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RISK ASSESSMENT FOR HALOGENATED SOLVENTS

By

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ABSTRACT

A recent development in the cancer risk area is the advent of biologically based pharmacokinetic and pharmacodynamic models. These models allow for the incorporation of biological and mechanistic data into the risk assessment process. These advances will not only improve the risk assessment process for halogenated solvents but will stimulate and guide basic research in the biological area.

Key Words: pharmacokinetics, pharmacodynamics, risk assessment

INTRODUCTION

There is increasing evidence that some chemicals cause cancer in animals through nongenotoxic mechanisms. The halogenated solvents appear to fall in this class. The existence of such chemicals poses a regulatory dilemma regarding the significance of low-level exposures to humans. It has historically been assumed that carcinogenesis is a non-threshold process. That is, no matter how low the exposure, there is some probability that cancer will result. However, if the halogenated solvents actually work through a nongenotoxic mechanism, it is likely that they have a threshold at low doses. Regulatory agencies have been slow to adopt this perspective into the risk analysis process. If risk analysis is to be scientifically based, it is important that mechanistic data be incorporated into the process when available. A recent development in the cancer risk area is the advent of biologically based pharmacokinetic and pharmacodynamic models. Pharmacokinetic models relate applied dose to effective dose at the target tissue, while pharmacodynamic models relate effective dose with biological effect. These models provide a basis for improving the risk analysis process for halogenated solvents.

PHARMACOKINETIC MODELING

Pharmacokinetics is the study of the absorption, distribution, metabolism, and elimination of chemicals in humans and animals. Predictive, biologically based pharmacokinetic models provide an effective approach for interpreting empirical data relating to pharmacokinetics. [1-4] These models utilize measured physiological

parameters, such as breathing rates, blood flow rates, tissue volumes, etc., to describe the metabolic process. These models can often quantitatively relate exposure concentrations (in air, water, or food) to concentrations of parent compound or metabolite in various tissues of the body, allowing prediction of the relationship between applied dose of a chemical and effective dose at the target tissue(s). A chief advantage of the pharmacokinetic model is that by simply using the appropriate physiological, biochemical, and metabolic parameters, the same model can describe the dynamics of chemical transport and metabolism in mice, rats, and humans.

No one pharmacokinetic model can be used to determine the pharmacokinetics of all chemicals. The number of compartments and the way they are connected will vary from chemical to chemical depending upon the chemical's metabolic behavior and the nature of the carcinogenic bioassay data available for the chemical. Tissue groups commonly used in a pharmacokinetic model include: (1) organs such as brain, kidney, and viscera; (2) muscle; (3) fat; and (4) metabolic organs (principally the liver). A pharmacokinetic model is described mathematically by a set of differential equations which calculate the rate of change in the amount of chemical partitioned into each compartment. Metabolism can be described using both a linear metabolic component and a Michaelis-Menten component describing saturable metabolism.

The parameters used in a pharmacokinetic model can be divided into three classes: physiological, biochemical, and metabolic. The physiological parameters (such as breathing rates, blood flow rates, and tissue volumes) are well-defined for mice, rats, and humans, and are

fixed before using the model. Biochemical parameters describe the partitioning of a given chemical between air and blood and blood and tissues and can be experimentally determined using a vial equilibration technique. Metabolic parameters for volatile compounds can be determined either through closed chamber studies or optimization techniques to establish the best metabolic parameters consistent with empirical data.

Pharmacokinetics and Animal Bioassay Data

Biologically-based pharmacokinetic models can be adapted to analyze data from animal bioassays. [5] Consider the case of tetrachloroethylene (perchloroethylene; PCE). Several long-term bioassays have studied the carcinogenicity of tetrachloroethylene administered through either gavage or inhalation. PCE was shown to be carcinogenic in B6C3F1 mice through both inhalation and gavage, but no statistically significant increases in cancer were observed in Fischer 344/N rats. The metabolic pathways of PCE are still uncertain, but there is convincing evidence that the principal site of metabolism is the hepatic microsomal cytochrome P-450 system. In addition, PCE is believed to be metabolized by a second linear pathway which acts by unidentified mechanisms to produce other metabolites.

