✓

Vinyl Chloride
Risk assessment
threshold

4225

:LLULAR RESPONSE
:ldez, John J. Miglio,

:ESE HAMSTER V79

:COUS CLEARANCE
R. K. Wolff, R. F.

HANGES INDUCED
:Y A 9-WEEK RECOV-

APPLICATION OF THE "FILTER MODEL" TO A RISK ASSESSMENT FOR VINYL CHLORIDE

Clark S. Olson

Illinois Environmental Protection Agency,
Springfield, Illinois

David J. Schaeffer

U.S. Army Construction Engineering Research Laboratory,
Champaign, Illinois

The filter model was used to estimate thresholds for the induction of cancer from many dose-response sets for inhalation and ingestion exposure to vinyl chloride for rat and inhalation exposure for mouse. Estimates for a variety of end-point combinations were log-normally distributed over about 2 decades from about 1 to 100 ppm for inhalation exposure to rat and 0.1 to 30 ppm for mouse. When the data is transformed to "dose" (milligrams per kilogram body weight per day), the estimates for inhalation and ingestion exposure and also for rat and mouse are similar. Estimates for different experiments carried out for different durations of time (single exposure to 1 yr) are comparable. Since the threshold is an intrinsic property of the biological system, the estimate, even from a protocol for short exposure and less than lifetime observation, can be used directly in a risk assessment as the maximum safe dose.

INTRODUCTION

A "threshold" is defined in *Webster's Seventh New Collegiate Dictionary* (1961) as the "point at which a physiological or psychological effect begins to be produced." Druckrey (1959), for example, found that hepatomas were induced by 10^{12} molecules, but not 4×10^{11} molecules, of dimethylaminoazobenzene. Most models of carcinogenesis use a risk-level approach involving one-to-one interactions ("hits") between the insult and target molecules. These theories assume that cells receiving even a single direct hit are affected, and do not consider the possibility of a threshold. However, up to 80% of cells do survive direct hits (by radiation) (Thompson, 1985). There appear to be two populations of cells, however: the sensitive, for which one hit is enough to cause a biological effect, and the nonsensitive. Victor P. Bond (Brookhaven), as quoted by Thompson (1985), notes that

> "A single hit by a charged particle can cause a single cell effect." The result is that "it dies or does not die." If it lives, "it is mutated or not."

We thank the reviewers for stimulating and thoughtful suggestions.

Requests for reprints should be sent to David J. Schaeffer, CERL (EN), P.O. Box 4005, Champaign, Illinois 61820.

Journal of Toxicology and Environmental Health, 17:1–22, 1986

> If it is mutated, "it is carcinogenic or not." There is no middle ground for any of these choices. "The response is a statistical phenomenon."

Because both the "hit" and the response are statistical events, even the one-hit model of chemical carcinogenesis allows for a threshold.

At another level, the existence of real-life thresholds is defined by two factors. One is the stochastic process of molecular interactions in a complex chemical milieu. The other aspect of real-life thresholds is the "no observable effect level" (NOEL) determined by the background noise of any detection system. [This detection requirement is similar to Von Borstal's (1982) concept of threshold.] These factors govern whether or not an effect can be observed in a *population* exposed to a carcinogenic insult, and not with the probability of a single molecule causing cancer in an individual.

One reason why most models used for extrapolation of carcinogen assays to low exposures (Hoel et al., 1983; Fishbein, 1980; Thompson and Funderlich, 1981) do not consider a threshold is the irreversibility of the initial biochemical reactions in carcinogenesis. This ignores the fact that reactions that are irreversible at one level may, through homeostatic mechanisms, be reversible at further steps in carcinogenesis and at higher levels of biological organization, such as through detoxication of activated products, repair of DNA, or control by the immune system.

Another reason why a threshold is not incorporated into extrapolation models is that it is simpler to ignore the large background challenge from thousands of xenobiotics and radiation in the environment through the diet and other modes of exposure (Ames, 1983). A response threshold level must be considered against the uncertainty of the magnitude of both the background challenge and the natural response to it. Thus, relatively few enzyme types of the mixed-function oxidase system (MFO) and other detoxication systems process both natural and human-made carcinogens without distinction (Jakoby, 1980; Jenner et al., 1981). When exposure to a substance of interest is low, these systems must handle the individual substance of interest along with the natural carcinogens already challenging the organism. Risk from exposures to industrial carcinogens must therefore include the magnitude of the added exposure to the background exposure and the added response to the background response.

A realistic extrapolation model would incorporate the homeostatic concept of challenge and response and use a threshold based on departure of the exposed population's response from the population's background response. The idea of approaching exposure-response relations from a homeostatic point of view is similar to that used in the formalism of the steady-state rate of mutation (Stover and Eyring, 1970a,b; Eyring and Stover, 1970; Eyring et al., 1971). These authors considered populations heterogeneous for genotypes and response

phenotypes. "Since biological processes are close to equilibrium most of the time," (Eyring and Stover, 1970), a steady-state theory of mutations was derived on the basis that a biological response (death, mutation, cancer) "ensues when the rate of insult has exceeded the rate of recovery to a sufficient extent that reserves are depleted and the steady-state can no longer be maintained" (Eyring and Stover 1970). Although the inbred colonies of rats and mice used in carcinogenesis studies have more homogeneous genotypes than do the wild populations considered by Stover and Eyring, these colonies are not isogenic. Further, they appear to evolve rapidly (Fitch and Atchley, 1985; Lewin, 1985).

One threshold model incorporating homeostatic mechanisms is the filter model (Schaeffer et al., 1980). The thermodynamic, first-order kinetic, and statistical theory of this model has been given (Janardan and Schaeffer, 1977; Schaeffer et al., 1983). The mathematical formula for the filter model is

$$(R\text{-}R_0) = a\,(\ln D) + b\,(\ln D)^2 \qquad (1)$$

where R is the response at the Dth level of exposure, R_0 is the response of the controls, and a and b are constants obtained by regression or maximum likelihood estimation. The threshold (D_T) for the filter model is estimated either as the point exp ($1\text{-}a/2b$) where the exposure-response curve changes slope or from the confidence limit on the extrapolated value (Schaeffer et al., 1981). Threshold estimates from this model have been compared with "virtual safe dose" (VSD) estimates from risk-level models (Schaeffer et al., 1982; Schaeffer, 1983).

We apply the filter model to over 100 data sets reported for vinyl chloride (VC) by several authors for various organisms and end-points. VC is an especially appropriate substance for evaluating extrapolation models because of its importance as an industrial compound and as an environmental contaminant (U.S. Environmental Protection Agency, 1975, 1980; Waxweiler et al., 1981). Furthermore, there is abundant data on the carcinogenicity, general toxicology, and pharmacology of VC in animals and data from epidemiology studies on humans. Other estimates of risk from VC permit comparisons between many models. VC is representative of a number of organic compounds that are low in chemical reactivity and acute toxicity but that are activated to a potentially carcinogenic form by the MFO system.

METHODS

Sources of Data

Data on frequencies of tumors resulting from VC exposure were obtained from published studies on several strains of rat and mouse involving exposure by inhalation and ingestion, short- and long-term

experiments, and different sacrifice schedules. These studies encompassed many end-points, including incidence of animals with various specific tumors or all tumors, or number of specific tumors or all tumors per animal. Latency data was not used. Each set used showed a well-defined exposure-response relationship, contained at least three exposures and a control, and usually was the complete set given by the author. In addition, some sets of Maltoni were combined. The protocols used in different experiments are summarized in Table 1.

Units Used in Exposures

Inhalation exposure is generally expressed as the concentration (ppm) in air. For VC, 1 ppm is equal to 2.56 mg/m^3 or 0.00256 mg/l (U.S. Environmental Protection Agency, 1975). The inhalation exposure concentration was transformed into an estimated dose (milligrams per kilogram body weight per day) by multiplying the concentration in the air by daily inhalation (liters) and by the fraction of a day used for exposure. For the rat, the conversion factor is obtained as (200 l/24 h $\times$ 0.00256 mg/l $\cdot$ ppm)/0.25 kg = 0.086 mg/kg body weight $\cdot$ ppm $\cdot$ h, in good agreement with the value 0.095 mg/kg $\cdot$ ppm $\cdot$ h obtained by an integrative method (Withey and Collins, 1976). The factor for the mouse is (40 l/24 h $\times$ 0.00256 mg/l $\cdot$ ppm)/0.025 kg = 0.171 mg/kg body weight $\cdot$ ppm $\cdot$ h.

Statistical Methods

After subtracting off control responses, data were fitted to the filter model using ordinary least squares constrained to pass through the origin. Some data sets, those with very shallow slopes or showing poor exposure-response behavior, were fitted to a model that constrained $a \geq 0$ and $b \geq 0$. The Rosen (1960) algorithm for a nonlinear gradient search, as implemented by Kuester and Mize (1973), was used. The algorithm proceeds by the user selecting a feasible starting point for the coefficients a and b and for the step size. The derivative of the objective function [Eq. (1)] with respect to the independent variable (ln D) is evaluated at this base point, and the normalized direction vector components are determined. If the derivative is less than or equal to the tolerance limit, the procedure is stopped because the required point has been found. Otherwise, a new point is located according to rules specified in the algorithm and the objective function is evaluated.

RESULTS

Rat

Threshold estimates for chronic inhalation experiments are 1–100 ppm (0.1–10 mg/kg body weight $\cdot$ d) (Table 2 and Fig. 1). Table 2 is arranged in ascending order of D_T. The table gives our identifying set

...udies encom-
...with various
...mors or all tu-
...used showed a
...d at least three
...te set given by
...combined. The
...d in Table 1.

...concentration
...0256 mg/l (U.S.
...exposure con-
...lligrams per ki-
...ntration in the
...a day used for
...d as (200 l/24 h
...ht · ppm · h, in
...obtained by an
...r for the mouse
...1 mg/kg body

...ted to the filter
...s through the
...showing poor
...at constrained
...linear gradient
...s used. The al-
...g point for the
...e of the objec-
...riable (ln *D*) is
...n vector com-
...r equal to the
...required point
...rding to rules
...valuated.

...nts are 1–100
...). Table 2 is
...dentifying set

TABLE 1. Summary of Experiments Used for Threshold Estimation

Reference	Strain[a]	Number of data sets	Dose range[b]	Exposure protocol	Observation at[c]	End-point
			(ppm)			
Rat (inhalation)						
Maltoni						
BT-1	S-D	12	50–10,000	4 h/d, 5 d/wk, 52 wk	D	Various cancer
BT-2	S-D	5	100–200	4 h/d, 5 d/wk, 52 wk	D	Various cancer
BT-15	S-D	3	1–25	4 h/d, 5 d/wk, 52 wk	D	Various cancer
Composite						
BT-1,2,6,9,15	S-D	11	1–30,000	4 h/d, 5 d/wk, 52 wk	D	Various cancer
BT-7,17	W	3	1–10,000	4 h/d, 5 d/wk, 52 wk	D	Various cancer
BT-3	S-D	5	50–10,000	4 h/d, 5 d/wk, 52 wk	D	Various cancer
Capuio	W	4	50–20,000	4 h/d, 5 d/wk, 52 wk	D	Various cancer
Hong	S-D	14	50–1000	6 h/d, 5 d/wk, 45 wk	+ 12 mo	Various cancer
Rat (ingestion)			(mg/kg · d)			
Maltoni[d]						
BT-11	S-D	5	3.3–50	5 × wk, 52 wk	D	Total angiosarcoma
BT-27	S-D	2	0.03–1	5 × wk, 52–59 wk	D	Total angiosarcoma
BT-11,27	S-D	5	0.03–50	5 × wk, 52–59 wk	D	Total angiosarcoma
feron[e]	W	6	1.7–14	4 h/d, 7 d/wk, 135–144 wk	D	Total angiosarcoma
Mouse (inhalation)			(ppm)			
Maltoni						
BT-4	Swiss	7	50–10,000	4 h/d, 5 d/wk, 30 wk	D	Various cancer
Lee	CD	3	50–1000	6 h/d, 5 d/wk, 26–52 wk	S	Various cancer
Hong	CD	16	50–1000	6 h/d, 5 d/wk, 26 wk	+ 12 mo	Pulmonary tumors
Hong	CD	16	50–1000	6 h/d, 5 d/wk, 13 wk	+ 12 mo	Various cancer
Suzuki	CD	4	1–100	5 h/d, 5 d/wk, 4 wk	+ 40 wk	Pulmonary tumors
Hong	CD	8	50–1000	6 h/d, 5 d/wk, 1 mo.	+ 12 mo	Pulmonary tumors
Hehir	ICR/Aj	1	50–50,000	1 h (single)	D	Pulmonary tumors

[a] S-D, Sprague-Dawley; W, Wistar.
[b] All included a control.
[c] D to death; S, sacrificed after exposure; +, additional observation time.
[d] Intubation.
[e] Fed polyvinyl chloride with vinyl chloride.

