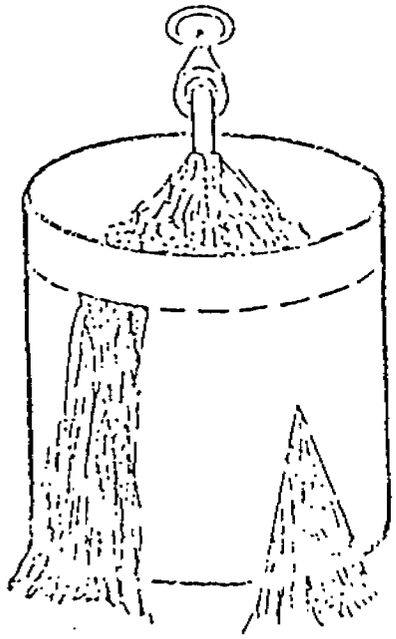
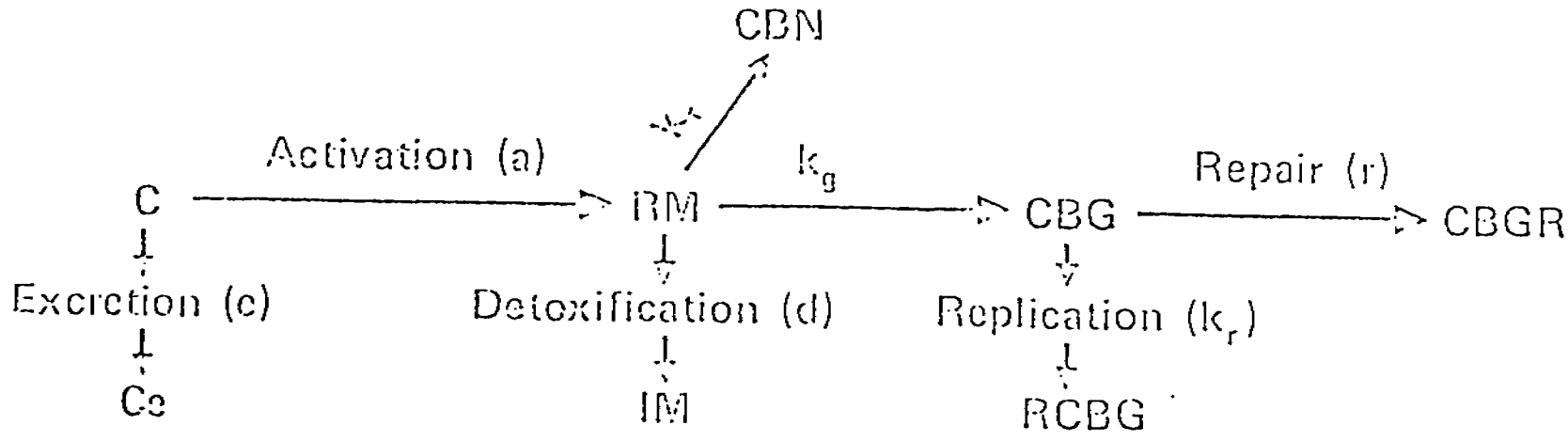
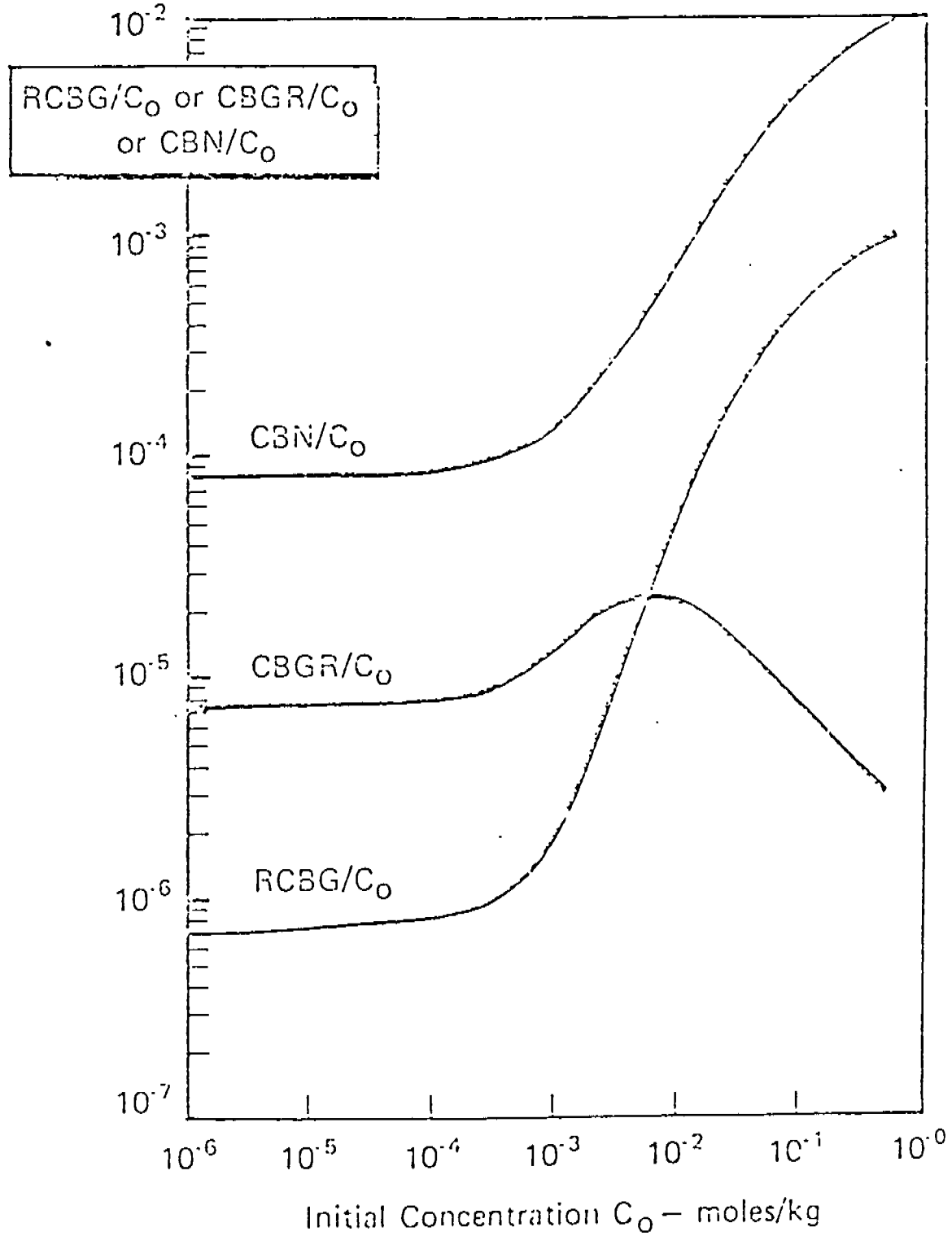