Figure 1 presents cancer incidence for mice and rats as a function of applied dose. This dose-response curve represents the classical approach to risk assessment in which risks (probability of cancer) are expressed in terms of applied dose. Notice that cancer incidence decreases in mice at high doses. In the classical approach (no consideration of pharmacokinetics), this decrease is explained in terms

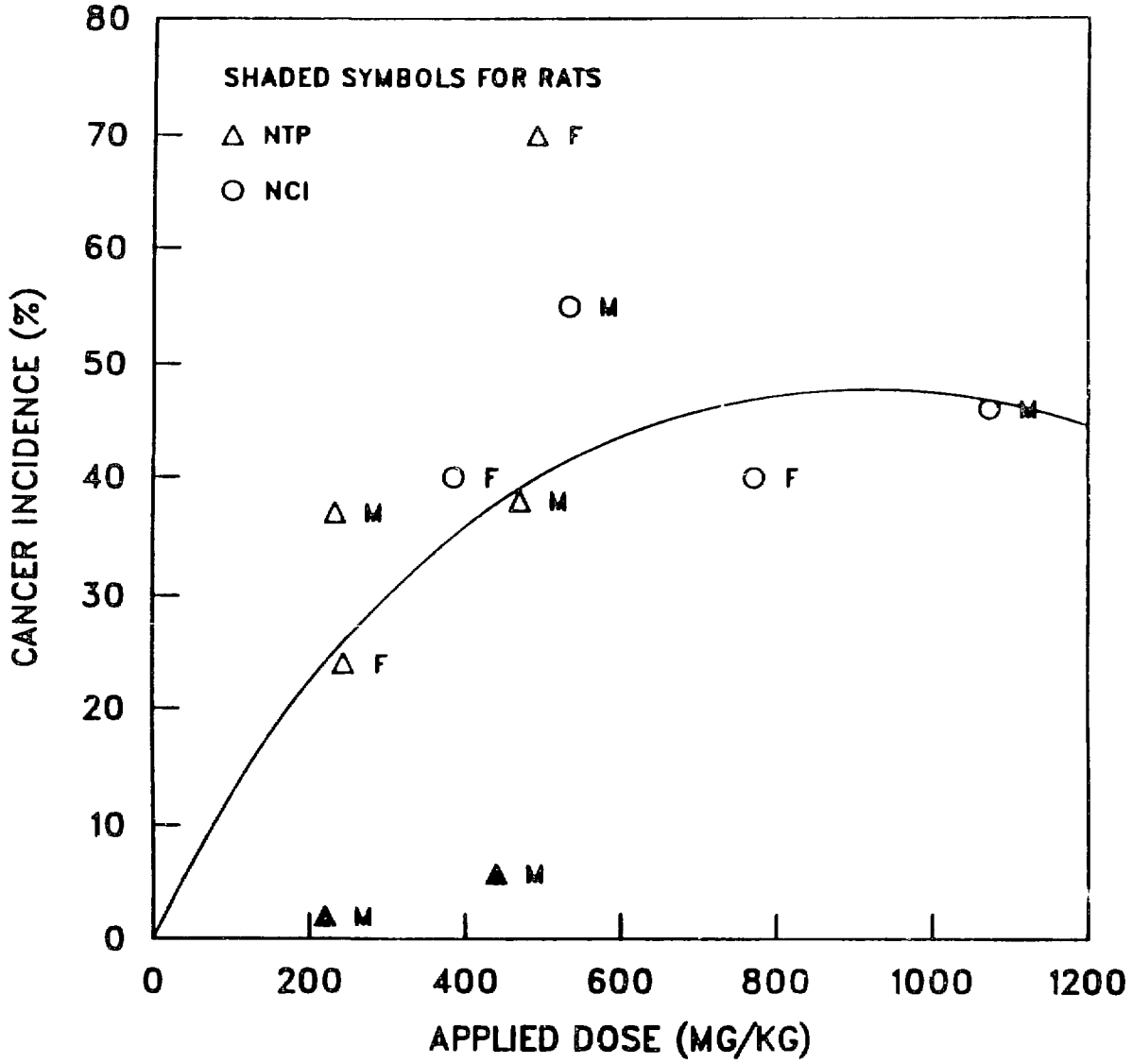
SHADED SYMBOLS FOR RATS
△ NTP
△ F
○ NCI
○ M
○ F
○ F
△ M
△ M
○ M
△ F
▲ M
▲ M
CANCER INCIDENCE (%)
0
10
20
30
40
50
60
70
80
0
200
400
600
800
1000
1200
APPLIED DOSE (MG/KG)

FIGURE 1.

of cellular toxicity at high doses and subsequent cell death. Also, notice that the cancer incidence obtained for rats is inconsistent with that for mice. In the classical approach, this is taken to mean that PCE does not appear to be carcinogenic in rats. We now attempt to use pharmacokinetics to analyze the animal bioassay data.