TABLE 2. Threshold Estimates (D_T) for Rat Inhalation and Ingestion Data Obtained with the Filter Model

Set number	End-point[a]	Number of d–r pairs	Input units	D_T estimate[b]		Correlation R^2	Reference[c]	Related set number
				ppm	mg/kg · d			
624	total #, F	4	ppm	0.0038	(0.0014)	0.89	4	625
611	nod. hyp.	7	mg	—	0.005	0.91	8;11,27	—
424	LAS	7	mg	(0.024)	0.006	0.93	7;1	573
452	total	5	mg	—	0.04[d]	0.99	6;15	—
423	total	7	mg	(1.3)	0.32	0.99	7;1	018
573	LAS	7	ppm	1.3	(0.32)	0.88	8;1	424
453	other	5	mg	(2.2)	0.53	0.97	6;15	—
454	total #	5	mg	—	0.55[d]	0.99	6;15	—
018	total	7	ppm	2.6	(0.64)	0.99	7;1	423
440	LAS	11	mg	(2.7)	0.66	0.94	6;C	—
600	total #	16	ppm	3.1	(0.76)	0.93	8;C	—
604	LA	15	ppm	3.6	(0.89)	0.92	8;C	—
549	LC, F	4	mg	—	1.2	0.99	2	—
020	kidney	7	ppm	5.3[d]	—	0.91	7;1	—
574	total mal., #	7	ppm	5.8	(1.4)	0.97	8;1	—
626	total #, M&F	4	ppm	6.8	(2.5)	0.99	4	627
628	total #, M&F, 6 mo	4	ppm	8.0[d]	—	0.99	4	629
627	total #, M&F	4	mg	(8.2)	3.0	0.99	4	626
439	total	11	mg	(9.4)	2.3	0.99	6;C	—
610	NP	7	mg	—	2.3	0.94	8;11,27	—
591	LAS	4	mg	—	2.5	0.99	8;27	—
553	total	7	ppm	11	(2.7)	0.99	7;1	428
609	LAS	7	mg	—	2.9	0.98	8;11,27	—
588	LAS	4	mg	—	2.9	0.99	8;11	415
414	total	4	mg	—	3.3	0.99	6;11	—
629	total #, M&F, 6 mo	4	mg	—	3.7[d]	0.99	4	628
586	total mal., #	4	mg	—	3.9	0.95	8;11	—
548	LC, M	4	mg	—	3.9	0.99	2	—
699	LAS, F	4	ppm	12	(4.4)	0.96	4	—
428	total #	7	mg	(16)	4.0	0.99	7;1	553
442	other	11	mg	(16)	3.9	0.95	6;C	—

591	LAS	7	ppm	11	2.9	0.98	8;11	415
553	total	7	mg	—	2.9	0.99	6;11	—
609	LAS	4	mg	—	3.3	0.99	4	628
588	LAS	4	mg	—	3.7[d]	0.99	8;11	—
414	total	4	mg	—	3.9	0.95	2	—
629	total #, M&F, 6 mo	4	mg	—	3.9	0.99	4	553
586	total mal., #	4	mg	—	(4.4)	0.96	7;1	—
548	LC, M	4	ppm	12	4.0	0.99	6;C	
699	LAS, F	7	mg	(11b)	3.9	0.95		
428	total #	11	mg	(16)				
442	other							

416	total #	4	mg	—	4.1	0.98	6;11	—
546	LNP, M	4	mg	—	4.1	0.99	2	—
412	LAS	7	mg	(17)	4.3	0.80	6;7	583
607	LAS	8	ppm	18	(4.4)	0.90	8;7	—
533	LAS, F	4	mg	(18)	6.8	0.96	4	699
583	LAS	7	ppm	19	(4.7)	0.90	8;7	412
443	total #	11	mg	(19)	4.6	0.99	6;C	—
551	LAS, F	4	mg	—	4.9	0.99	2	—
550	LAS, M	4	mg	—	5.0	0.99	2	—
578	total mal., #	7	ppm	21	(5.2)	0.93	8;3	—
576	total #	4	ppm	21	(5.2)	0.97	8;2	—
592	LAS	7	ppm	23	(5.7)	0.99	1	—
625	total #, F	3[e]	mg	—	8.2[e]	0.89	4	624
700	LAS,M&F	4	ppm	23	(8.5)	0.98	4	701
698	LAS, M	4	ppm	24	(8.8)	0.97	4	532
622	total #, M	4	ppm	25	(9.2)	0.99	4	623
581	total mal., #	7	ppm	27	(6.6)	0.92	8;7,17	—
417	total	7	mg	(27)	6.6	0.96	6;3	—
427	other	7	mg	(32)	7.9	0.92	7;1	022
413	total #	7	mg	(32)	7.8	0.97	6;7,17	—
605	Zymbal	15	ppm	33	(8.0)	0.81	8;C	—
419	total #	7	ppm	33	(8.1)	0.96	6;3	—
022	other	7	ppm	34	(8.3)	0.93	7;1	427
580	Zymbal	7	ppm	35	(8.6)	0.98	8;3	418
021	Zymbal	7	ppm	36	(9.0)	0.84	7;1	426
606	total mal., #	8	ppm	38	(9.5)	0.87	8;7,17	—
438	total #	4	mg	(41)	10	0.99	6;2	—
589	total mal., #	4	mg	—	12	0.58	8;27	—
623	total #, M	4	mg	(41)	15	0.99	4	622
532	LAS	4	mg	(41)	15	0.98	4	698
701	LAS, M&F	4	mg	(41)	15	0.98	4	700

(See footnotes on page 8)

TABLE 2. Threshold Estimates (D_T) for Rat Inhalation and Ingestion Data Obtained with the Filter Model (*Continued*)

Set number	End-point[a]	Number of d–r pairs	Input units	D_T estimate[b] ppm	D_T estimate[b] mg/kg · d	Correlation R^2	Reference[c]	Related set number
595	total #	7	ppm	53	(13)	0.89	1	—
593	lung ad.	7	ppm	54	(13)	0.89	1	—
436	LAS	4	mg	(57)	14	0.99	6;2	—
437	other	4	mg	(57)	14	0.99	6;2	—
594	skin	7	ppm	61	(15)	0.85	1	—
418	Zymbal	7	mg	(73)	18	0.99	6;3	580
426	Zymbal	7	mg	(77)	19	0.85	7;1	021
441	Zymbal	11	mg	(93)	23	0.89	6;C	—

[a] LAS, liver angiosarcoma; NP, neoplasia; LC, liver carcinoma; total mal., total malignant; lung ad., lung adenoma; #, number of tumors per individual, all others are incidence; M, male; F, female; nod, hyp., nodular hyperplasia of liver.

[b] Those marked with dash—from ingestion experiment.

[c] First number is reference as follows: (1) Caputo et al. (1974); (2) Feron et al. (1981); (3) Hehir et al. (1981); (4) Hong et al. (1981); (5) Lee et al. (1978); (6) Maltoni (1979); (7) Maltoni and Lefemine (1975); (8) Maltoni (1981); (9) Suzuki (1983). Second number gives Maltoni experiment number(s). C, composite, S-D rat, inhalation.

[d] D_T very large; threshold estimate obtained by confidence interval method.

[e] D_T very large for original data set; only first three values of dose-response set used.

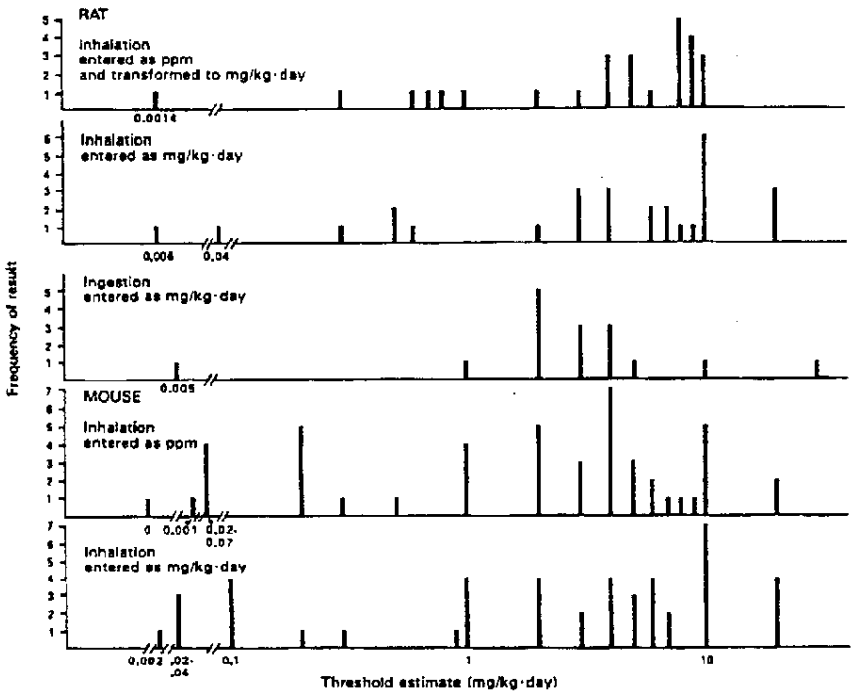


FIGURE 1. Frequency distribution of threshold estimates obtained for rat and mouse data.

number, the biological end-point(s) for the study, the number of exposure-response pairs used in calculations, the input units of exposure (ppm or milligrams per kilogram body weight per day), the estimated threshold (in ppm and milligrams per kilogram body weight per day), the coefficient of determination (R^2), the literature reference used, and a modeled set based on the same or similar data. For example, the data from Maltoni BT-1 for incidence of animals with any type of tumor was entered into the computation as milligrams per kilogram body weight per day in set 423 and as ppm in set 018. Exposure values given as ppm in the original reference were also transformed to estimated dose (milligrams per kilogram body weight per day) prior to the modeling. Values in parentheses in this table are unit conversions made post modeling. The rationale and significance of these unit conversions are given in the discussion section. Figure 1 shows that similar estimates of the threshold are obtained overall using either metric in the modeling.

numbers). (c, composite; SD rat, mouse
^{d} D_1 very large; threshold estimate obtained by confidence interval method.
^{e} D_1 very large for original data set; only first three values of dose-response set used.

The protocols for Maltoni's (Maltoni and Lefemine, 1975; Maltoni, 1979; Maltoni et al., 1981) inhalation experiments BT-1, -2, -6, -9, and -15 using Sprague-Dawley (S-D) rats and BT-7 using Wistar (W) rats, and Caputo's (Caputo et al., 1974) study using Wistar rats (4 h/d × 5 d/wk for 12 mo) were virtually identical. The Hong et al. (1981) experiment differed only in having 1 h more per day and a shorter (10 mo) total exposure period. Threshold estimates for all of these studies are similar irrespective of strain used and exposure duration (17 to >52 wk). D_T estimates for Zymbal-gland tumors are consistently at the high end of the range. Estimates of D_T for other tumors and neoplasia are distributed throughout the range, which implies that the threshold level must be about the same for all affected organs.