Figure 8



C = Chemical
RM = Reactive Metabolite
Ce = Excreted Chemical
IM = Inactive Metabolite
CBN = Covalent Binding, Nongenetic
CBG = Covalent Binding, Genetic
CBGR = Repaired Covalently Bound Genetic Material
RCBG = Retained Genetic Program, Critical & Noncritical

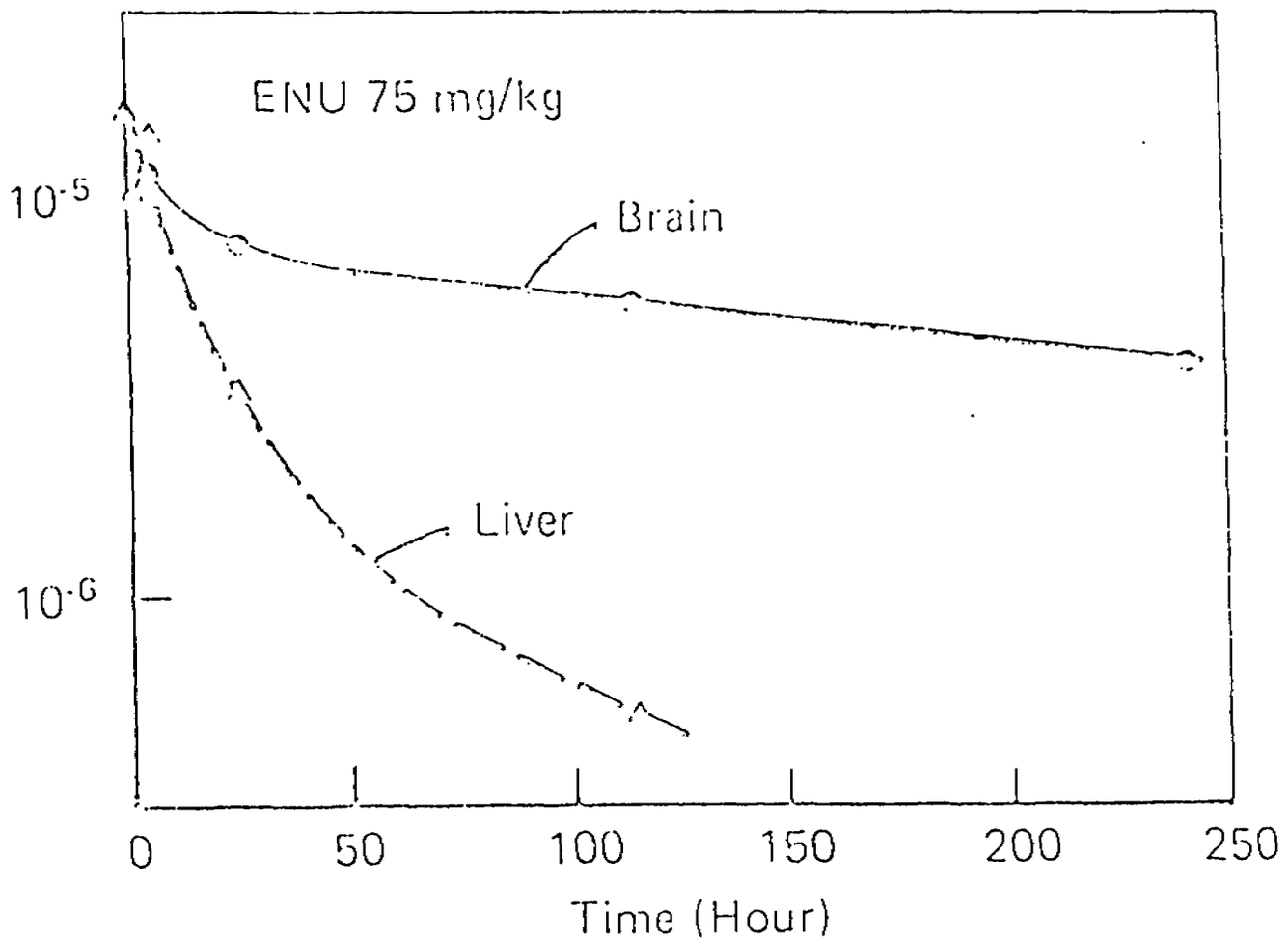
Figure 9



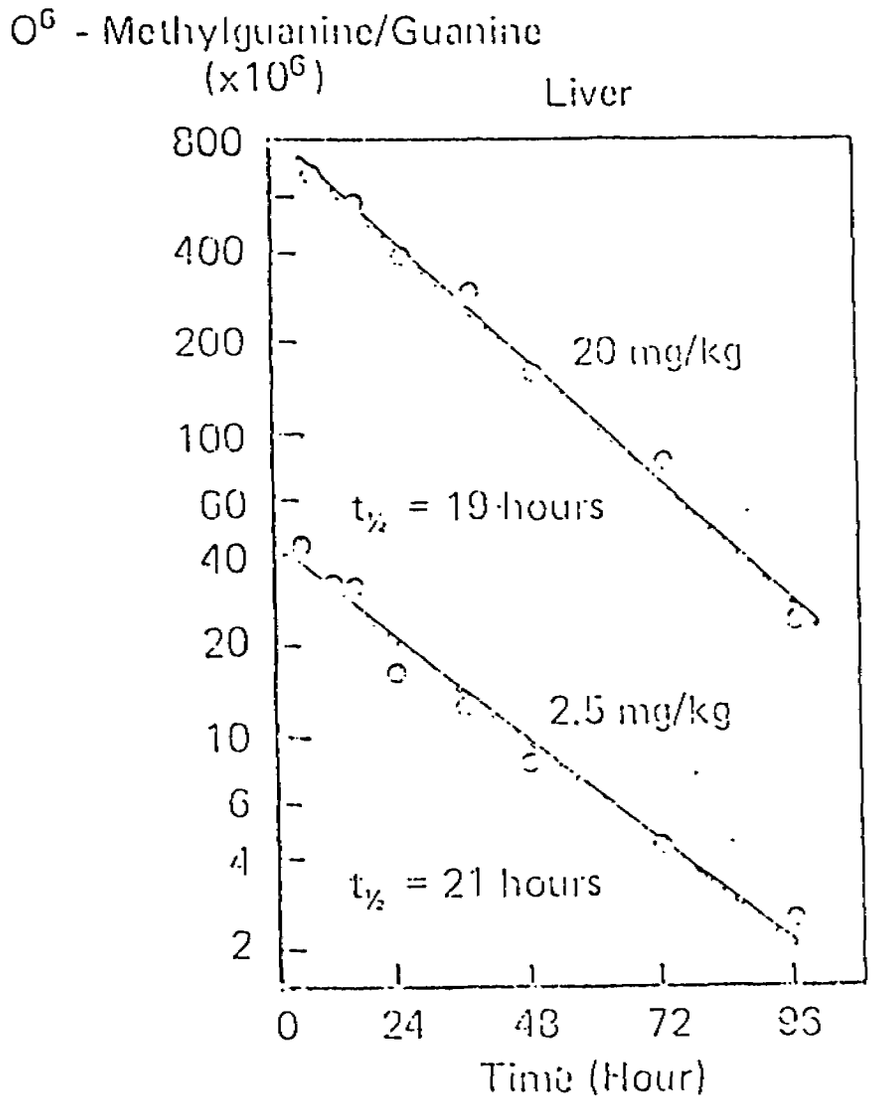
R&S 101238

Figure 10

O^6 - Ethylguanine/Guanine



Goth and Rajewsky (1974)