Figure 2 shows cancer incidence versus total metabolite production in the liver for both the inhalation and gavage bioassays. The incidence rate is not well-linearized when plotted against total metabolite production. However, the dose-response curve is increasing (that is, it does not decrease at high doses) and the cancer incidence for rats is more consistent with that for mice.

Figure 3 displays cancer incidence versus metabolite production along the linear pathway. In this case, cancer incidence appears to be independent of dose, which suggests that the metabolite produced via the linear pathway is not carcinogenic. That is, there does not appear to be a relationship between the amount of metabolite produced via the linear pathway and cancer incidence in the animal bioassays.

Figure 4 shows cancer incidence versus metabolite production along the nonlinear MFO pathway and presents a much better correlation between cancer incidence and metabolite production. The dose-response function is now nearly linear and the cancer incidence in rats is consistent with that in mice.

These results indicate that the tumor incidence rates observed in animal bioassays are more clearly correlated with the amount of the intermediate, reactant metabolite produced by the nonlinear metabolic pathway of PCE than with either the applied dose of parent compound or the metabolite produced via the linear pathway. We can conclude that:

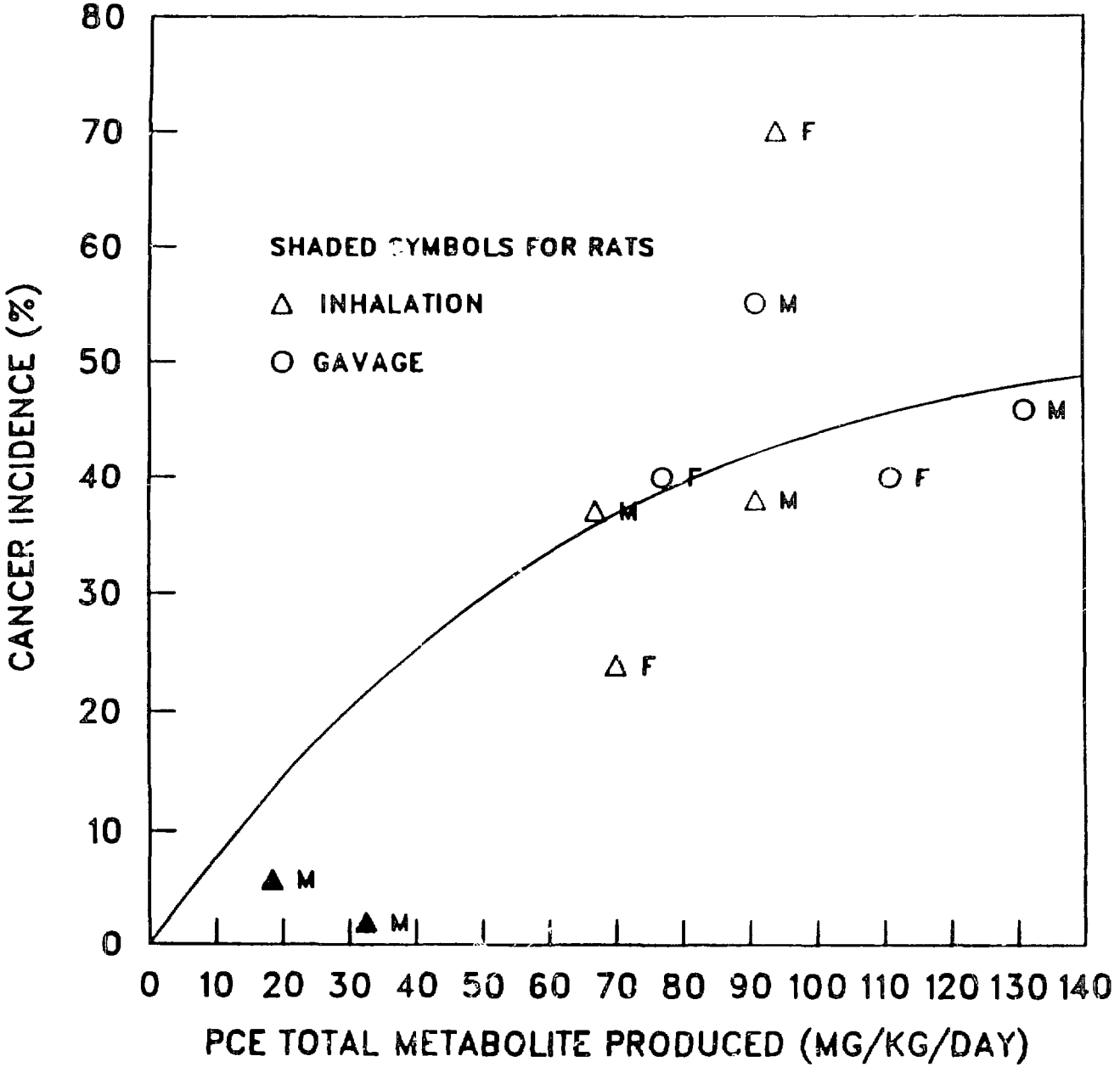
80
70
60
50
40
30
20
10
0
CANCER INCIDENCE (%)
SHADED SYMBOLS FOR RATS
△ INHALATION
○ GAVAGE
△ F
○ M
○ M
○ F
△ M
○ F
△ M
△ F
▲ M
▲ M
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
PCE TOTAL METABOLITE PRODUCED (MG/KG/DAY)