Thresholds were estimated for ingestion experiments using gavage [BT-11 and BT-27 (Maltoni, 1979; Maltoni et al., 1981)] and feeding (Feron et al., 1981). Values of D_T for several end-points are randomly distributed within the range found for inhalation experiments (Table 2 and Fig. 1). Results for BT-11 (S-D rat) and Feron (W rat) are similar in spite of the somewhat different protocols and strains of rat.

Mouse

Most of the threshold estimates for various inhalation experiments fall in a range of 0.1 to 10 mg/kg body weight · d (Table 3, Figs. 1 and 2). The arrangement of data in Table 3 follows the arrangement in Table 2. Pre- or postmodeling conversion of units (ppm or milligrams per kilogram body weight per day) made no difference in the distributions of estimates expressed in the same units (Fig. 2).

Two of the studies were for long-term chronic exposure. Experiment BT-4 of Maltoni and Lefemine (1975) exposed Swiss mice using almost the same protocol as they used with the rat (4 h/d, 5 d/wk, 30 wk, and observed until death). The other experiment (Hong et al., 1981) exposed CD mice for 6 h/d, 5 d/wk for 26 wk and observed them for an additional 12 mo. D_T values for these two studies overlap completely.

In addition to the long-term chronic experiments, there are several other experiments for the mouse with exposure periods from one session to several months and with the observation period terminated by sacrifice or lasting for varying times after exposure. Figure 2 shows that a similar range of threshold estimates was obtained from data for experiments of varying protocols. Threshold estimates for pulmonary tumor (adenoma) are represented in each line of each experiment of Fig. 2. The estimates for this specific end-point fall in the narrow range of 1–10 mg/kg body weight · d with the exceptions of two sets (527, 642) of Hong et al. (1981) 13-wk inhalation exposure and the Suzuki (1983) 4-wk experiment (in sets 640, 642).

TABLE 3. Threshold Estimates (D_T) for Mouse Inhalation Data Obtained with Filter Model

Set number	Weeks of exposure	Weeks of observation[a]	End-point[b]	Number of d-r pairs	Input units	D_T estimate ppm	D_T estimate mg/kg · d	Correlation R^2	Reference[c]	Related set number
634	4	+52	total #, M&F	4	ppm	<10[-6]	(<10[-6])	0.85	4	635
640	13	+52	pul, F	4	ppm	0.001	(0.001)	0.99	4	527
648	13	+52	total #, M&F	4	mg	(0.0027)	0.002	0.98	4	647
647	13	+52	total #, M&F	4	ppm	0.02	(0.015)	0.98	4	648
642	13	+52	pul, M&F	4	mg	(0.027)	0.02	1	4	641
669	26	+52	total #, M	4	mg	(0.027)	0.02	0.91	4	668
662	4	+40	pul	6	mg	(0.051)	0.044	0.98	9	661
641	13	+52	pul, M&F	4	ppm	0.06	(0.044)	1	4	642
661	4	+40	pul.	6	ppm	0.086	(0.074)	0.98	9	662
668	26	+52	total #, M	4	ppm	0.09	(0.066)	0.91	4	669
529	26	+52	pul, M	4	mg	(0.15)	0.11	0.91	4	702
654	18–26	0	pul, M	4	mg	(0.20)	0.15	1	5	653
526	13	+52	pul, M	4	mg	(0.25)	0.18	0.99	4	639
429	30	D	all	7	mg	(0.29)	0.14	0.97	7	023
702	26	+52	pul, M	4	ppm	0.29	(0.21)	0.91	4	529
683	31–39	0	AS #, M	4	mg	(0.31)	0.23	1	5	682
653	18–26	0	pul, M	4	ppm	0.32	(0.24)	1	5	654
659	4	+40	pul	6	ppm	0.34[d]	(0.29)[d]	1	9	660
639	13	+52	pul, M	4	ppm	0.36	(0.27)	1	4	526
660	4	+40	pul	6	mg	—	0.31[d]	1	9	659
682	31–39	0	AS #, M	4	ppm	0.44	(0.32)	1	5	683
023	30	+43	all	7	ppm	0.52	(0.25)	0.97	7	429
024	30	+43	LAS	7	ppm	—	(0.5)[e]	—	7	430
644	13	+52	total #, M	7	mg	(1.2)	0.89	0.97	4	643
643	13	+52	total #, M	4	ppm	1.4	(1.0)	0.97	4	644
673	26	+52	total #, M&F	4	mg	(1.8)	1.3	0.91	4	672
504	30	0	total #	7	ppm	1.8[d]	(2.5)[d]	0.95	8	—
672	26	+52	total #, M&F	4	ppm	20	(1.5)	0.93	4	673
650	4–13	0	pul, M	4	mg	(2.0)	1.5	1	5	649
649	4–13	0	pul, M	4	ppm	2.2	(1.6)	1	5	650
689	31–39	0	total #, F	4	mg	(2.4)	1.8	1	5	688

(See footnotes on page 13.)

TABLE 3. Threshold Estimates (D_1) for Mouse Inhalation Data Obtained with Filter Model (*Continued*)

Set number	Weeks of exposure	Weeks off observation[a]	End-point[b]	Number of *df* pairs	Input units	D_1 estimate		Correlation R^2	Reference[c]	Related set number
						ppm	mg/kg · d			
434	30	+43	all	7	mg	(2.4)	1.2	0.97	7	552
552	30	+43	all	7	ppm	2.4	(1.2)	0.97	7	434
527	13	+52	pul, F	4	mg	—	2.2[e]	1	4	640
688	31–39	0	total #, F	4	ppm	3.1	(2.3)	1	5	689
690	31–39	0	total #, M&F	4	ppm	3.2	(2.4)	1	5	691
646	13	+52	total #, F	4	mg	—	2.4[d]	0.99	4	645
691	31–39	0	total #, M&F	4	mg	(3.3)	2.4	1	5	690
645	13	+52	total #, F	4	ppm	3.4[d]	(2.5)[d]	0.99	4	646
670	26	+52	total #, F	4	ppm	3.6	(2.7)	0.93	4	671
671	26	+52	total #, F	4	mg	(3.6)	2.6	0.93	4	670
463	31–39	0	pul	4	mg	(4.4)	3.2	0.97	5	675
686	31–39	0	total #, M	4	ppm	5.0	(3.7)	1	5	687
632	4	+52	total #, F	4	ppm	(3.8)[f]	—	1	4	633
687	31–39	0	total #, M	4	mg	(5.3)	3.9	1	5	686
657	18–26	0	pul, M&F	4	ppm	5.4	(4.0)	0.99	5	658
655	18–26	0	pul, F	4	ppm	5.6	(4.1)	1	5	656
676	31–39	0	pul, F	4	ppm	5.6	(4.1)	1	5	464
658	18–26	0	pul, M&F	4	mg	(6.0)	4.4	0.99	5	657
656	18–26	0	pul, F	4	mg	(6.1)	4.5	1.0	5	655
464	31–39	0	pul	4	mg	(6.1)	4.5	0.99	5	676
684	31–39	0	AS #, F	4	ppm	6.5	(4.8)	0.94	5	685
685	31–39	0	AS #, F	4	mg	(6.9)	5.1	0.94	5	684
663	26	+52	LAS, M&F	4	ppm	7.0	(5.2)	0.94	4	664
664	26	+52	LAS, M&F	4	mg	(7.5)	5.5	0.94	4	663
679	31–39	0	LAS, M	4	ppm	7.7	(5.7)	1	5	465
677	31–39	0	pul, M&F	4	ppm	8.0	(5.9)	1	5	678
025	30	43	pul	7	ppm	8.1	(4.0)	0.95	7	432
027	30	43	other	7	ppm	8.3	(4.1)	0.85	7	433
465	31–39	0	LAS	4	mg	(8.6)	6.3	0.99	5	679
665	26	+52	pul, M&F	4	ppm	8.7	(6.4)	0.88	4	667
616	30	D	ELAS	5	ppm	9.0	(4.4)	0.95	8	619
678	31–39	0	pul, M&F	4	mg	(9.0)	6.6	1	5	677
674	4	+52	pul, M&F	4	ppm	9.1	(6.9)	0.97	4	524
667	26	+52	pul, M&F	4	mg	(9.4)	6.9	0.88	4	665
432	30	43	pul	7	mg	(9.4)	4.6	0.95	7	025

027	30	43	other	7	ppm	8.1	(4.1)	0.81		[illegible]
465	31–39	0	LAS	4	mg	(8.6)	6.3	0.99	5	679
665	26	+52	pul, M&F	4	ppm	8.7	(6.4)	0.88	4	667
616	30	D	ELAS	5	ppm	9.0	(4.4)	0.95	8	619
678	31–39	0	pul, M&F	4	mg	(9.0)	6.6	1	5	677
674	4	+52	pul, M&F	4	ppm	9.3	(6.9)	0.97	4	524
667	26	+52	pul, M&F	4	mg	(9.4)	6.9	0.88	4	665
432	30	43	pul	7	mg	(9.4)	4.6	0.95	7	025
630	4	+52	pul, M&F	4	ppm	9.6	(7.1)	0.91	4	631
631	4	+52 [f]	pul, M&F	4	mg	(10.0)	7.7	0.91	4	630
524	4	+52	pul	4	mg	(10)	7.5	0.97	4	674
619	30	D	ELAS	5	mg	(11)	5.3	0.95	8	616
433	30	43	other	7	mg	(12)	6.0	0.85	7	027
675	31–39	0	pul, M	4	ppm	13	(9.6)	0.99	5	463
651	4–13	0	pul, M&F	4	ppm	15	(11)	0.98	5	652
666	26	+52	LAS, F	4	ppm	16	(12)	0.98	4	528
635	4	+52	total #, M&F	4	mg	—	12[f]	1	4	634
652	4–13	0	pul, M&F	4	mg	(18)	13	0.98	5	651
528	26	+52	LAS	4	mg	(18)	13	0.98	4	666
617	30	D	pul	4	ppm	18	(8.7)	0.99	8	620
597	—[g]	D	pul	5	ppm	18	(3.1)	0.96	3	—
636	13	+52	LAS, F	4	ppm	19	(14)	1	4	525
680	31–39	0	LAS, M&F	4	ppm	19	(14)	0.99	5	681
681	31–39	0	LAS, M&F	4	mg	(22)	16	0.99	5	680
525	13	+52	LAS	4	mg	(22)	16	0.99	4	636
637	13	+52	LAS, M&F	4	ppm	23	(17)	0.98	4	638
620	30	D	pul	4	mg	(25)	12	0.99	8	617
703	26	+52	pul, F	4	ppm	25	(18)	0.88	4	530
430	30	43	LAS	7	mg	—	18[d]	0.88	7	—
638	13	+52	LAS, M&F	4	mg	(27)	20	0.98	4	637
633	4	+52	total #, F	4	mg	—	21[d]	0.95	4	632
530	26	+52	pul	4	mg	(30)	22	0.88	4	703
618	30	D	skin	6	ppm	38	(18)	0.99	8	621
621	30	D	skin	6	mg	(55)	27	0.99	8	618

[a] +, After exposure; +0 sacrificed after exposure; D, to death.
[b] pul, Pulmonary; AS, total angiosarcoma, liver and elsewhere; ELAS, extraliver angiosarcoma; other abbreviations as in Table 2.
[c] Literature citation given in Table 2, footnote c.
[d] D_T very large; threshold estimate obtained by confidence interval method.
[e] $D_t = <10^{-6}$; threshold estimate obtained by Rosen technique (see methods section).
[f] D_T very large with original dose-response-set; this D_T obtained with set of three $d-r$ pairs.
[g] Single exposure.
[h] Sets 661 and 662 include spontaneous tumors, while sets 659 and 660 do not. Data from Suzuki (1983).