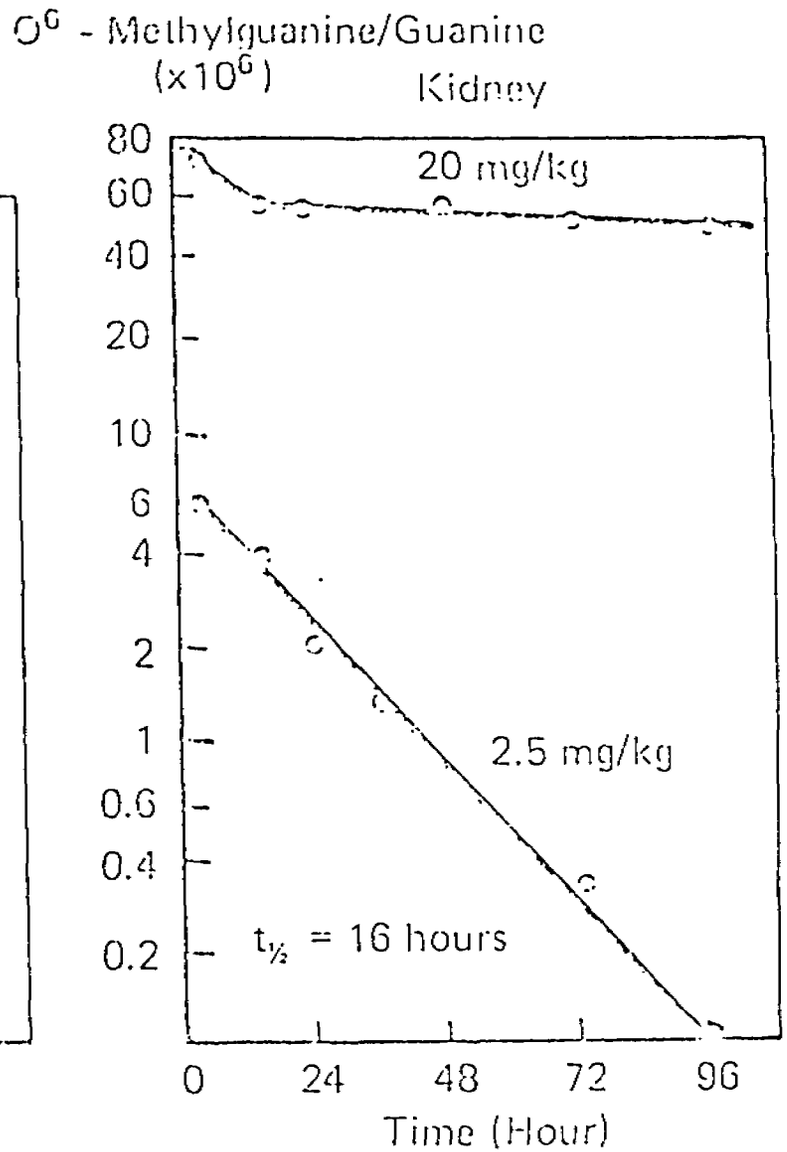


Figure 11

Pegg et al (1976)