Figure 2.

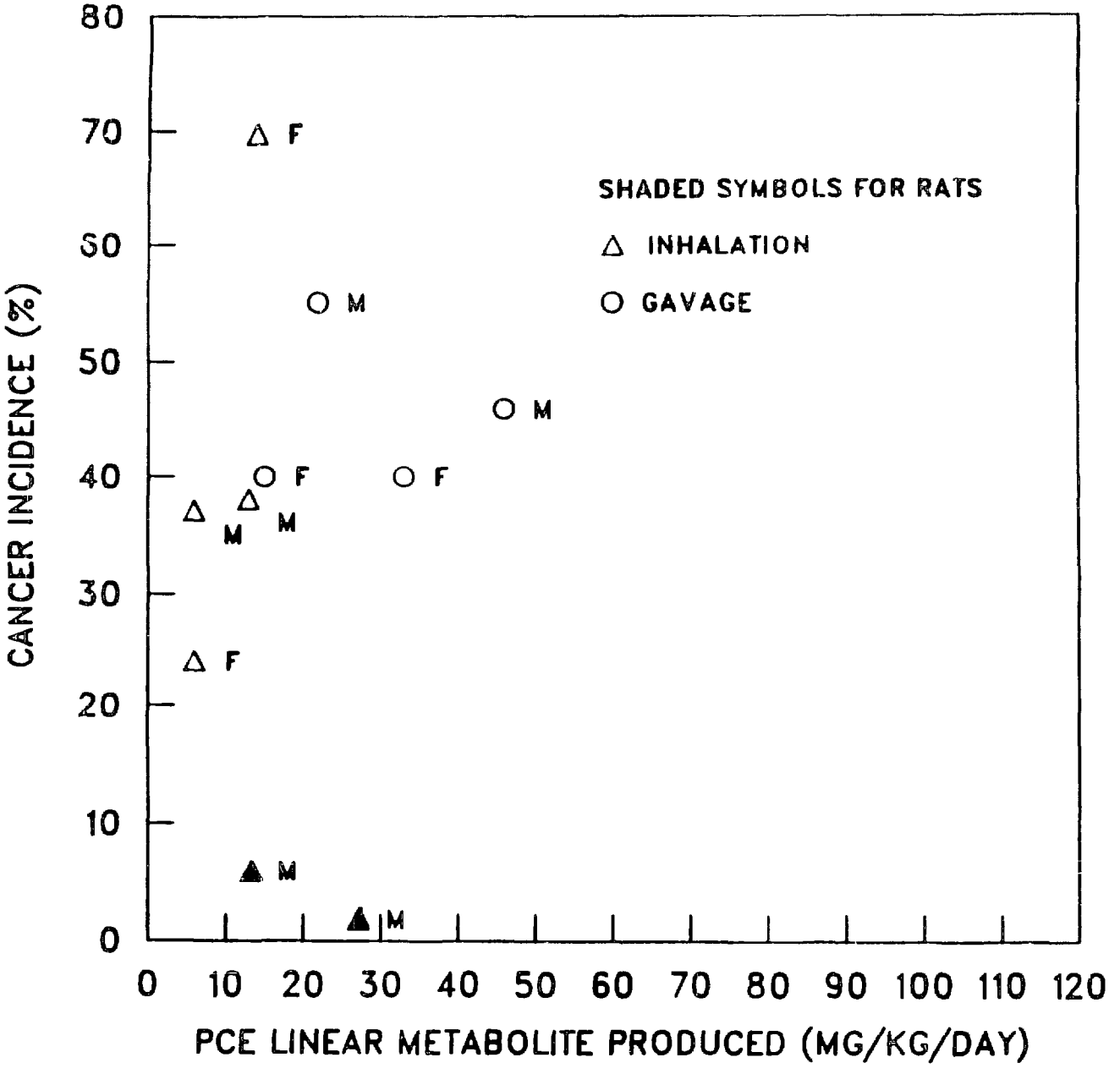
80
70
60
50
40
30
20
10
0
CANCER INCIDENCE (%)
SHADED SYMBOLS FOR RATS
INHALATION
GAVAGE
F
M
M
F
F
M
M
F
M
M
0 10 20 30 40 50 60 70 80 90 100 110 120
PCE LINEAR METABOLITE PRODUCED (MG/KG/DAY)