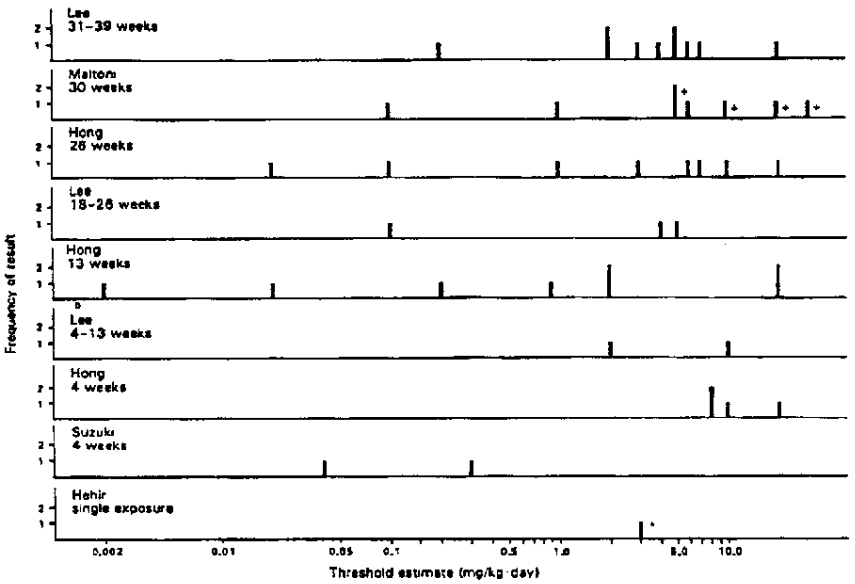
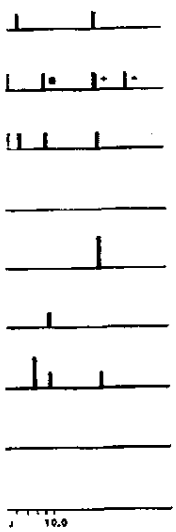


FIGURE 2. Frequency distribution of threshold estimates for mouse experiments of different exposure duration. All data except that of Hehir et al. (1981) were obtained by input in milligrams per kilogram per day; + indicates data obtained at 41 wk (Maltoni and Lefemine, 1975).

When compared on the basis of exposure (ppm), D_T values for the mouse are clearly less than those for the rat. The median D_T is 22 ppm for the rat and 8.2 ppm for the mouse, and 92% of the mouse values are less than the rat median. However, when compared on the basis of estimated dose (milligrams per kilogram body weight per day), the distribution of D_T values are similar and the median for each is about 4 mg/kg body weight · d.

DISCUSSION

The filter model has been used to make a large number of threshold estimates from the extensive literature on VC. In general, these estimates are plausible in terms of the original data set. For instance, the estimate is clearly less than the first obviously positive result in the set. Also, a conservative estimate of D_T of 1 mg/kg body weight · d, given as the 10-percentile of the distribution, is small compared with the intake of about a hundred mg/kg body weight · d of natural carcinogens estimated by Ames (1983). Furthermore, the potency, and thus the hazard, of VC is not unusually large compared to potencies of other



nts of different ex-
nput in milligrams
ine, 1975).

values for the
D_T is 22 ppm
mouse values
d on the basis
t per day), the
each is about

er of threshold
ral, these esti-
r instance, the
sult in the set.
ight · d, given
pared with the
ral carcinogens
, and thus the
ncies of other

human-made and natural carcinogens (Crouch and Wilson, 1979; Crouch et al., 1983; Sugimura, 1982; Gold et al. 1984).

Threshold estimates are also plausible when compared with measured and estimated levels of exposure leading to binding of VC with macromolecules. Thus there is measurable protein binding at an exposure level of 1 ppm for 6 h (= 0.5 mg/kg body weight · d) in the rat liver (Watanabe et al., 1978). From their dose-response data, we estimate with the filter model that the threshold for binding to protein is 0.1 mg/kg body weight · d. Since the level of binding to DNA is about one-hundredth that for cytoplasmic protein for a given exposure (Bolt et al., 1980), a threshold for DNA binding would presumably only occur at a higher exposure level. However, binding to DNA has been measured in rats exposed to 10 ppm for 6 h (= 5 mg/kg body weight · d) (Guengerich and Watanabe, 1979) and is approximately linear from 0 to 250 ppm. An interpolation of this data to a dose of 1 mg/kg body weight · d gave an estimate of 40 adducts per cell. Estimates were similarly made from two other studies that had used single dose exposures of VC to rat (Bolt et al., 1980) and mouse (Bergman, 1982). Binding levels of 13,000 and 260 adducts per cell, respectively, were estimated for doses of 1 mg/kg body weight · d. (This calculation used the conversion factor from the methods section and assumed 10 g for the weight of liver in rat, 10^9 cells per liver in rat, and 10×10^{-12} g DNA per cell for rat and mouse.) These calculations indicate that the evidence is mixed on whether there will be a significant level of adduct formation at 1 mg/kg body weight · d in comparison with the hundreds to thousands of lesions formed as "background" events in a cell each day (Stott and Watanabe, 1982). Such adducts as there are could be further reduced by repair before the possibility of fixation by replicative DNA synthesis. Also, the threshold estimate we give may reflect reversal of the process at later stages in a multistep process of carcinogenesis.

Estimates of D_T for rats or mice cover a range of two to three decades. The large number of estimated values for each species defines the distribution of D_T in detail and brackets the portion of the infinite range between the lowest exposure and zero, which should be used for risk assessment. The variability in the threshold estimates should also be compared to that for biological data in general. Incidence for cancer in different concurrent control groups can vary from 0 to 28% (Society of Toxicology, Task Force of Past Presidents, 1982), while differences in enzyme activity can vary by a factor of 150 (Harris et al., 1982). Tumor rates in replicate groups in the ED01 study differed by up to 3- to 6-fold for controls and treatment groups, and Hartley-Sielkin risk estimates for "virtually safe dose" at 30 mo differed by 0.04–2 ppm for replicates (Society of Toxicology, 1981).

The results for the mouse experiments of various durations of ex-

posure and/or observation, along with the rat experiment BT-3, suggest that the instantaneous level of exposure is more important than the cumulative effects over time. A practical conclusion from these results is that in some cases a carcinogen assay need be carried out only until there is a noticeable exposure-response relationship over several exposures. This is in contrast to the recommendations of the ED01 group (Gaylor, 1980).

In all the experiments used for estimating threshold, exposure started when the animals were young. Groth et al. (1981) exposed rats of different ages for a short time at a single dose. Based on lifetime observation, they concluded that older animals were more susceptible than younger animals. From their work, one would predict that animals exposed for longer periods of time would show a lower threshold simply due to aging. However, Drew et al. (1983) point out that Groth et al. (1981) did not really let the group of animals exposed at a younger age live long enough after the exposure period to develop the typical VC-induced cancer—liver angiosarcoma. In fact, Drew et al. (1983) concluded from their own single-dose experiment that younger animals are more susceptible. They also noted that similar results have been obtained for other substances. Younger animals are known to have higher cell-proliferation rates and higher levels of endogenous promoters. The mouse series shown in Fig. 2 probably shows the threshold for younger animals. It is unfortunate that these workers did not do a dose-response study at different ages, since the susceptibility (total incidence of cancer at an above-threshold dose) could be age-dependent with a threshold that was age-independent. The scatter in the threshold estimates makes it difficult to decide if there is a lower threshold for older animals or animals exposed chronically for a longer period into old age. Since these estimates are averaged over age and other factors, they are population, not individual, estimates. Hence, if additional variables could be controlled, distinguishable estimates might be developed for different subgroups, although these must all be within the range developed here for the population. The conclusion that the threshold is a function of exposure level, not duration, is consistent with the meaning of "threshold" and contrasts sharply with duration-dependent incidence rates (Druckrey, 1959) at higher exposures.

Recent studies note that when rat and mouse (and also hamster) data are compared over a number of chemicals, there is rather good agreement in the carcinogenic potency of the chemicals (Schach von Wittenau and Estes, 1983; Crouch and Wilson, 1979; Gold et al., 1984). D_T estimates follow this pattern; the ranges of D_T estimates for rat and mouse overlap considerably when scaled by using standard respiratory constants. Because the method used to estimate D_T is nonlinear, there is no a priori reason why the distributions of D_T values estimated from data transformed pre- and postmodeling (Fig. 1) should be the same if

the estimates only reflect mathematical relationships. If the estimates reflect biology, however, a D_T value obtained from data expressed in one set of units must be linearly transformable to other units. The fact that this linear transformation is possible with D_T values strengthens our assertion that the filter model is a valid mathematical expression of the net biochemical events that are scored as exposure-responses.

Comparisons of D_T With Other Estimates of Risk

The D_T data base can be compared with the carcinogenicity potency (TD50) (Peto et al., 1984) values reported for vinyl chloride (Gold et al., 1984). The shapes of the distributions of D_T values given in Figs. 1 and 2 are very similar to the shapes of the distributions of TD50 reported by Gold et al. (1984) for vinyl chloride. The similarity between the distributions of the two measures is consistent with our assertion that D_T values are legitimate extrapolations from the observable range to very low exposures. Additional support for the assertion is given by the multiple correlation ($r = 0.78$; $p = 0.0008$) for the regression through the origin for log(TD50) and log(D_T + 1) values for nine sets of data.

In evaluating the significance of our results, it was instructive to compare D_T values with other estimates of lifetime risk for VC, as we have done for other compounds (Schaeffer et al., 1982). One formal comparison was made previously by solving the model [Eq. (1)] as a Lagrange-gamma multihit dose-response model (Janardan and Schaeffer, 1984). The point estimate of 0.001 ppm for the "virtual safe dose" at an excess risk of 10^{-6} for the set used by Rai and Van Ryzin (1979) compares favorably with their estimate of 0.00056 ppm made using a generalized K-hit dose-response model. Significance of D_T estimates can also be judged by comparing them with the numerous published estimates of 0–0.1 for the excess lifetime risk to rodents and humans exposed to 1 ppm VC (OSHA 1980, p. 5200). Additional comparisons for specific data sets for most of the models commonly used for risk analysis can be made from the data in Tables 2–4.

Risk to Humans

D_T estimates for rat and mouse demonstrate that the procedure used here provides consistent estimates of the threshold, taken over all routes of exposure and end-points. Because the threshold is an intrinsic property of the biologic system with a basis in thermodynamics, interspecies conversions of D_T values should not be subject to the uncertainties in conversions of exposure-response data. While the delivered dose is dependent on metabolic and kinetic factors that differ with species and individuals, thermodynamic requirements for the reaction of a sufficient delivered dose are determined by the structures of the carcinogen and target molecules.

Under conditions of homeostatic equilibrium (Eyring and Stover,

TABLE 4. Low-Dose Extrapolations Using Data of Maltoni[a]

Model	Experiment	Data	Risk	VSD[b]
Linear				
Gaylor and Kodell (1980)	BT-1	50–6000	10^{-6}	7×10^{-4}
Kuzmack and McGaughy (1975)	BT-1		10^{-6}	2×10^{-4}
Extreme value				
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	4×10^{-7}
Logit				
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	2×10^{-6}
Schneiderman et al. (1975)	BT-1	50–500	10^{-6}	0.119
Probit				
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	5×10^{-3}
Schneiderman et al. (1975)	BT-1	50–500	10^{-6}	0.073
Weibull				
Carlborg (1981)	BT-1	50–6000	10^{-6}	6×10^{-8}
Gamma multihit				
Rai and Van Ryzin (1979)	BT-1	50–500	10^{-6}	5×10^{-4}
Cornfield et al. (1980)	BT-1	50–6000	10^{-4}	3×10^{-5}
Van Ryzin and Rai (1980)	BT-1	50–6000	10^{-4}	0.12
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	8.4×10^{-8}
Haseman, (1981)	BT-1	50–6000	10^{-6}	3.9×10^{-10}
Food Safety Council (1978)	BT-1	50–6000	10^{-6}	3.9×10^{-10}
Multistage				
Crump et al. (1977)	BT-1	50–500	10^{-6}	5×10^{-3}
Guess et al. (1977)	BT-1	50–2500	10^{-6}	10^{-1}–10^{-3}
Food Safety Council (1978)	BT-1	50–6000	10^{-6}	0.02
Gaylor and Kodell (1980)	BT-1	50–6000	10^{-6}	5.2×10^{-4}
Cornfield et al. (1980)	BT-1	50–6000	10^{-4}	2
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	0.2
Haseman et al. (1981)	BT-1	50–6000	10^{-6}	0.02
U.S. Environmental Protection Agency (1980)	BT-1	50–2500	10^{-6}	0.005

[a] Maltoni et al., 1981.