Figure 12

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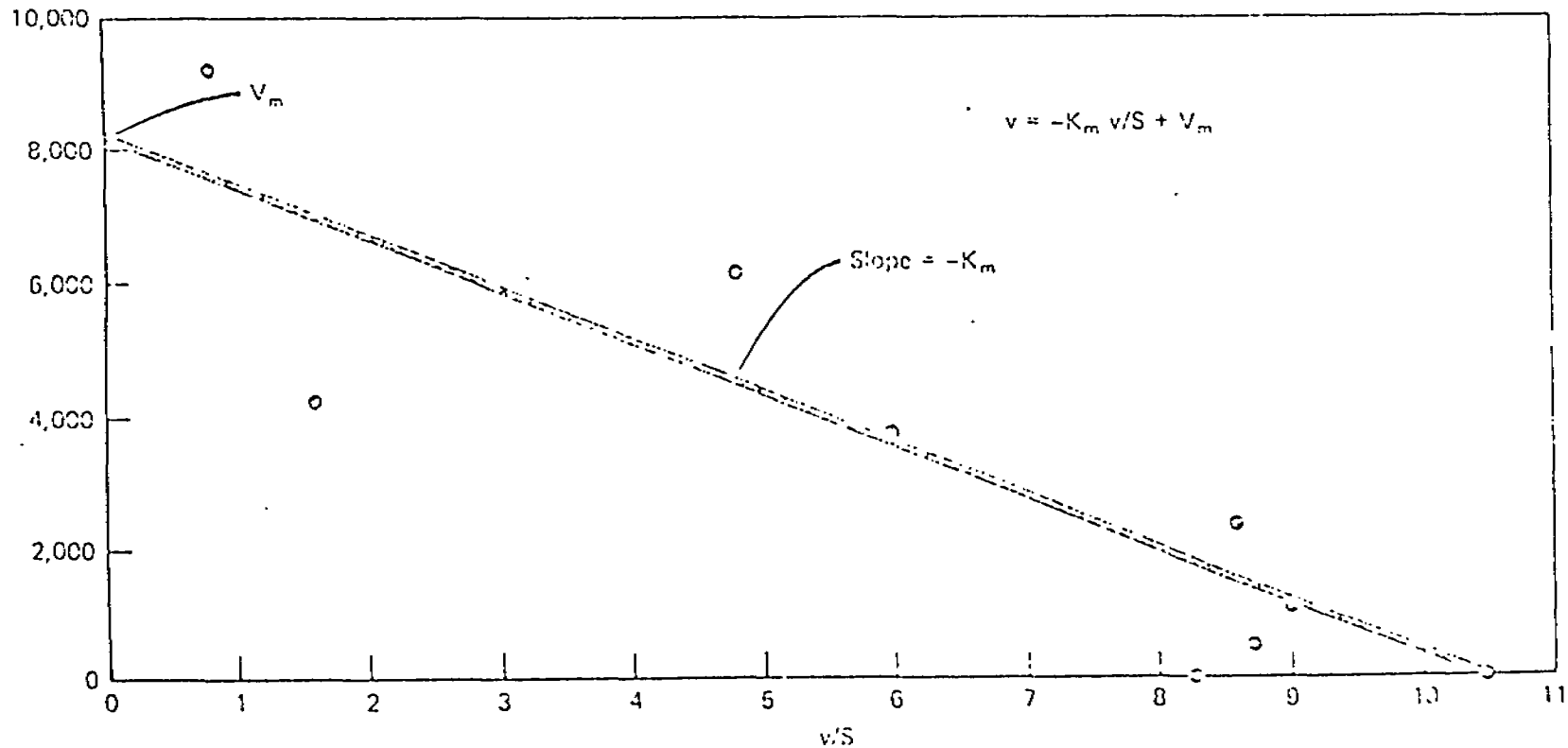