Figure 3

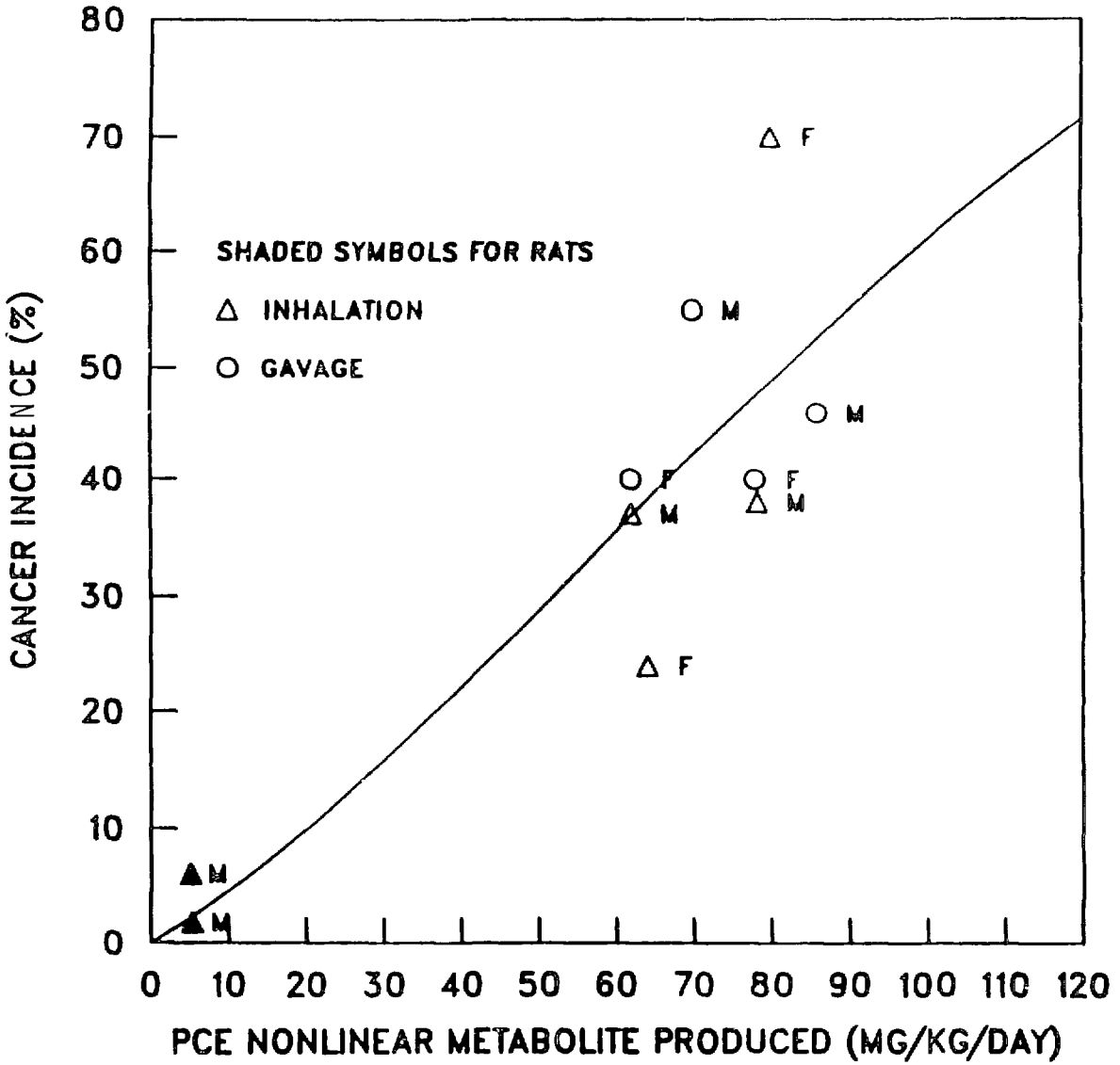
80
70
60
50
40
30
20
10
0
CANCER INCIDENCE (%)
SHADED SYMBOLS FOR RATS
△ INHALATION
○ GAVAGE
△ F
○ M
○ M
○ F
○ F
△ M
△ M
△ F
▲ M
▲ M
0 10 20 30 40 50 60 70 80 90 100 110 120
PCE NONLINEAR METABOLITE PRODUCED (MG/KG/DAY)