[b] VSD, virtual safe dose.

1970), the net free energy for the reaction of a carcinogen with a target molecule in in vitro systems, hence the threshold, should not vary appreciably between species. This was found in the agreement of threshold estimates (in milligrams per kilogram body weight per day) for mice and rats, and we expect that thresholds for humans are also about the same. Thus, although the measured metabolic rate for VC in humans is about 20% that for the rat (Bolt et al., 1981), which is about the same fraction for basal metabolism (Oser, 1981), the DNA repair rate for human cells is 5–9 times faster than rat [Hart and Setlow (1974); based on exposure to a different xenobiotic]. The National Academy of Sciences (1975) estimated that workers exposed to 280 mg/kg body weight · d VC based on exposure of 150 ppm for 8 h/d, 5 d/wk, 50 wk/yr for 5 yr, suffered a risk of 0.2% excess angiosarcoma. Using linear extrapolation, a risk of 10^{-6} corresponds to 0.14 mg/kg body weight · d (~20 ppm), in excellent agreement with this expectation.

The question remains how large data sets such as ours or that of Gold et al. (1984) should be used for risk assessment. One suggestion is to apply the same methods that are used to set safety exposures for toxic substances. These exposures are often estimated as one-hundredth the median toxic dose (e.g., LD50, LC50, etc.). Ratios of the median of all D_T estimates to the median of all TD50 values are: 0.010 (rat), 0.04 (mouse), 0.012 (rat + mouse). These ratios suggest that TD50 values might be similarly adjusted using a factor of 0.01, at least as a first attempt.

A different problem concerns the scatter observed in estimates made using various data. When only a few values are available, the median of the D_T estimates could be used. When enough values to characterize the (log-normal) distribution of D_T estimates are available, some reasonable probability point might be chosen. We suggest, for reasons based on the expected scatter in observations from a log-normal distribution (Vysocanskii and Petunin, 1980), that this point be the lower three-standard-deviations bound from the mean of the logarithms of the values.

In conclusion, we have used the filter model with a large number of dose-response sets for VC. This model considers the damage/response aspects of homeostasis. Threshold estimates at the lower end of the range (1 mg/kg body weight · d) are at a level where there is an approximate threshold for DNA adduction in one rodent study but not in two others. The threshold estimate can be used in a risk assessment even when the experimental protocol is for short exposure and less than lifetime observation.

REFERENCES

Ames, B. N. 1983. Dietary carcinogens and anticarcinogens. *Science* 221:1256–1264.

Bergman, K. 1982. Reactions of vinyl chloride with RNA and DNA of various mouse tissues in vivo. *Arch. Toxicol.* 49:117–129.

[redacted]

[illegible]	BT-1	[illegible]	[illegible]	[illegible]
Gaylor and Kodell (1980)	BT-1	50–4000	10^{-4}	5.2×10^{-4}
Cornfield et al. (1980)	BT-1	50–6000	10^{-4}	2
Krewski and Van Ryzin (1981)	BT-1	50–6000	10^{-5}	0.2
Haseman et al. (1981)	BT-1	50–6000	10^{-6}	0.02
U.S. Environmental Protection Agency (1980)	BT-1	50–2500	10^{-6}	0.005

[a] Maltoni et al., 1981.
[b] VSD, virtual safe dose.

Bolt, H. M., Filser, J. G., Laib, R. J., and Ottenwalder, H. 1980. Binding kinetics of vinyl chloride and vinyl bromide at very low doses. *Arch. Toxicol. Suppl.* 3:129–142.

Bolt, H. M., Laib, R. J., Filser, J. G., Ottenwalder, H., and Buchter, A. 1981. Vinyl chloride and related compounds: Mechanisms of action on the liver. In *Frontiers in Liver Disease*, eds. P. D. Berk and T. C. Chalmers, pp. 80–92. New York: Thieme-Stratton.

Caputo, A., Viola, P. L., and Bigotti, A. 1974. Oncogenicity of vinyl chloride at low concentrations in rats and rabbits. *IRCS Med. Sci: Libr. Compend.* 2:1582.

Carlborg, F. W. 1981. Dose-response functions in carcinogenesis and the Weibull model. *Food Cosmet. Toxicol.* 19:255–263.

Cornfield, J., Rai, K., and Van Ryzin, J. 1980. Procedures for assessing risk at low levels of exposure. *Arch. Toxicol. Suppl.* 3:295–303.

Crouch, E., and Wilson, R. 1979. Interspecies comparison of carcinogenic potency. *J. Toxicol. Environ. Health* 5:1095–1118.

Crouch, E., Wilson, R., and Zeise, L. 1983. The risks of drinking water. *Water Resour. Res.* 19:1359–1375.

Crump, K. S., Guess, H. A., and Deal, K. L. 1977. Confidence intervals and test of hypothesis concerning dose response relations inferred from animal carcinogenicity data. *Biometrics* 33:437–451.

Drew, R. T., Boorman, G. A., Haseman, J. K., McConnell, E. E., Busey, W. M., and Moore, J. A. 1983. The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice and hamsters. *Toxicol. Appl. Pharmacol.* 68:120–130.

Druckrey, H. 1959. Pharmacological approach to carcinogenesis. In *Ciba Foundation Symposium on Carcinogenesis: Mechanisms of Action*, eds. G. E. W. Wolstenholme and M. O'Connor, p. 110. Boston: Little, Brown.

Eyring, H., and Stover, B. J. 1970. The dynamics of life. II. The steady-state theory of mutation rates. *Proc. Natl. Acad. Sci. U.S.A.* 66:441–444.

Eyring, H., Stover, B. J., and Brown, R. A. 1971. The dynamics of life. V. Applying the steady-state theory of mutations to human cancer. *Proc. Natl. Acad. Sci. U.S.A.* 68:1670–1672.

Feron, V. J., Hendriksen, C. F. M., Speck, A. S., Til, H. P., and Spit, B. J. 1981. Lifespan oral toxicity study of vinyl chloride in rats. *Food Cosmet. Toxicol.* 19:317–333.

Fishbein, L. 1980. Overview of some aspects of quantitative risk assessment. *J. Toxicol. Environ. Health* 6:1275–1296.

Fitch, W. M., and Atchely, W. R. 1985. Evolution in inbred strains of mice appears rapid. *Science* 228:1169–1175.

Food Safety Council. 1978. Quantitative risk assessment. *Food Cosmet. Toxicol.* 16:109–136.

Gaylor, D. W. 1980. The ED_{01} study: Summary and conclusions. *J. Environ. Pathol. Toxicol.* 3(3):179–183.

Gaylor, D. W., and Kodell, R. L. 1980. Linear interpolation algorithm for low dose risk assessment of toxic substances. *J. Environ. Pathol. Toxicol.* 4:305–312.

Gold, L. S., Sawyer, C. B., Magaw, R., Backman, G. M., de Veciana, M., Levinson, R., Hooper, N. K., Havender, W. R., Bernstein, L., Peto, R., Pike, M. C., and Ames, B. N. 1984. A carcinogenic potency database of the standardized results of animal bioassays. *Environ. Health Perspect.* 56:9–319.

Groth, D. H., Coate, W. B., Ulland, B. M., and Homung, R. W. 1981. Effects of aging on the induction of angiosarcoma. *Environ. Health Perspect.* 41:53–57.

Guengerich, F. P., and Watanabe, P. G. 1979. Metabolism of [^{14}C] and [^{36}Cl]-labeled vinyl chloride in vivo and in vitro. *Biochem. Pharmacol.* 28:589–596.

Guess, H., Crump, K., and Peto, R. 1977. Uncertainty estimates for low-dose-rate extrapolations of animal carcinogenicity data. *Cancer Res.* 37:3475–3483.

Harris, C. C., Trump, B. F., Grafstrom, R., and Autrup, H. 1982. Differences in metabolism of chemical carcinogens in cultured human epithelial tissues and cells. *J. Cell. Biochem.* 18:285–294.

Hart, R. W., and Setlow, R. B. 1974. Correlation between DNA excission-repair and life-span in a number of mammalian species. *Proc. Natl. Acad. Sci. U.S.A.* 76:2169–2173.

Haseman, J. K., Hoel, D. G., and Jennrich, R. I. 1981. Some practical problems arising from use of the gamma multihit model for risk estimation. *J. Toxicol. Environ. Health* 8:379–386.

Hehir, R. M., McNamara, B. P., McLaughlin, J., Jr., Willigan, D. A., Bierbower, G., and Hardisty, J. F. 1981. Cancer induction following single and multiple exposures to a constant amount of vinyl chloride monomer. *Environ. Health Perspect.* 41:63–72.

Hoel, O. G., Kaplan, N. L., and Anderson, M. W. 1983. Implication of nonlinear kinetics on risk estimation in carcinogenesis. *Science* 219:1032–1037.

Hong, C. B., Winston, J. M., Thornburg, L. P., and Lee, C. C. 1981. Follow-up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: Tumor incidence and mortality subsequent to exposure. *J. Toxicol. Environ. Health* 7:909–924.

Jakoby, W. B. 1980. Detoxication enzymes. In *Enzymatic Basis of Detoxication*, ed. W. B. Jakoby, pp. 1–6. New York: Academic.

Janardan, K. G., and Schaeffer, D. J. 1977. Models for the analysis of chromosomal aberrations in human leukocytes. *Biom. J.* 19:599–612.

Janardan, K. G., and Schaeffer, D. J. 1984. Risk assessment of toxic substances using a Lagrange-gamma multi-hit dose response model. *Proc. Social Stat. Section Am. Statistical Assoc.* 1984:244–249, Philadelphia, Penna., Aug 13–16, 1984.

Jenner, P., Testa, B., and DiCarlo, F. J. 1981. Xenobiotic and endobiotic metabolizing enzymes: An overstretched discrimination. *Trends Pharmaceut. Sci.* 2:135–137.

Krewski, D., and Van Ryzin, J. 1981. Dose response models for quantal response toxicity data. In *Statistics and Related Topics. Proceedings of a Conference, Ottawa, May 5–7, 1980*, eds. M. C. Sorgo, D. R. Dawson, J. N. K. Rao, and A. K. M. E. Salch, pp. 201–231. New York: North Holland.

Kuester, J. L., and Mize, J. H. 1973. *Optimization Techniques with FORTRAN.* New York: McGraw-Hill.

Kuzmack, A. M., and McGaughy, R. E. 1975. *Quantitative Risk Assessment for Community Exposure to VC.* U.S. Environmental Protection Agency report, December.

Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L., and Woods, J. C. 1978. Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. Environ. Health* 4: 15–30.

Lewin, R. 1985. Why do inbred mice evolve so quickly? *Science* 228:1187.

Maltoni, Cesare. 1979. Vinyl chloride carcinogenicity: An experimental model for carcinogenesis studies. In *Origins of Human Cancer*, book A, eds. H. H. Hiatt, J. D. Watson, and J. A. Winston, pp. 119–146. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.

Maltoni, C., and Lefemine, G. 1975. Carcinogenicity bioassays of vinyl chloride: Current results. *Ann. N.Y. Acad. Sci.* 246:195–218.

Maltoni, C., Lefemine, G., Cilibert, A., Cotti, G., and Carretti, D. 1981. Carcinogenicity bioassays of vinyl chloride monomer: A model of risk assessment on an experimental basis. *Environ. Health Perspect.* 41:3–29.

National Academy of Sciences. 1975. *Pest Control: An Assessment of Present and Alternative Technologies. Vol. I. Contemporary Pest Control Practices and Prospects. A Report of the Executive Committee*, p. 77. Washington, D.C.: National Academy of Sciences.