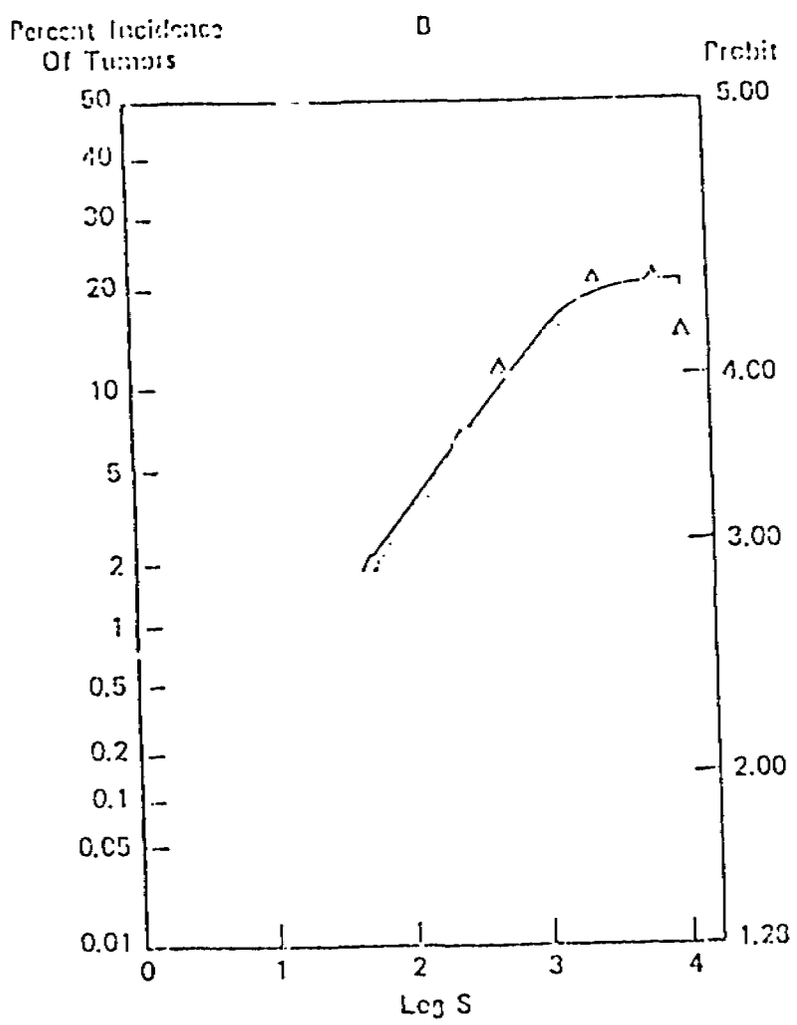
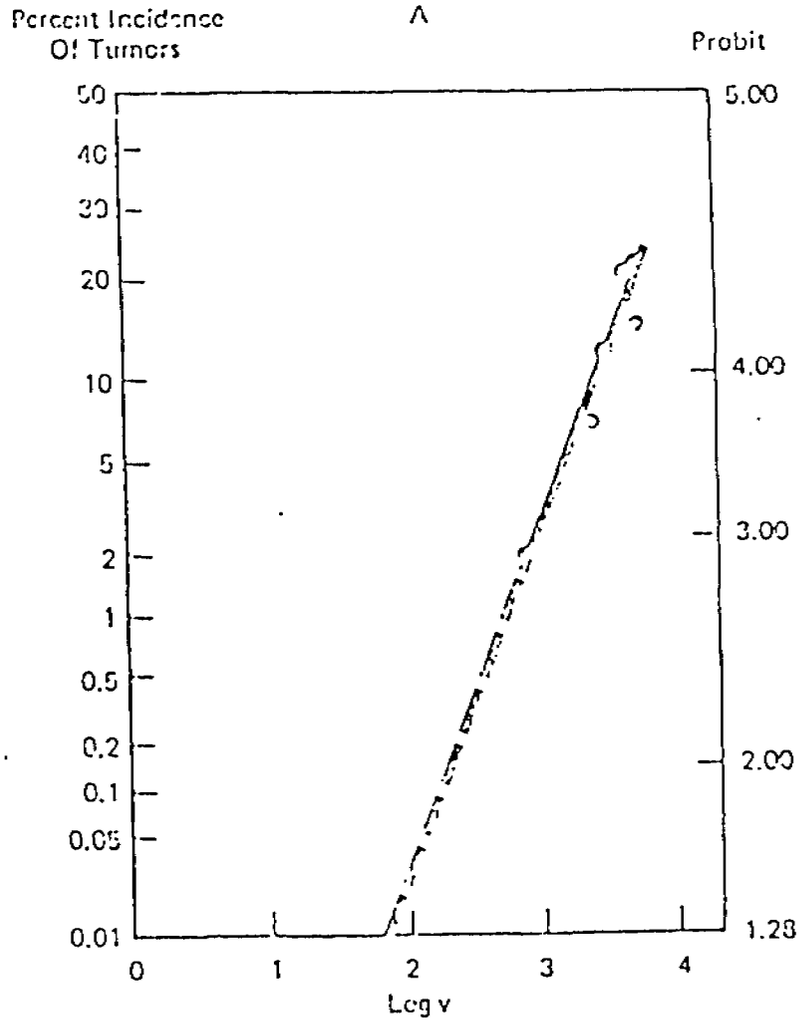


