The Carcinogenic Potency of Ethylene Dichloride in Two Animal Bioassays: A Comparison of Inhalation and Gavage Studies

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As part of our project on carcinogenic potency (Ames et al., this volume), we have analyzed the two long-term cancer bioassays of ethylene dichloride (EDC; 1,2-dichloroethane) that are presently available—the gavage study by the National Cancer Institute (NCI 1978) and the inhalation study by Maltoni (this volume). The NCI bioassay indicated significant increases in a variety of tumors in rats and mice of both sexes, whereas the preliminary conclusion from the Maltoni study was that there were no significant carcinogenic effects in either rats or mice. Thus, the results of cancer tests on EDC apparently were different when the dose was administered by inhalation rather than by gavage. We discuss seven factors that alone or in combination might account for this apparent discrepancy, including:

1. impurities in the test chemicals;
2. contaminants in air from other chemicals tested in the same room in the gavage bioassay;
3. contaminants in diet and effect of vehicle (corn oil) in gavage study;
4. dose levels and patterns of dosing;
5. routes of exposure;
6. strain differences in test animals;
7. statistical considerations of the effect of intercurrent mortality: potency analysis of NCI gavage bioassay, and preliminary potency analysis of Maltoni's inhalation bioassay.

From our evaluation of these factors, we conclude that the difference in experimental results may be due in part to several of these factors, and that an adequate comparison requires life-table data from the inhalation study to assess the effects of intercurrent mortality on tumor incidence. The information that would permit such a life-table analysis was not available at the time of writing of this paper. A future communication will contain an analysis of this data.

PURITIES OF TEST CHEMICALS

The EDC preparations used in the gavage and inhalation bioassays were of equivalent high purity, and it seems unlikely that impurities played an important role in the outcomes. This was not clear when the meeting started. Although the NCI report stated, not very precisely, that the sample tested in the bioassay was a technical grade chemical with a purity "greater than 90%," the NCI Chemical Repository indicated a purity of greater than 99.9% (NCI Chemical Repository, Midwest Research Institute [Tracor Jitco subcontract #74-24-106002], IIT Research Institute, 3441 South Federal Street, Chicago, Illinois 60616. EDC data sheet, June 8, 1978, from James Keith [October 29, 1979]).

We obtained a sample, and reanalysis of this material by R. Reitz of Dow Chemical and H. Plotnick of the National Institute for Occupational Safety and Health (NIOSH) indicated a purity by gas chromatography of between 98.5% and 99.8% for the 7-year-old stock samples (see Appendix). A minor impurity of chloroform of 0.02% was found, as well as 14 other trace contaminants.

(The analysis of purity of EDC by Dow Chemical Corporation was an average of 4 determinations and gave 99.3 ± 0.23% purity, with impurities of $CHCl_3$ (2200 ppm), acetone (350 ppm), and seven halogenated compounds at less than 100 ppm (Reitz, this volume). Dibromochloropropane [DBCP; 1,2-dibromo-3-chloropropane] was present at 40 ppm, possibly indicating that test samples of EDC and DBCP were stored in the same room. The analysis of purity of EDC performed by NIOSH was a peak-area analysis indicating greater than 98% purity, with $CHCl_3$ as the major contaminant and 12 other minor contaminants [Plotnick, this volume].)

Maltoni reported a purity of 99.8% for the EDC used in his inhalation bioassay, which is virtually identical to the purity of the NCI chemical. Although no chloroform was found in the Maltoni sample, minor impurities of trichloroethylene (TCE; 1,1,2-trichloroethane), perchloroethylene (PCE; tetrachloroethylene), carbon tetrachloride, and benzene were present.

CONTAMINANTS IN AIR IN THE GAVAGE BIOASSAY

In the NCI bioassay, several compounds were tested simultaneously in the same room as EDC. Some of these chemicals were carcinogens. We estimate that the exposure to each of these contaminants would likely be less than 0.002% of the highest dose administered in each test and therefore would be unlikely to contribute appreciably to the observed tumor incidence.