OSHA. Occupational Safety and Health Administration. 1980. Identification, Classification and Regulation of Potential Occupational Carcinogens. *Fed. Reg.* 45(15):5001–5296 (Jan. 22).

Oser, B. L. 1981. The rat as a model for human toxicological evaluation. *J. Toxicol. Environ. Health* 8:521–542.

Peto, R., Pike, M. C., Bernstein, L., Gold, L. S., and Ames, B. N. 1984. The TD_{50}: A proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments. *Environ. Health Perspect.* 58:1–8.

Rai, K., and Van Ryzin, J. 1979. Risk assessment of toxic substances using a generalized multi-hit dose response model. In *Energy and Health*, eds. N. E. Breslow and A. S. Whittenmore, pp. 99–117. Philadelphia: SIAM Institute for Mathematics and Society.

Rosen, J. B. 1960. The gradient projection method for nonlinear programming. Part I. Linear constraints. *J. Soc. Ind. Appl. Math.* 8:181–217.

Schach von Wittenau, M., and Estes, P. C. 1983. The redundancy of mouse carcinogenicity bioassays. *Fundam. Appl. Toxicol.* 3:631–639.

Schaeffer, D. 1983. Thresholds for carcinogenesis and their significance to medical practice. *Med. Hypotheses.* 10:175–184.

Schaeffer, D. J., Janardan, K. G., and Kerster, H. W. 1980. Threshold and dose response estimation using the "filter model." *Environ. Int.* 4:157–162.

Schaeffer, D. J., Janardan, K. G., and Kerster, H. W. 1981. Threshold estimation from the linear dose-response model: Method and radiation data. *Environ. Manage.* 5:515–520.

Schaeffer, D. J., Olson, C., Kerster, H. W., and Janardan, K. G. 1982. The low dose extrapolation problem: a review and a new model. *Am J. Math. Manage. Sci.* 2:223–251.

Schaeffer, D. J., Janardan, K. G., Kerster, H. W., and Clarke, A. C. M. 1983. Statistically estimated free energies of chromosome aberration production from frequency data. *Biom. J.* 25:275–282.

Schneiderman, M. A., Mantel, N., and Brown, C. C. 1975. From mouse to man—Or how to get from the laboratory to Park Avenue and 59th St. *Ann. N.Y. Acad. Sci.* 246:237–248.

Society of Toxicology. 1981. Re-examination of the ED_{01} study. *Fundam. Appl. Toxicol.* 1:26–128.

Society of Toxicology, Task Force of Past Presidents. 1982. Animal data in hazard evaluation: Paths and pitfalls. *Fundam. Appl. Toxicol.* 2:101–107.

Stott, W. T., and Watanabe, P. G. 1982. Differentiation of genetic versus epigenetic mechanisms of toxicity and its application to risk assessment. *Drug Metab. Rev.* 13:853–873.

Stover, B. J., and Eyring, H. 1970a. The dynamics of life. I. Death from internal irradiation by 231 Pu and 226 Ra, aging, cancer, and other diseases. *Proc. Natl. Acad. Sci. U.S.A.* 66:132–139.

Stover, B. J., and Eyring, H. 1970b. The dynamics of life. III. Mechanisms of nonsurvival and the relation of dose size. *Proc. Natl. Acad. Sci. U.S.A.* 66:672–676.

Sugimura, T. 1982. A view of a cancer researcher on environmental mutagens. In *Environmental Mutagens and Carcinogens,* eds. T. Sugimura, S. Konda, and H. Takebe, pp. 737–741. New York: Liss.

Suzuki, Y. 1983. Neoplastic effect of vinyl chloride in mouse lung—Lower doses and short term exposure. *Environ. Res.* 32:91–103.

Thompson, D. E. 1985. Heavy radiation and mammalian cells. *Science News* 127:394–395.

Thompson, W. A., Jr., and Funderlic, R. E. 1981. A simple threshold model for the classical bioassay problem. In *Measurement of Risks,* eds. G. C. Berg and H. D. Maillie, pp. 521–533. New York: Plenum.

U.S. Environmental Protection Agency. 1975. Scientific and Technical Assessment Report on VC and PVC. EPA-600/6-75-004 STAR series, U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.

U.S. Environmental Protection Agency. 1980. Ambient Water Quality Criteria for VC. EPA 440/5-080-078 Oct. 1980. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Criteria and Standards Division, Washington, D.C.

Van Ryzin, J., and Rai, K. 1980. The use of quantal response data to make predictions. In *The Scientific Basis of Toxicity Assessment,* ed. H. Witschi, pp. 273–290. New York: Elsevier.

Von Borstal, R. C. 1982. Thresholds and negative slopes in dose-mutation curves. In *Environmental Mutagens and Carcinogens,* eds. T. Sugimura, S. Konda, and H. Takebe, pp. 737–741. New York: Liss.

Vysocanskii, D. F., and Petunin, J. I. 1980. Justification of the 3-sigma rule for unimodal distributions. *Theory of Probability and Mathematical Statistics (USSR)* 21:25–35.

Watanabe, P. G., Zempel, J. H., Pegg, D. G., and Gehring, P. J. 1978. Hepatic macromolecular binding following exposure to vinyl chloride. *Toxicol. Appl. Pharmacol.* 44:571–579.

Waxweiler, R. J., Landrigan, P. J., Infante, P., and Shapiro, R. 1981. Conference to reevaluate the toxicity of vinyl chloride, poly(vinyl chloride) and structural analogs. *Environ. Health Perspect.* 41:1–234.

Withey, J. R., and Collins, B. T. 1976. A statistical assessment of the quantitative uptake of VCM from aqueous solution. *J. Toxicol. Environ. Health* 2:311–321.

Received September 28, 1984
Accepted January 28, 1985

BIOASSAY
VINYL CHLORIDE
GD EC.

4243

ONCOGENIC RESPONSE OF STRAIN A/J MICE TO INHALED CHEMICALS

Bernard Adkins, Jr.

Northrop Services, Inc., Research Triangle Park, North Carolina

Ethard W. Van Stee, Jane Ellen Simmons, Scot L. Eustis

National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Strain A/J mice were exposed by inhalation for 6 h/d, 5 d/wk, for 6 mo to carbon disulfide, 1,2-dibromoethane, ethylene oxide, naphthalene, nitrogen dioxide, or vinyl chloride. Significant increases in pulmonary adenoma formation were observed following exposure to 300 ppm carbon disulfide; 20 and 50 ppm 1,2-dibromoethane; 70 and 200 ppm ethylene oxide; 10 ppm nitrogen dioxide; and 50, 200, and 500 ppm vinyl chloride comapred to control animals. Repeated studies with 1,2-dibromoethane, ethylene oxide, and vinyl chloride gave similarly significant results. Exposure of mice to 30 ppm naphthalene did not elicit a significant adenoma response. Histopathological examination of lungs from animals in these studies revealed multiple alveolar adenomas. Results from earlier studies with these chemicals, using strain A mice and Swiss mice, and bioassay information with rats and mice were compared with these data. These results provide further information for the validation of this in vivo model as a tool for predicting oncogenic potential following chemical exposure.

INTRODUCTION

The strain A mouse lung tumor bioassay, originally described in 1940 (Shimkin, 1940; Andervont and Shimkin, 1940) and refined over the last two decades, has been under extensive investigation as a short-term in vivo model for predicting the potential carcinogenicity of numerous chemicals (Shimkin and Stoner, 1975; Stoner and Shimkin, 1982; Stoner et al., 1984). With the exception of one report (Leong et al., 1971), testing of chemicals from occupational exposures to date has employed noninhalation routes of administration of test chemicals. Several studies have compared tumor response in strain A mice following various routes of chemical treatment (Schut et al., 1983; Stoner

This effort was supported by National Institute of Environmental Health Sciences (NIEHS) contract NO1-ES-79-0009. The authors gratefully acknowledge the excellent technical assistance of the Northrop Services, Inc., staff, including Robert W. O'Connor, Andrew K. Miller, Clinton R. Moorman, Rebecca C. Hamrick, Patricia A. Sams, Pamela Y. Seabrook, and Kathy S. Micor, and the NIEHS staff, including Richard A. Sloane and Michael P. Moorman.

Requests for reprints should be sent to Bernard Adkins, Jr., Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, North Carolina 27709.

Journal of Toxicology and Environmental Health, 17:311–322, 1986

et al., 1984), tumor response in 2-yr chronic bioassays (Maronpot et al., 1983), and interaction with other test chemicals (Witschi, 1983; Witschi et al., 1981; Witschi and Doherty, 1984). Previous reports have also characterized the urethane–strain A mouse response (Dourson and Baxter, 1981; Shimkin and Stoner, 1975) and urethane–Swiss mouse pulmonary adenogenesis (Dourson and O'Flaherty, 1982; Sichak and O'Flaherty, 1984).

The present study was designed to test the oncogenic response of the strain A mouse animal model with various chemicals administered via inhalation. All chemicals selected were tested at or below their threshold limit value (TLV) (American Conference of Governmental Industrial Hygienists, 1983) and were either known to be carcinogens or potential carcinogens (genetic toxicity information), or were of unknown carcinogenic potential due to an absence of testing data. The potent hepatocarcinogen, vinyl chloride, was tested by inhalation in this sytem and compared to previous results reported with strain CD1 mice (Hong et al., 1981; Lee et al., 1978; Suzuki, 1978, 1983). Similarly, chemicals of increasing occupational/environmental concern, 1,2-dibromoethane and ethylene oxide, were tested by inhalation in this system. Three other chemicals (carbon disulfide, naphthalene, and nitrogen dioxide) were also consequently tested during other inhalation studies with these chemicals. Representative lungs from treated and control groups of animals were also examined histopathologically to characterize the adenogenic response to the chemicals tested.

METHODS

Animals

Six to 8-wk-old strain A/J male and female mice (15–25 g) obtained from Jackson Laboratories, Bar Harbor, Me., were used throughout the study. Female mice were used predominantly in the study, except for one study conducted to compare the adenogenic response of both sexes of strain A/J mice following vinyl chloride treatment. The mice were maintained on hardwood bedding (Sani-chips, P.J. Murphy Forest Products Corp., Rochell, N.J.) in temperature-controlled (21–23°C) and humidity-controlled (40–60%) exposure rooms with a 12-h light/dark cycle. NIH-31 certified rodent diet (Zeigler Brothers, Gardner, Pa.) or AIN-76 purified diet (Bio-Serv, Inc., Frenchtown, N.J.) and deionized water were provided ad libitum during nonexposure periods. The health status of all animals was observed twice daily, 7 d/wk. All healthy animals were randomly incorporated into treatment and control groups within 2 wk after receipt.

Chemicals

Reagent grade (98–99% purity) carbon disulfide, 1,2-dibromoethane, and naphthalene were obtained from Fisher Scientific Com-

pany, St. Louis, Mo. The ethylene oxide and nitrogen dioxide were supplied by National Welding Company, Raleigh, N.C., as carrier-grade (≥99.7% purity) cylinder gases. The purity of the vinyl chloride used was not available.

Exposure System

All chemical treatments, except urethane (positive control), were performed by inhalation for 6 h/d, 5 d/wk, for 6 mo in 1330-l glass/stainless-steel exposure chambers. Operating conditions included ventilation with conditioned air at 300–500 lpm, static pressure of −0.3 to −0.5 in of water, temperature of 20–26°C, relative humidity of 40–60%, and 15- to 30-min exhausting prior to unloading animals. Each gas/vapor phase chemical was metered into the main chamber air supply through Koch mixing elements (Koch Engineering Company, New York, N.Y.) prior to introduction into the exposure chambers. Chamber chemical concentrations were monitored with Miran infrared spectrophotometers (Model 80, Foxboro Analytical Instruments, South Norwalk, Conn.) and computer-adjusted (DEC PDP 11/34, Digital Equipment Corp.) to desired exposure levels via a software feedback loop arrangement similar to that described by O'Connor and Adkins (1984) and Van Stee and Moorman (1984a,b). Animals were exposed in stainless-steel wire-mesh exposure cages by treatment group. Animal cages were randomly rotated within exposure chambers each week both between and within the three or four tiers in the chambers. Chemicals were tested at the following inhalation exposure doses: carbon disulfide, 300 ppm; 1,2-dibromoethane, 50 and 20 ppm; ethylene oxide, 200 and 70 ppm; naphthalene, 30 and 10 ppm; nitrogen dioxide, 10, 5, and 1 ppm; and vinyl chloride, 500, 200, and 50 ppm. Replicate studies were conducted with 1,2-dibromoethane, ethylene oxide, and vinyl chloride to test for consistency with this animal model.