Figure 4.

1. The carcinogenic metabolite is produced via the nonlinear cytochrome P-450 pathway;

2. The decrease in the classical dose-response curve at high doses is a result of saturation of the carcinogenic nonlinear metabolic pathway and not cell death;

3. Carcinogenic response to PCE in mice appears to be about equal between sexes and across routes of administration (inhalation vs. gavage).

4. PCE may be carcinogenic in *both* rats and mice. (The reason rats did not develop cancer in the bioassays was that the metabolized doses were too small).

PHARMACODYNAMIC MODELING

Biologically based pharmacodynamic models relate fundamental cellular processes to the epidemiology of cancer in animal and human populations. Several authors have developed pharmacodynamic models based on the assumption that normal cells require two genetic alterations to become cancer cells. [6-8] These genetic alterations are rare and result from a mutation, translocation, or other event at a specific gene. (The background rate of such genetic events in Fischer rat liver tissue is about 1 per 10 million cell divisions). A cell which has undergone a single genetic alteration is termed initiated. An initiated cell is not malignant, nor will it necessarily progress to malignancy. Initiated cells expand through clonal proliferation to form islands (foci) of initiated cells. When one of these initiated cells

undergoes a second genetic alteration, a cancer cell is formed. The vast majority of initiated cells never undergo the second genetic alteration necessary to become cancerous.

This two-stage process is clearly observed in rat liver. Initiated cells are arranged in foci of hepatocytes displaying alterations in phenotype which can be identified by histochemical staining. Initiated cells also have higher rates of proliferation than normal hepatocytes. (In control Fischer rats, initiated cells can proliferate up to 80 times faster than normal hepatocytes). Consider the case of the carcinogen diethylnitrosamine (DEN). A single dose of DEN to rats produces a reproducible number of foci in the liver (each island representing one originally initiated cell). Figure 5 shows the number of foci in rat liver as a function of time after induction by a single dose of 10 mg DEN/kg in rats given a partial hepatectomy 24 hours before DEN application. The partial hepatectomy is performed to enhance cell proliferation so that all initiated cells will clonally expand into visible foci. Figure 5 shows that the number of foci remains constant from the sixth week after induction.

Figure 6 presents the dose-response curve for island induction and indicates that a direct relationship between the number of foci induced and dose of DEN exits. These data demonstrate a consistency and predictability in the early stages of the cancer process. Ongoing research in the pharmacodynamic area is attempting to quantitatively relate the age-specific incidence of initiated cells in rat liver to the age-specific incidence of hepatocellular carcinomas. It is hoped that insights gained from experience with liver carcinogenesis will enable