(In the NCI bioassay with rats, four other chemicals [1,1-dichloroethane, DBCP, TCE, and carbon disulfide] were under test in the same room with EDC. A rough calculation indicates that exposures to these contaminants should be quite low. The significant exposure to these contaminants should be by inhalation of volatiles in the room atmosphere, which arise largely from the exhaled breath of dosed rats [there should be no volatilization from feed because all were administered by gavage]. There were 200 rats [50 animals of each sex for

each of two dosed groups] in each bioassay. The air changed 300 times/day [12–15 times/hr] [NCI 1978]. Rats exhale 10% of the gavage dose [Yllner 1971; Sopikof and Gorshunova 1979], and rats breathe in about 0.2 m^3/day. We estimate that the average daily exposure by inhalation to each contaminant would be about 0.002% of the highest gavage dose administered in each test. Mice in this study were housed with mice used in the gavage bioassays of 17 other chemicals. However, ventilation conditions were similar, and thus it seems unlikely that these contaminants contributed appreciably to the observed tumor incidences.)

Synergism is hard to evaluate between EDC and the low levels of these impurities, but appears improbable. It is also difficult to estimate the probability that any mix-up of chemicals occurred during the administration of dose.

CONTAMINANTS IN DIET—EFFECT OF VEHICLE IN GAVAGE STUDY

The Maltoni and NCI studies differ in the lab chow, and only the NCI study used a corn oil vehicle. Although vehicle controls were run in the gavage study, we cannot exclude the possibility that corn oil or various unanalyzed factors in the chow (such as antioxidants, aflatoxin, pesticides, or nitrosamines) could have had promoting effects on tumor frequency. We calculate the amount of corn oil as about 1 ml/(day · rat) at the NCI high dose (100 mg/kg EDC was dissolved in about a 6% solution in corn oil). The NCI lab chow was Wayne Lab-Blox® (Allied Mills); Maltoni used a different chow.

DOSE LEVELS AND PATTERNS OF DOSING

A possible explanation for the discrepancy between the results of the two bioassays is that the nonpositive inhalation study used doses that were much lower than those used in the NCI gavage study. Calculations show, however, that the two highest dose levels in the inhalation study were entirely comparable on a mg/(kg · day) basis to those yielding a strongly positive result in the NCI study (Table 1).

(We calculate daily doses [mg/kg body weight] extrapolated over the lifetime of the animals in the experiment, as determined by the age of the last survivor. For all dose routes, we calculate a daily administered dose, without adjusting for any differences in efficiency of absorption or retention by the various routes.

The calculated doses in the gavage study and in the two highest dose groups in the inhalation study were quite similar, each with two roughly equivalent dose levels within the dose range where significant increases in tumor incidences were observed in the gavage bioassay. Although the inhalation study by Maltoni was of more thorough design, employing four dose levels and a larger number of animals per dose group, two of the inhalation dose levels were very

Table 1
Number of Animals and Average Daily Lifetime Doses in EDC Experiments

	mg/(kg · day)	
	gavage (NCI)[a]	inhalation (Maltoni)[b]
Rats		1.6
		3.2
	24.0	16.0
	48.0	48.0
Mice	60.0[c]	5.6
	120.0[c]	11.0
	92.5[d]	56.0
	185.0[d]	171.0

Data for males and females unless otherwise indicated.
[a]50 Animals/dosed group; 20 animals/matched vehicle controls; 60 animals/pooled vehicle controls; dosed for 5 days/wk for 78 wk; daily dose calculated for 90-wk experiment period for mice and 110 wk for rats; B6C3F1 mice and Osborne-Mendel rats of both sexes.
[b]90 Animals/dosed group; 45 animals/in-chamber control group; 45 animals/out-of-chamber control group; dosed 7 hr/day, 5 days/wk for 78 wk; observed for life; Swiss mice and Sprague-Dawley rats of both sexes.
[c]Male.
[d]Female.

much lower [one-tenth to one-fifth] than the NCI gavage low dose [see Table 1]. The gavage low dose produced significant increases of tumors at some sites, but it is unlikely that significant responses would be produced at inhalation dose levels an order of magnitude lower.

The doses shown in Figure 1 are calculated from the following data. We assume that rats weigh 0.5 kg, eat 5% of their weight per day, and breathe 0.14 liter/min; that mice weigh 0.03 kg, eat 10% of their weight per day, and breathe 0.03 liter/min. In the NCI gavage study, the animals are exposed 5 days each week for 78 weeks, and the lifetimes are taken as 90 weeks for mice and 110 weeks for rats. In rats, for the last 34 weeks of the exposure period, a cycle of 4 weeks of dose alternating with one dose-free week was maintained.)