Pulmonary Tumor Bioassay

The assay was performed according to the standardized protocol of Shimkin and Stoner (1975) with the exception that chemical treatment was via inhalation. Control groups consisted of conditioned air chamber-exposure (sham) animals and urethane-treated animals (non-exposed, positive controls), which received single intraperitoneal injections (1000 mg/kg). Lungs were removed from animals following 6 mo of treatment and fixed for 24 h in Tellyesniczky's fixative (Shimkin and Stoner, 1975). Enumeration of the typical pearly white nodules on individual lung lobes was performed in replicate by three or more different technicians. Sections of paraffin-embedded lungs having nodules and stained with hematoxylin and eosin were examined histopathologically.

Statistical Analysis

Lung adenoma frequencies, expressed as the number of adenomas per mouse, were evaluated statistically by comparing experimental with control means of each data point using a one-way analysis of variance of Kruskal–Wallis test, as appropriate. When F values indicated significance ($p < 0.05$), comparisons were made of each group with the sham control using Duncan's new multiple-range test (Freund et al., 1960).

RESULTS

Pulmonary Tumor Bioassay

Table 1 presents results on the prevalence of pulmonary adenomas in control and treated animals for all chemicals tested via inhalation exposure for 6 mo. Treatment-related mortality was observed in the 20 and 50 ppm 1,2-dibromoethane (first study) and 500 ppm vinyl chloride (first study, both sexes) groups of strain A/J animals. Significant increases in the frequency (tumors per mouse) and incidence (tumors per tumor-bearing mouse lung) of adenoma formation during the testing period were observed with animals exposed to carbon disulfide, 1,2-dibromoethane (two studies), ethylene oxide (two studies), nitrogen dioxide, and vinyl chloride (two studies). Specifically, exposure of mice to 300 pm carbon disulfide resulted in a small, but statistically significant ($p < 0.05$), increase in the frequency of adenoma formation. The results of the two studies with 1,2-dibromoethane indicated a concentration-related increase in the frequency of adenoma formation. These later results were significant and reproducible at 50 ppm. All animals in the first study responded with slightly more tumors than their counterparts in the second study. The results of the two studies with ethylene oxide indicated a small, but statistically significant ($p < 0.05$), increase in the frequency and incidence of adenoma formation. The results of the two studies with 1,2-dibromoethane indicated a concentration-related increase in the frequency and incidence of adenoma formation. These latter results were significant and reproducible at 50 ppm. All animals in the first study responded with slightly more tumors than their counterparts in the second study. The results of the two studies with ethylene oxide indicated a small, but statistically significant ($p < 0.05$), increase in the frequency and incidence of adenoma formation. The exposure of mice to naphthalene did not cause any significant change in tumor frequency, but did show a significant increase in tumor incidence following treatment at 10 and 30 ppm. Mice exposed to nitrogen dioxide showed a small, but statistically significant ($p < 0.05$), increase in tumor frequency and incidence. The results of the two studies with vinyl chloride indicated a concentration-

related increase in frequency and incidence of adenoma formation. The response was significant and reproducible and was observed in both sexes. The male mice exposed to 50 ppm in the first study developed significantly more tumors than the female mice ($p < 0.001$) exposed to the same concentration in both studies. The female mice exposed to 50 ppm in the second study developed slightly, but statistically significant ($p < 0.05$), more tumors than similar female mice in the first study (controls were not statistically different).

The results of the studies showing little or no change in frequency and incidence of adenoma formation following the regimen of chemical exposure (6 h/d, 5 d/wk, for 6 mo) were further compared with the pooled, negative control response from all experiments. Animals exposed to carbon disulfide (300 ppm) or to ethylene oxide (70 to 200 ppm) showed a significant increase in a mean tumor frequency, even though the increases were relatively small compared to vinyl chloride and 1,2-dibromoethane. Similarly, 200 ppm ethylene oxide (first study) exposure resulted in a significant tumor incidence when compared to the pooled, negative control response. Exposure to naphthalene was without significant effect on tumor frequency, but was significant when the incidence of tumor formation was compared with the pooled, negative control response.

The frequencies of adenoma formation from the negative control animals exposed to conditioned air are also presented in Table 1. The mean tumor count among the 30 female mice tested in the first study with 1,2-dibromoethane indicated a statistically significant ($p < 0.05$) increase from that observed in all studies. The mean tumor counts from the other studies did not differ significantly from each other. The incidence of tumor formation was different in the negative control mice in the naphthalene and first 1,2-dibromoethane studies. The incidence of tumor formation from the other studies did not differ significantly from each other.

The frequency and incidence of adenoma formation from positive control animals given a single intraperitoneal injection of urethane (1000 mg/kg) is summarized in Table 2. Data are presented by study group for both male and female mice surviving through the 26-wk holding period. The positive control mice from the first study with ethylene oxide showed a significantly lower frequency of adenoma formation than the mice from studies with other chemicals, even though there was 100% tumor incidence. The tumor frequencies observed in positive control animals from other experiments were not significantly different from one another.

Histopathologic Evaluation of Lung Adenomas

Histopathological examination of lungs from animals used in these studies revealed multiple alveolar adenomas in treated animals and

TABLE 1. Pulmonary Tumor Bioassay in Strain A/J Mice

Chemical	Inhalation exposure dose (ppm)[a]	Survivors[b]	Surviving animals			
			Total number of tumors (average)	Percent of animals with tumors	Tumors per mouse[c]	Tumors per tumor-bearing mouse lung[c]
Carbon disulfide	300	30/30	23	39	0.71 ± 0.63[d,e]	1.98 ± 0.18[d]
	0	29/30	11	28	0.37 ± 0.38	1.34 ± 0.23
1,2-Dibromoethane	50	11/30	187	100	17.0 ± 4.09[d,e]	17.0 ± 0.53[d,e]
	20	11/30	72	100	6.51 ± 3.67[d,e]	6.51 ± 0.66[d,e]
	0	30/30	29	51	0.97 ± 1.07[f]	1.91 ± 0.29[f]
1,2-Dibromoethane (study 2)	50	57/60	920	100	15.3 ± 5.01[d,e]	15.6 ± 1.51[d,e]
	20	57/60	72	68	1.28 ± 1.18[d,e]	1.87 ± 0.11[d]
	0	58/60	18	26	0.31 ± 0.54	1.27 ± 0.06
Ethylene oxide	200	29/30	63	87	2.14 ± 0.49[d,e]	2.47 ± 0.14[d,e]
	70	28/30	24	56	0.86 ± 0.45[d,e]	1.53 ± 0.10
	0	30/30	11	28	0.46 ± 0.38	1.62 ± 0.27
Ethylene oxide (study 2)	200	28/30	21	42	0.73 ± 0.98[d,e]	1.76 ± 0.22[d]
	0	29/30	11	28	0.22 ± 0.38	1.12 ± 0.12

Naphthalene	30	29/30	11	80	0.37 ± 0.55	1.25 ± 0.07[d]
	10	27/30	10	29	0.35 ± 0.55	1.25 ± 0.07[d]
	0	29/30	6	21	0.21 ± 0.39	1.00 ± 0.00[f]
Nitrogen dioxide	10	30/30	914	130	0.45 ± 0.60[e]	1.55 ± 0.00[d]
	5	30/30	3	11	0.13 ± 0.21	1.17 ± 0.29
	1	29/30	5	12	0.16 ± 0.30	1.39 ± 0.24
	0	29/30	12	80	0.40 ± 0.02	1.39 ± 0.10
Vinyl chloride	500(M)	33/70	2864	100	86.6 ± 7.16[d,e]	86.8 ± 3.48[d,e]
	500	47/70	3809	100	70.3 ± 6.74[d,e]	70.4 ± 2.67[d,e]
	50(M)	72/72	185	88	2.55 ± 0.73[d,e]	2.92 ± 0.23[d,e]
	50	69/70	109	74	1.59 ± 0.57[d,e]	2.13 ± 0.10[d,e]
	0(M)	66/70	26	34	0.39 ± 0.28	1.16 ± 0.03
	0	70/70	33	38	0.47 ± 0.30	1.22 ± 0.06
Vinyl chloride	200	30/30	1124	100	43.9 ± 7.83[d,e]	44.1 ± 1.26[d,e]
(study 2)	50	28/30	59	87	1.97 ± 1.32[d,e]	2.42 ± 0.23[d,e]
	0	30/30	11	26		1.45 ± 0.29

[a] Inhalation exposure: 6 h/d, 5 d/w, 6 mo. Data presented for female mice, except for three groups of male mice (M) tested with vinyl chloride.

[b] Survivors: number of survivors at end of study/number of animals at start of study.

[c] Mean ± standard deviation.

[d] Significantly different ($p < 0.05$) from the corresponding negative control response within the treatment.

[e] Significantly different ($p < 0.05$) from pooled negative control tumor response for all treatments.

[f] Significantly different ($p < 0.05$) from the negative control tumor responses from all other treatment groups.

TABLE 2. Strain A Mouse Positive Control Response to Urethane (1000 mg/kg)[a]

Study group	Sex	Survivors[b]	Surviving animals: Total number of tumors (average)	Surviving animals: Percent of animals with tumors	Surviving animals: Tumors per mouse[c]
Carbon disulfide	F	20/20	460	100	22.9 ± 2.55
Ethylene oxide (study 1)	F	19/20	380	100	20.1 ± 1.77[d]
Ethylene oxide (study 2)	F	19/20	447	100	23.5 ± 6.49
Vinyl chloride	M	20/20	574	100	28.7 ± 3.40
(study 1)	F	17/20	452	100	26.6 ± 2.40
Vinyl chloride (study 2)	F	20/20	282	100	24.1 ± 5.36
1,2-Dibromoethane (study 1)	F	19/20	496	100	26.1 ± 4.40
1,2-Dibromoethane (study 2)	F	29/30	797	100	27.5 ± 6.46
Naphthalene	F	20/20	578	100	28.9 ± 7.10
Nitrogen dioxide	F	39/40	924	100	23.7 ± 3.42

[a] Urethane was administered in a single intraperitoneal injection followed by 26 wk of observation.
[b] Survivors: number of survivors at end of study/number of animals at start of study.
[c] Mean ± standard deviation.
[d] Significantly different ($p < 0.05$) from other counts.

single alveolar adenomas in untreated animals. The alveolar adenomas were morphologically similar in nearly all mice and consisted of large cuboidal or columnar epithelial cells supported by a sparse fibroblastic stroma and arranged in poorly defined acinar structures with papillary formations. An adenoma in a control mouse from the first vinyl chloride study had a primarily solid pattern of growth with uniform polygonal-shaped cells arranged in small clusters separated by capillaries. There was no apparent difference in the occurrence of pattern of anatomic distribution of alveolar adenomas occurring in urethane-treated mice and treated mice of each study group examined.

Alveolar epithelial hyperplasia was observed in lungs examined from treated and positive control (urethane) animals and consisted of focal proliferation of cubiodal cells comprising the alveolar epithelium but without effacement of normal alveolar architecture. This lesion may precede the development of the alveolar adenoma; it was present in the lungs of most treated mice with adenomas and was not observed in examined control lungs.