R&S 101242

Figure 13





developed for biologically active agents. Since metabolism and other physiological processes involved in detoxification are more active in smaller animals, doses of a biologically active chemical producing equivalent responses increase with decreasing size of the animal when expressed per unit of mass but remain relatively constant when expressed per unit of surface area. However, for a chemical requiring activation to the toxic form, the rate of transformation to the toxic form will be roughly proportional to the body surface area. Since toxicity is a function of the concentration of the active form in tissue, the total amount transformed must then be normalized for mass to estimate an equivalent response.

Using the aforementioned rationale, the maximum velocity, V_m, for a 70 kg man can be estimated by a calculation using the V_m value obtained for a 0.250 kg rat. The V_m of man for VC will be:

$$V_m \text{ (man)} = V_m \text{ (rat)} \left(\frac{1.85 \text{ sq m}}{0.045 \text{ sq m}}\right)$$

where the values 1.85 and 0.045 sq m are the body surface areas of a 70 kg man and a 0.250 kg rat, respectively. For an 8-hour exposure, the value is 469105 µg/8 hr. In order to use this number to theoretically estimate the response in man using data collected in rats, the V_m for man must be adjusted to a mass equivalent to that of rats since toxicity is a function of concentration in tissue. To do this the number is divided by 70 kg/0.250 kg or 280. The resulting V_m for man on a mass equivalent basis to that of rats is 1675 µg/8 hr. Using this value, the amount of VC transformed to a reactive form by man on a mass equivalent basis to rat is given by the equation:

$$v\ \mu g/8\ hr = \frac{1675\ \mu g/hr \cdot S\ \mu g/\ell}{860\ \mu g/\ell + S\ \mu g/\ell}\ .$$

Using this equation, the amount of VC transformed by man on a mass equivalent basis to rats was calculated as a function of exposure concentration. The expected incidence of angiosarcoma was estimated from the probit equation (Table 3).