In both tests the exposure time was the same: doses were administered 5 days per week for 78 weeks. In the NCI bioassay there were two minor adjustments in dose levels for each dose group in rats and female mice, and one change in male mice. The dose we report is a time-weighted arithmetic average of these dose levels (see Table 1). The pattern of dosing was also changed in the NCI bioassay in rats. During the final half (34 weeks) of the exposure period, the dosing was interrupted for 1 week every fifth week. In the inhalation study, there was one reduction in dose level in the highest dose groups of both rats and mice.

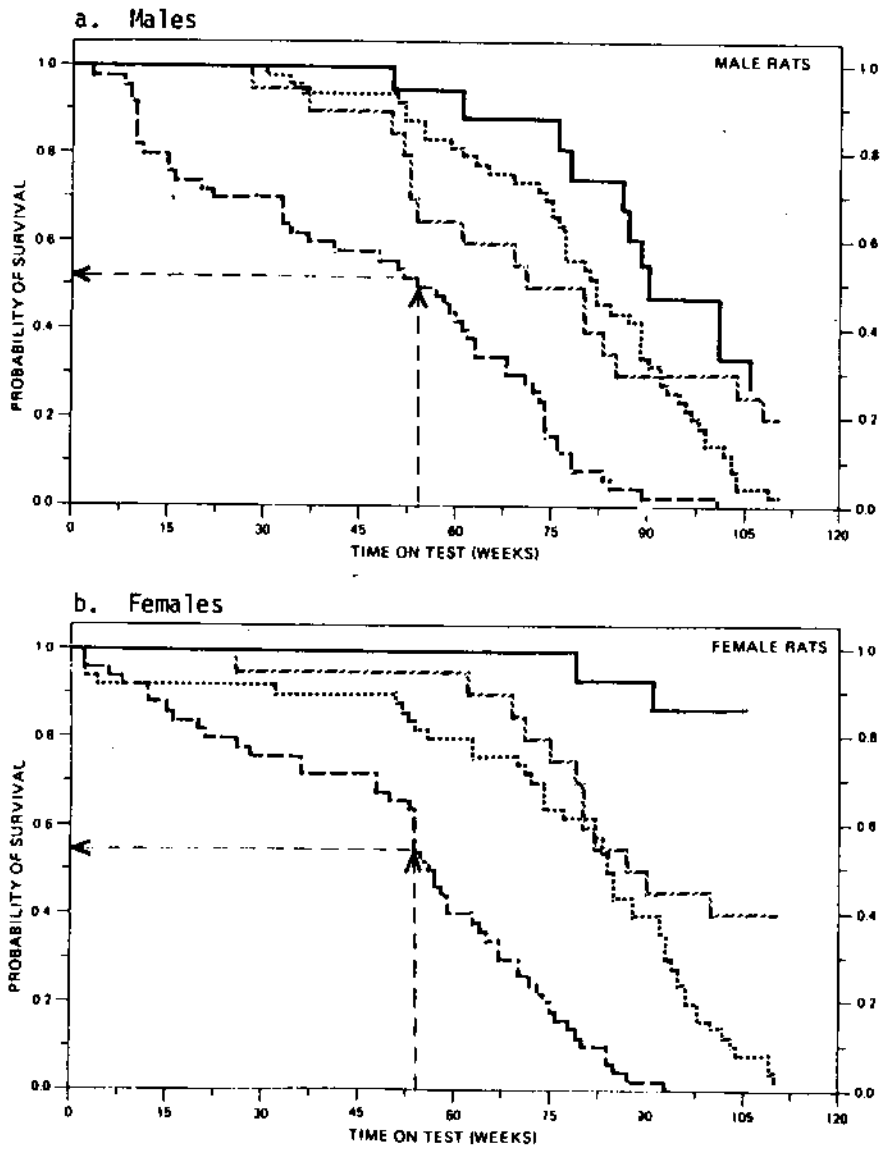

Figure 1

Survival comparisons of EDC chronic study rats (by gavage; NCI); (*a*) males, (*b*) females. We have not reproduced here the survival data for mice; premature mortality among mice was less than for rats. (——) Untreated control; (/ – / – /–) vehicle control; (· · · · ·) low dose; (– – – –) high dose. (Reprinted from NCI 1978.)

ROUTES OF EXPOSURE

Would the different routes of exposure used in the two bioassays give different effective whole-body doses of EDC, i.e., would doses administered by inhalation be less effective than those given orally or by gavage? Published evidence available at the date of this meeting indicates that the effective doses of EDC delivered to tissues would be similar whether the chemical was administered by gavage or by inhalation, except for a transient high concentration in the liver in the gavage exposure due to the first-pass effect.