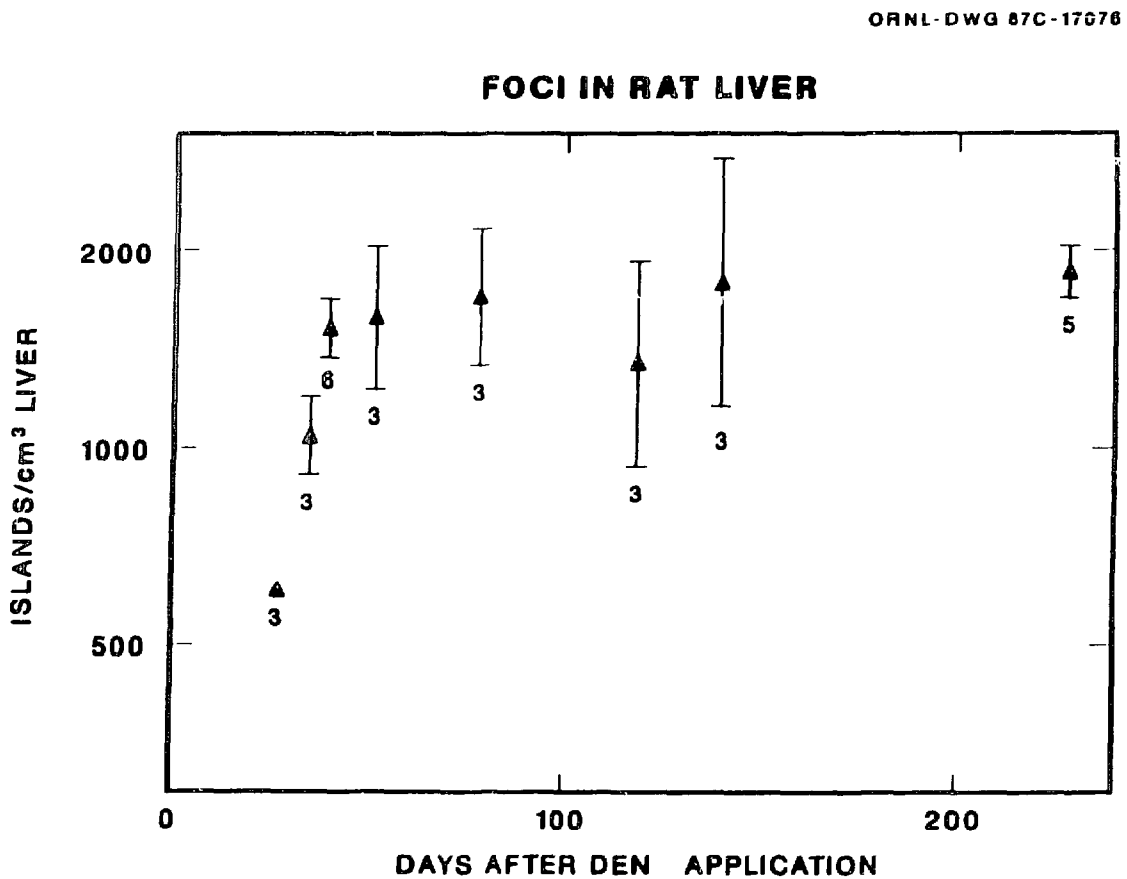
ORNL-DWG 87C-17076
FOCI IN RAT LIVER
ISLANDS/cm³ LIVER
2000
1000
500
0
100
200
DAYS AFTER DEN APPLICATION
3
3
6
3
3
3
3
5

Figure 5.