Several changes in the lungs of mice treated with 1,2-dibromoethane clearly distinguished them from others examined. A single bronchiolar adenoma with a prominent papillary pattern was observed,

consisting of tall columnar epithelial cells. In each of the 1,2-dibromoethane-treated mice examined, multifocal hyperplasia and cellular atypia of the bronchiolar epithelium were observed. These changes consisted of focal stratification of bronchiolar epithelial cells or small papillary formations protruding into the bronchiolar lumens and occasional cells with enlarged hyperchromatic nuclei. Minimal chronic inflammation accompanied these changes and was characterized by a few widely scattered mononuclear cells in the bronchiolar lamina propria. In addition, alveolar epithelial hyperplasia in 1,2-dibromoethane-treated mice was frequently parabronchiolar in location and affected alveoli that abut the bronchiolar walls.

In summary, there were no apparent differences in pattern of distribution or morphologic appearance of alveolar adenomas occurring in urethane-treated mice and mice treated with ethylene oxide, vinyl chloride, or 1,2-dibromoethane. Bronchiolar epithelial hyperplasia and cellular atypia occurred only in 1,2-dibromoethane-treated mice, and one mouse had a bronchiolar adenoma. No carcinomas were observed in the tissues examined.

DISCUSSION

This inhalation study was conducted to test first a variety of chemicals of occupational concern in the strain A/J lung adenoma bioassay system. Second, as has been reported earlier for different chemicals and testing regimen (Maronpot et al., 1983),this study was designed to provide additional information as a continuing effort to evaluate this animal model system as a short-term test for predicting in vivo oncogenesis. Our findings indicate that strain A/J mice develop lung adenomas in a dose-related manner with 1,2-dibromoethane, ethylene oxide, and vinyl chloride. These data agree with previous reports involving various laboratory animals, including mice, that show positive carcinogenic responses for these same chemicals (National Cancer Institute, 1982; Garman et al., 1985; Snellings et al., 1984; Maltoni, 1977; Suzuki, 1983; Viola et al., 1971). Other chemicals tested in this study, including carbon disulfide and nitrogen dioxide, showed significant increases in frequency and incidence of adenoma formation compared to corresponding control responses. These latter findings, if interpreted to indicate oncogenic potential, are supported in part by a previously reported in vitro carcinogenesis study [nitrogen dioxide: Sone et al. (1983)]. Carbon disulfide and napththalene are currently planned for testing in 2-yr National Toxicology Program carcinogenesis bioassays.

Comparison of the control strain A/J mouse tumor response with previous reports showed some similarities with our data. The average frequency of adenomas found in our control mice was 41.7% (Table 1),

with 30.4% of the animals having tumors after the 26-wk duration. These data agree with other 26-wk studies reported by Schut et al. (1983), Shimkin and Stoner (1975), and Stoner et al. (1984), and with a short study duration (4 mo) reported by Witschi et al. (1981). Positive control data expressed as the number of animals developing lung adenomas after a single intraperitoneal injection of urethane (1000 mg/kg) and the frequency of tumor formation in our studies (Table 2) also agree with previous reports (Schut et al., 1983; Shimkin and Stoner; 1975; Stoner et al., 1984). In addition, our data were similar to the control data reported by Maronpot et al. (1983). Histopathological characterization of the multiplicity of the alveolar adenoma response in our studies agrees with the adenogenic response described by Shimkin and Stoner (1975).

The lack of congruity with this model system reported by Maronpot et al. (1983) is evident in data presented in this effort. No reports have demonstrated significant oncogenic potential from animals studies for carbon disulfide or nitrogen dioxide, which did elicit significant adenoma formation in our study. Similarly, naphthalene did not display a significant tumor frequency response, but did, however,show a significant tumor incidence at 10 and 30 ppm, which may have been expected considering the formation of unique lesions of nonciliated pulmonary bronchiolar epithelial cells (Clara cells) of the mouse (Reid et al.,1973; Mahvi et al.,1977; Tong et al., 1982). Ethylene oxide (70 ppm), 1,2-dibromoethane (20 ppm), and vinyl chloride (50 ppm) did elicit a positive tumor frequency and incidence, as shown in Table 1. These data are congruent with the positive carcinogenic findings that report the lowest effective levels for these chemicals to be 10 ppm (Garman et al., 1985), 10 ppm (National Cancer Institute, 1982), and 25 ppm (Maltoni, 1977), respectively.

The lack of consistency of results from different testing laboratories cited by Maronpot et al. (1983) is somewhat evident in our findings. Nonreproducibility between duplicate sudies is shown in Table 1: treatment with 20 ppm 1,2-dibromoethane and treatment with 200 ppm ethylene oxide. Similarly, a lack of consistency in the negative control response is also evident in Table 1. The positive control response to urethane (Table 2) was consistent, except for the tumor response cited during the first ethylene oxide study. On the other hand, the positive findings for 1,2-dibromoethane, ethylene oxide, and vinyl chloride in Table 1 are consistent with positive carcinogenesis data from other animal studies cited for these chemicals. These discrepancies in tumor response may be explained as follows. Our studies involved inhalation treatment, whereas other studies cited have typically involved other routes of exposure. Thus, the dose, metabolism, and mode of exposure of target cells were different from those of noninhalation studies; also, the strains, sources, diet, nutrition, etc. vary between studies in different laboratories.

Further validation of this short-term in vivo model for predicting oncogenic potential of chemicals would be required to permit conclusions relative to the validity and sensitivity of this test. This process should take into account the wealth of information being gathered through both prechronic and chronic testing of these and structurally related chemicals, as well as genetic toxicity data. Treatment of animals with an inert gas (i.e., nitrogen), should also be considered in further characterizing the negative control response. Only after a thorough examination and evaluation can the usefulness of this pulmonary adenoma bioassay be assessed.

REFERENCES

American Conference of Governmental Industrial Hygienist. 1983. *Threshold Limit Values for Chemical Substances and Physical Agents in the Word Environment*, first printing. pp. 10–43. Cincinnati, Ohio: ACGIH.

Andervont, H. B., and Shimkin, M. B. 1940. Biologic testing of carcinogens, II. Pulmonary tumor induction technique. *J. Natl. Cancer Inst.* 1:225–239.

Dourson, M. L., and Baxter, C. S. 1981. Reduced prevalence and growth rate of urethane-induced lung adenoma in aging adult strain A mice. *Toxicology* 20:164–172.

Dourson, M. L., and O'Flaherty, E. J. 1982. Relationship of lung adenoma prevalence and growth rate to acute urethane dose and target cell number. *J. Natl. Cancer Inst.* 69:851–857.

Freund, J. E., Livermore, P. E., and Miller, I. 1960. *Manual of Experimental Statistics*, p. 74. Englewood Cliffs, N.J.: Prentice-Hall.

Garman, R. H., Snellings, W. M., and Maronpot, R. R. 1985. Brain tumors in F344 rats associated with chronic inhalation exposure to ethylene oxide. *Neurotoxicology* 6:117–138.

Hong, C. B., Winston, J. M., Thornburg, L. P., Lee, C. C., and Woods, J. S. 1981. Follow-up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: Tumor incidence and mortality subsequent to exposure. *J. Toxicol. Environ. Health* 7:909–924.

Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L., and Woods, J. S. 1978. Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. Environ. Health* 4:15–30.

Leong, B. K. J., Macjarland, H. N., and Reese, W. H., Jr. 1971. Induction of lung adenomas by chronic inhalation of bis(chloromethyl) ether. *Arch. Environ. Health* 22:663–666.

Mahvi, D., Bank, H., and Harley, R. 1977. Morphology of a naphthalene-induced bronchiolar lesion. *Am. J. Pathol.* 86:559–572.

Maltoni, C. 1977. Vinyl chloride carcinogenicity: An experimental model for carcinogenesis studies. In *Origins of Human Cancer*, Book A, eds. H. H. Hiatt, J. D. Watson, and J. A. Winsten. pp. 119–146. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.

Maronpot, R. R., Witschi, H. P., Smith, L. H., and McCoy, J. L. 1983. Recent experience with the strain A mouse pulmonary tumor bioassay model. *Environ. Sci. Res.* 27:341–349.

National Cancer Institute. 1982. Carcinogenesis bioassay of 1,2-dibromoethane (inhalation study). TR-210(CAS No. 106-93-4). Carcinogenesis Testing Program. Department of Health and Human Services publication (NIH) 81-1766.

O'Connor, R. W., and Adkins, B., Jr. 1984. A computer-asisted system for inhalation toxicology. *Toxicologist* 4(1):126.

Reid, W. D., Hett, K. F., Glick, J. M., and Krishma, G. 1973. Metabolism and binding of aromatic hydrocarbons in the lung. *Am. Rev. Respir. Dis.* 107:539–551.

Schut, H. A. J., Loeb, T. R., Grimes, L. A., and Stoner, G. D. 1983. Distribution, elimination, and test for carcinogenicity of 2,6-dinitrotoluene after intraperitoneal and oral administration to strain A mice. *J. Toxicol. Environ. Health* 12:659–670.

Shimkin, M. B. 1940. Induced pulmonary tumors in mice, II. Reaction of lungs of strain A mice to carcinogenic hydrocarbons. *Arch. Pathol.* 29:239–255.

Shimkin, M. B., and Stoner, G. D. 1975. Lung tumors in mice. Application to carcinogenesis bioassay. *Adv. Cancer Res.* 21:1–58.

Sichak, S. P., and O'Flaherty, E. J. 1984. Consideration of the mechanism of pulmonary adenogenesis in urethane-treated Swiss mice. *Toxicol. Appl. Pharmacol.* 76:397–402.

Snellings, W. M., Weil, C. S., and Maronpot, R. R. 1984. A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* 75:105–117.

Sone, S., Brennan, L. M., and Creasia, D. A. 1983. *In vivo* and *in vitro* NO_2 exposures enhance phagocytic and tumoricidal activities of rat alveolar macrophages. *J. Toxicol. Environ. Health* 11:151–163.

Stoner, G. D., and Shimkin, M. B. 1982. Strain A mouse lung tumor bioassay. *J. Am. Coll. Toxicol.* 1:145–169.

Stoner, G. D., Greisinger, E. A., Schut, H. A. J., Pereiara, M. A., Loeb, T. R., Klaunig, J. E., and Branstetter, D. C. 1984. A comparison of the lung adenoma response in strain A/J mice after intraperitoneal and oral administration of carcinogens. *Toxicol. Appl. Pharmacol.* 72:313–323.

Suzuki, Y. 1978. Pulmonary tumors induced in mice by vinyl chloride monomer. *Environ. Res.* 16:285–301.

Suzuki, Y. 1983. Neoplastic effect of vinyl chloride in mouse lung—lower doses and short-term exposure. *Environ. Res.* 32:91–103.

Tong, S. S., Lowe, M. C., Trush, M. A., Mimnaugh, E. G., Ginsburg, E., Hirokata, Y., and Gram, T. E. 1982. Bronchiolar epithelial damage and impairment of pulmonary microsomal monooxygenase activity in mice by naphthalene. *Exp. Mol. Pathol.* 37:358–369.

Van Stee, E. W., and Moorman, M. P. 1984a. Overview of a system for the computer-assisted operation of a small animal inhalation facility. *Environ. Health Perspect.* 54:299–310.

Van Stee, E. W., and Moorman, M. P. 1984b. Calibration of a system for the computer-assisted operation of a small animal inhalation facility. *Environ. Health Perspect.* 54:311–320.

Viola, P. L., Bigotti, A., and Caputo, A. 1971. Oncogenic response of rat skin, lungs and bones to vinyl chloride. *Cancer Res.* 31:516–522.

Witschi, H. P. 1983. Promotion of lung tumors in mice. *Environ. Health Perspect.* 50:267–273.

Witschi, H. P., and Doherty, D. G. 1984. Butylated hyroxyanisole and lung tumor development in A/J mice. *Fundam. Appl. Toxicol.* 4:795–801.

Witschi, H. P., Hakkinen, P. J., and Kehrer, J. P. 1981. Modification of lung tumor development in A/J mice. *Toxicology* 21:37–45.

Received October 10, 1985
Accepted November 1, 1985