In uptake studies of vinyl chloride in Sprague-Dawley rats (Table 2), it was calculated that similar doses administered orally (20 ppm in drinking water for 24 hours; 0.9 mg/kg) or by inhalation (2 ppm for 24 hours; 1.7 mg/kg) would produce the same concentration-time dependence of the chemical in blood (Withey 1976). In a separate experiment, after intravenous administration of EDC and three related compounds (methylene chloride, chloroform, and TCE) to Wistar rats, the concentration in all tissues except adipose tissue was very similar to that found in blood (Withey and Collins 1980).

Gut flora might produce carcinogenic metabolites from doses of EDC administered by gavage that would not be produced (or produced in lesser amounts) in the lung from doses of EDC administered by inhalation. From existing data we cannot evaluate the likelihood of this occurring. However, the doses of EDC required to produce similar toxic effects by the two routes are quite similar. The acute toxic doses from a single gavage or a short inhalation exposure are roughly equivalent using rats of the same strain (Table 3). In addition, daily chronic exposures at equivalent dose levels over a longer period of time by the two routes also produced comparable toxic effects (8-week exposure period with Osborne-Mendel and Wistar rats; 52–64 week exposures with Osborne-Mendel and Sprague-Dawley rats; see Tables 4 and 5).

Thorough studies by Reitz and Spreafico (both, this volume), which directly address the question of effective doses by different routes, conclude that the blood concentration, metabolism, and tissue distribution of EDC are approximately the same for a given administered dose by gavage or inhalation.

Table 2

Doses by Inhalation and Gavage Give the Same Concentration of Vinyl Chloride in Blood

Route	Dose	AUC[a] (min-μg/ml)
Gavage[b]	0.9	19.2
Inhalation[c]	1.7	19.5

[a] Area under curve of plot of blood concentration of vinyl chloride versus time.
[b] Withey and Collins (1976).
[c] Withey (1976).

Table 3
Acute Toxicity of EDC: Wistar Rats, Single Exposure

	LD_{50}[a] (mg/kg · day)
Gavage[b]	680
Inhalation[c]	
1000 ppm, 7 hr	519
3000 ppm, 2.7 hr	611
12,000 ppm, 0.5 hr	444

[a]Median lethal dose.
[b]McCollister et al. (1956).
[c]Spencer et al. (1951).

Table 4
8-Week EDC Exposure: Rats

	Mortality	
	males (%)	females (%)
Gavage dose[a]	60	20
134 mg/(kg · day)	(3/5)	(1/5)
Inhalation dose[b]	100	100
148 mg/(kg · day)	(15/15)	(15/15)

[a]Osborne-Mendel rats, NCI bioassay (1978).
[b]Wistar rats, Spencer et al. (1951).

Table 5
52–64-Week EDC Exposure: Rats

	Mortality	
	males (%)	females (%)
Gavage dose[a]	48	46
37 mg/(kg · day)	(24/50)	(23/50)
Inhalation dose[b]	67	40
53.6 mg/(kg · day)	(60/90)	(36/90)

[a]Osborne-Mendel rats, NCI bioassay (1978); mortality at 52 wk of experiment.
[b]Sprague-Dawley rats, Maltoni (this volume); expected mortality at 64 wk of experiment.

STRAINS OF TEST ANIMALS

Different strains of test animals were used in the two cancer tests. The NCI used Osborne-Mendel rats and B6C3F1 mice. Maltoni used Sprague-Dawley rats and Swiss mice. Both strains of mice are responsive to tumor induction by chlorinated compounds. The B6C3F1 mouse is responsive to aldrin; chlordane; heptachlor; DDE (1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene); kepone; chloroform; carbon tetrachloride; hexachloroethane; 1,1,2,2-tetrachloroethane; PCE; TCE; and 1,1,2-trichlorethane by oral route (R. A. Griesemer and C. Cueto, in prep.). The Swiss mouse is responsive to vinyl chloride (VC) and vinylidene chloride (VDC; 1,1-dichloroethene) by inhalation. In general, the rat is less responsive to chlorinated chemicals than the mouse. The Osborne-Mendel rat is responsive to chloroform, chlordane, and kepone by oral route but not to the other chlorinated compounds listed above. The Sprague-Dawley rat is responsive to VC by inhalation but not to VDC. It is not responsive to TCE by gavage (Maltoni 1980).