For men exposed to greater than 200 ppm VC, the incidence of angiosarcoma has been reported to be 0.02% (Fox and Collier, 1977). Hence, the theoretical calculated incidence of 1.0%, using data from rats, (Table 3) exceeds that currently detected in man by approximately 50-fold. This may indicate that people are less sensitive than rats to the induction of angiosarcoma or it may indicate the existence of a practical threshold for the induction of angiosarcoma. The value of v on a mass equivalent basis compared to rats for men exposed to 200 ppm is 625. This number lies below that for rats exposed to 50 ppm and is consistent with the possibility of a practical threshold. As indicated previously, angiosarcoma observed in rats exposed to 50 ppm occurred only in a rat that lived 135 weeks. Further, this rat did not die as a result of angiosarcoma but was killed. Thus, as indicated previously, projection of the data collected in rats below the range of exposures producing an observable response may overestimate the incidence.

In spite of the likelihood of overestimating the incidence of angiosarcoma in rats or man, the incidence predicted for people exposed to 1 ppm, the current upper limit of the Occupational Safety and Health Act (OSHA) standard, is very small (1.5 per 100 million). This value is

below the expected incidence of spontaneous angiosarcoma, reported to be 20 to 25 cases in the U.S. annually (Makk et al, 1976).

It is sad and unfortunate that 22 people in the U.S. developed angiosarcoma in response to exposures to hundreds of parts per million vinyl chloride. Such exposures have ceased rightfully. Whether reduction of exposures to less than 1 ppm is justifiable is debatable. However, based on the information available, further reduction is not justifiable. Admittedly, any level of exposure to vinyl chloride is not without risk. Elimination of risk from chemical carcinogenesis requires elimination of exposure to essentially all man-made chemicals, as well as those which existed prior to man's tinkering.

Many people have advocated using risk/benefit analysis to determine whether an important chemical such as vinyl chloride should continue to be utilized once found to be carcinogenic. To this should be added risk/risk analysis because modern technology seldom adds to the sea of carcinogens to which we are exposed without decreasing exposures to others in the sea. Is the risk of working in a vinyl chloride plant at exposures of less than 1 ppm greater than or less than that of working in the wood products industry where exposures to other carcinogens occur such as sawdust or the polycyclic aromatics produced by trees and other plants? How does the risk compare with that incurred by working in a foundry or manufacturing cement? To get rid of vinyl chloride would necessitate increased employment in these other industries to provide substitute products.

Has modern technology led to development of greater risks or lesser risks? I submit that if we had been as eager to elucidate the potential risks of antiquated technology they would have been revealed to be as bad, and often worse, then those we now are fretting over.

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TABLE I

Parameters for Describing the Metabolism of Inhaled Vinyl Chloride (VC) Using Michaelis-Menten Kinetics

S (ppm) VC)	S (µg VC/ℓ air)[a]	v µg VC metabolized[b] / g hr	v/S
1.4	3.6	30±3[c]	8.33
9	23.0	242±25	10.52
25	64.0	557±42	8.70
51	130.6	1181±93	9.04
109	279.0	2405±173	8.62
250	640.0	3826±345	5.98
511	1308.2	6263±355	4.79
1020	2611.2	4257±765	1.63
4600	11776.0	9255±1467	0.79

[a] 1 ppm VC = 2.56 µg VC/ℓ air.

[b] Determined from the total radioactivity in the carcass.

[c] Mean ± standard deviation.

TABLE 2

Correlation Between Exposure Concentration of Vinyl Chloride, Metabolism and Induction of Heaptic Angiosarcoma in Rats

Exposure Concentration S (ppm) VC	S (μg VC/ℓ air)[a]	$v \frac{\mu g \text{ VC metabolized}}{4 \text{ hr}}$ [b]	Log v	Percent Incidence of Hepatic Angiosarcoma[c]
10,000	25,000	5,521	3.742	15
6,000	15,360	5,403	3.733	22
2,500	6,400	5,030	3.702	22
500	1,280	3,413	3.533	13
250	640	2,435	3.386	7
50	128	739	2.869	2

[a] 1 ppm VC = 2.56(μg VC/ℓ air).