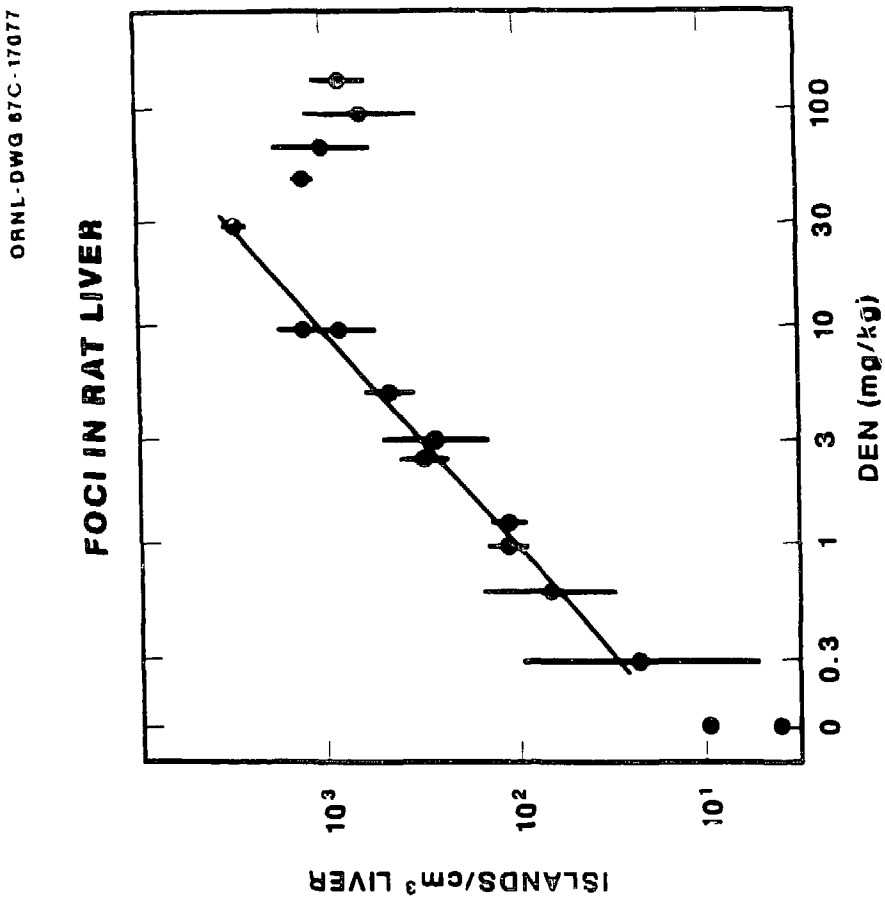
ORNL-DWG 87C-17077
FOCI IN RAT LIVER
ISLANDS/cm³ LIVER
10³
10²
10¹
0
0.3
1
3
10
30
100
DEN (mg/kg)

Figure 6.

identification of the proper experimental research necessary to understand pharmacoydnamics and assist in producing more realistic estimates of risk associated with exposure to environmental carcinogens.

CONCLUSION

To date, a biologically based approach to cancer risk assessment has been used for only a few compounds. Nevertheless, since these approaches incorporate more realistic biological data, they promise to vastly improve the risk assessment process, especially for nongenotoxic compounds which may have a threshold for carcinogenic action. The tetrachloroethylene example illustrated earlier shows the usefulness of pharmacokinetic models in formulating a hypothesis for the mechanism of tumorigenicity (the nonlinear metabolite) of a compound. It also illustrates the benefit of using pharmacokinetic models in extrapolating pharmacokinetic responses across species and across routes of administration. The application of pharmacodynamic models in risk assessment also holds great promise. Pharmacodynamics represents the interface between the applied aims of risk assessment and the large storehouse of biological data on the cellular and molecular nature of cancer. These particular models should improve risk assessments for halogenated solvents, which appear to work through a nongenotoxic promotional mechanism.

I believe we stand on the threshold of great advances in quantitative risk assessment. Within three to four years, we will see classical methods in risk assessment replaced by a second generation of biologically based approaches. These advances will not only improve the risk assessment process for halogenated solvents, but will stimulate and

guide basic research in the biological area. Rather than being a stepchild of biology, the multidisciplinary area of risk assessment will provide a theoretical framework to guide and consolidate basic biological research with the end result of providing more realistic estimates of the carcinogenic risk of exposure to environmental chemicals.

FIGURE LEGENDS

Figure 1. Cancer incidence for mice and rats from gavage (O) and inhalation (Δ) studies plotted as a function of applied dose (mg/kg/day). (M=Male, F=Female)

Figure 2. Cancer incidence for mice and rats from gavage (O) and inhalation (Δ) studies plotted as a function of total metabolites produced (mg/kg/day). (M=Male, F=Female)

Figure 3. Cancer incidence for mice and rats from gavage (O) and inhalation (Δ) studies plotted as a function of PCE metabolites produced along the linear pathway (mg/kg/day). (M=Male, F=Female)

Figure 4. Cancer incidence for mice and rats from gavage (O) and inhalation (Δ) studies plotted as a function of PCE metabolite produced along the nonlinear pathway (mg/kg/day). (M=Male, F=Female)

Figure 5. Number of islands (foci) as a function of time after a single application of 10 mg DEN/kg to rats.

Figure 6. Number of islands in rat liver as a function of dose after single application of DEN.

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