In the inhalation study using Sprague-Dawley rats, three dose groups had significantly higher incidence of mammary tumors than controls (Fisher exact $p < 0.05$). The majority of these tumors were fibromas and fibroadenomas. Because of the high incidence of these tumors in the control group (36/90 = 40%) and their variability in historical controls, intrepretation of this finding is difficult. In the NCI gavage study in Osborne-Mendel rats, the incidence of mammary fibroadenomas and adenocarcinomas in the control group was 0/20, and a significantly positive result was found in this tissue.

There was widespread murine pneumonia among rats in the NCI gavage study that was not present in the inhalation study. This may have had some effect on the bioassay for rats.

EFFECT OF INTERCURRENT MORTALITY ON NUMBER OF ANIMALS AT RISK

In the gavage study there was a significant degree of premature mortality among the test animals (as shown in Fig. 1) and this is also apparently the case in the inhalation study. This could significantly alter the determination of carcinogenicity. Because most tumors arise very late in the natural lifetimes of test animals, animals that die early will not have lived long enough to manifest tumors that otherwise might have developed. Premature mortality tends to be most prevalent in the highest dose groups. In a bioassay in which significant early mortality occurred in the high-dose group, the calculated proportion of tumor-bearing animals, based on the initial number of animals in the experiment, would be too low and thus would lead to an underestimate of the carcinogenic effect of the chemical.

Where significant mortality has occurred during any experiment, as in the case of the gavage study and apparently in the case of the inhalation study of EDC, life-table analysis will correct for its effects and therefore should be used

in the proper statistical interpretation of the experimental results (Pike and Roe 1963; Peto 1979). Life-table (actuarial) analysis relates the occurrence of a tumor to the number of animals still alive at the time the tumor appears, rather than to the number that started the experiment.

Most published animal cancer tests report only the final percentage of tumor-bearing animals in each dose group. This data is insufficient for an actuarial analysis.

Potency Analysis of NCI Gavage Bioassay

NCI concluded that EDC was a carcinogen in its bioassay and that the chemical significantly increased tumor incidence in both sexes of Osborne-Mendel rats and B6C3F1 mice at the following sites: squamous cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male rats; mammary adenocarcinomas in female rats; mammary adenocarcinomas and endometrial tumors in female mice; and lung alveolar-bronchiolar adenomas in mice of both sexes (see Ward, this volume). Moreover, there were a number of nonstomach metastatic tumors produced in both species (hemangiosarcomas in male rats, lung adenocarcinomas in both sexes of mice, and endometrial adenocarcinomas in female mice). EDC is clearly a carcinogen in these strains of test animals under the conditions of this experiment.

Our potency project (Ames et al., this volume) has analyzed the NCI data and the preliminary Maltoni data to estimate the carcinogenic potency of EDC. As described by Ames et al., we calculate a TD_{50}, the daily dose in mg/(kg · day) which if administered over a standard lifetime would decrease by half the probability that an animal remains tumor-free.

The estimate of TD_{50} is subject to both life-table and summary analysis. Depending upon the data available, two types of estimates of carcinogenic potency can be made, one more accurate than the other. Summary TD_{50} values are calculated when only tumor incidences are reported for each dose group. Life-table TD_{50} values are calculated when there is information on the time of death of each animal and the type of tumor, if any, present at death (Ames et al., this volume).

The potency analysis of the NCI gavage study is shown in Figure 2 where we have plotted the TD_{50} values for the combined tumor incidences of those tissues found significant by NCI, as mentioned above (see Fig. 2 legend for details of plot). In this plot of the summary data in Figure 2 (enclosed by single-dot confidence limits), the TD_{50} values of EDC in Osborne-Mendel rats lie to the left of the TD_{50} values plotted for B6C3F1 mice, indicating that the chemical is more potent in rats than in mice. Thus, EDC is more potent in Osborne-Mendel rats by gavage than in B6C3F1 mice, and more potent in female rats than in male rats.

In the life-table analysis of the same experiment (enclosed by double-dot confidence limits in Fig. 2), it is evident that in all cases the life-table TD_{50} values are to the left of the corresponding summary TD_{50} values, at three- to

tenfold more potent values—a shift to lower doses in mg/(kg · day) from 180 to 53 in female mice; from 256 to 90 in male mice; from 55 to 5 in female rats; and from 74 to 11 in male rats. These dramatic increases in the calculated potency are due to significant early mortality which occurred in the experiments. The mortality in the high-dose group in rats was nearly 50% at 52 weeks and 90% at 78 weeks; this compares to the mortality in the control group of 5% and 40%, respectively (see Fig. 1). Thus, only one-half of the high-dose animals remained at risk for the crucial second half of their 2-year life-span; it is during this time that we would expect most tumors to appear because of the higher-order dependence of cancer incidence upon age. Using the life-table data and averaging the fairly similar results from males and females, our estimates of potency of EDC by gavage are about 70 mg/(kg · day) in B6C3F1 mice and about 7 mg/(kg · day) in Osborne-Mendel rats.