[b] V_m corrected for 4 hour exposure, $8558(\frac{\mu g \text{ VC metabolized}}{6 \text{ hr}})$. $4/6 = 5706\ (\frac{\mu g \text{ VC metabolized}}{4 \text{ hr}})$

$$v = \frac{5706(\frac{\mu g \text{ VC}}{4 \text{ hr}}) \cdot S(\mu g/\ell)}{860\ (\mu g/\ell + S(\mu g/\ell)}$$

[c] From Maltoni and Lefemine (1975).

TABLE 3

Theoretical Amounts of Reactive Product Formed from VC by a 70 kg Man Exposed Continuously for 8 Hours and the Corresponding Expected Incidence of Angiosarcoma as Predicted from Data Collected in Rats Assuming no Threshold

Exposure Concentration		v[b] mg VC metabolized / 8 hr.	Log v	Probit Response[c]	Theoretical Percent Incidence of Angiosarcoma[c]
ppm	ug/l[a]				
200	512	625	2.79	2.68	1.62
50	128	217	2.34	1.98	0.11
5	12.8	24.6	1.39	0.52	3.74×10^{-4}
1	2.56	4.97	0.70	-0.54	1.5×10^{-6}

[a] 1 ppm = 2.56 ug/l.

[b] v has been calculated using the Michaelis-Menten equation after calculating the V_{max} for man from the V_{max} determined for rats. This calculation was made by assuming V_{max} is proportional to body surface area and subsequently adjusting it to a mass equivalent to that of rats. See t

[c] The expected probit response was calculated from probit equation 3. Subsequently the theoretical percent incidence of angiosarcoma was determined from the respective probit.

LEGENDS

Figure 1) Age-adjusted cancer death rates in the United States for men.

Figure 2) Age-adjusted total cancer death rates in the United States for men.

Figure 3) Age-adjusted cancer death rates in the United States for women.

Figure 4) Age-adjusted total cancer death rates in the United States for women.

Figure 5) Simulated percent of individuals responding adversely to the logarithm of selected doses. Measurable responses are represented by Triangles. The sigmoid curve (— —) represents a population described by normal distribution; in theory the percent responding never reaches zero on the low end or 100% on the high end. The other curves (-- and — - —) represent a threshold for the response. The boxed in portions represent regions in which prediction of incidence depends on stochastic, statistical projection.

Figure 6) A simplified diagram of the plausible fate of chemical in the body. C is the chemical, RM is a reactive intermediate bound irreversibly to a macromolecule.

Figure 7) Diagrammatic representation of the elimination of a chemical via a primary saturable pathway and by a secondary pathway and by a secondary pathway whose significance increases saturation of the primary pathway.

Figure 8) Hypothetical model of the fate of a chemical carcinogen in the body requiring, for expression of carcinogenicity, activation to a reactive electrophilic metabolite and subsequent irreversible covalent reaction with a genetic receptor. In this model, Excretion (e), Activation (a), Detoxification (d), and Repair (r) occur in accordance with dose-dependent (Michaelis-Menten) kinetics while the remaining reactions are represented by apparent first-order kinetics.

Figure 9) As a function of dose, the hypothetical amounts normalized for dose of the chemical bound covalently to nongenetic (CBN/C_0), bound covalently to genetic material but repaired ($CBGR/C_0$), and bound covalently to genetic material and not repaired ($RCBG/C_0$). These values were obtained using the foregoing model together with realistic but arbitrarily selected rate constants for the reactions designated by the model in Figure 8. Projection of the incidence of cancer in response to high doses to predict accurately the incidence of low doses requires a straight line parallel to abscissa.

Figure 10) Mole fraction of O-6-ethylguanine/guanine in the DNA of brain and liver as a function of time following a 76 mg/kg intraperitoneal injection of ethylnitrosourea in 10-day old rats.

Figure 11) Mole fraction of O-6-methylguanine/guanine in DNA of liver and kidney as a function of time following 2.5 or 20 mg/kg intraperitoneal injection of dimethylnitrosamine in rats.

Figure 12) Metabolism of vinyl chloride analyzed in accordance with Woolf-Augustinson-Hofstee linearized form of the Michaelis-Menten equation. Vales of v and v/S were taken from Table 1. The line was fit by linear regression analysis. The correlation coefficient, R, was 0.88.

Figure 13) (a) Metabolism of vinyl chloride expressed as log v ($\frac{\mu g \text{ VC metabolized}}{4 \text{ hr}}$) versus percent incidence of hepatic angiosarcoma (probability scale). (b) Exposure concentration expressed as log S(ppm) versus the percent incidence of hepatic angiosarcoma. The probit equivalents of the percent incidence are shown on the right hand ordinant. The solid line is the best fit for experimentally observed responses while the dashed line represents extrapolation below those doses producing an observable response assuming no threshold.