In addition to influencing the TD_{50}, life-table analysis can influence the determination of whether the data is compatible with a linear model. This effect can be seen in Figure 2. The life-table analysis of a number of the NCI experiments indicates that more tumors were observed in the high-dose group than would be expected on the basis of a linear model.

Potency Analysis of the Inhalation Bioassay (Maltoni)

The preliminary data from the inhalation study only allow us to perform a summary analysis. A comparison of the inhalation study and the gavage study in terms of their calculated potencies is presented in Figure 3. TD_{50} values from the inhalation study are plotted for those sites that had at least some response, although that response was judged nonsignificant. For comparison we plot life-table and summary TD_{50} values from the NCI data for similar single tissue sites and pathology. None of the summary TD_{50} values from the inhalation study were significant for tissues that were significant in the NCI gavage study. This agrees with Maltoni's conclusion that there is no significant dose-related increase in tumor incidence for any tissues in rats or mice.

We have plotted the symbol (· >) for the inhalation results, which indicates that based on this test we are 97.5% confident that a dose level which might be calculated to induce tumors in 50% of otherwise tumor-free animals would have to be greater than the value plotted, in this case, greater than 86.0 mg/(kg · day). This dose value is greater than the TD_{50} values calculated for the same tissues in the NCI study (i.e., less potent). Thus, even with the potency analysis, the results of the gavage and inhalation studies cannot be reconciled. However, the analysis here is based on preliminary summary data only. If there were a high rate of early mortality in the high-dose group, then it is possible that a life-table analysis might shift the location of this lower confidence limit downward and might also result in significant tumor incidences in some groups of animals. Early mortality may be expected here, based on results from the NCI gavage bioassay and other toxicity studies (Tables 3, 4, 5).

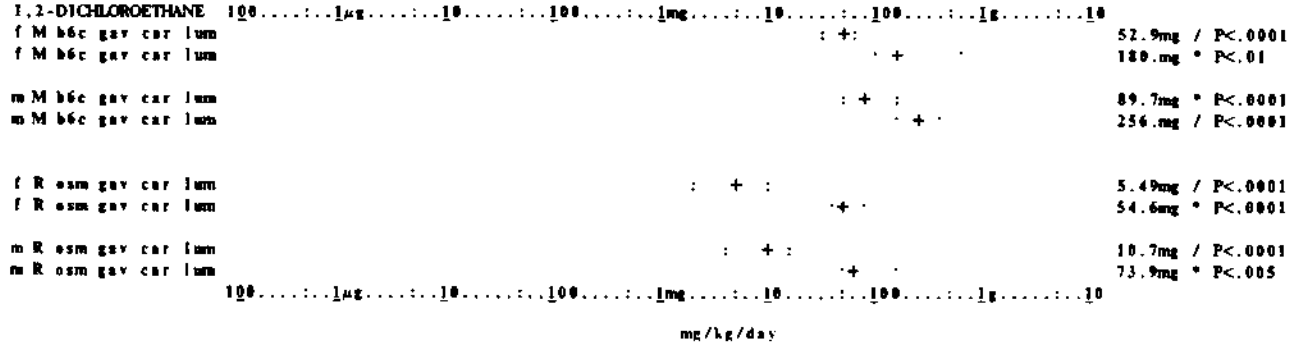

Figure 2

In this figure each experiment is contained in a single line, with information presented in the following sequence. (*Left*): Sex, species, and strain of test animal; route of exposure; and tumor pathology (tissue and tumor type). (gav) Gavage; (inh) inhalation; (f) female; (m) male; (M) mice; (R) rats; (b6c) B6C3F1; (swi) Swiss; (osm) Osborne-Mendel; (sda) Sprague-Dawley; (car lum) TD_{50} is calculated for the combined tumor incidences of all sites which were considered statistically and biologically significant by the NCI for that test group. (*Middle*): The TD_{50}, together with its 95% confidence interval, is plotted to the right on a logarithmic scale of dose, ranging from 100 mg/(kg · day) to 10 gm/(kg · day). (+) TD_{50} when $p < 0.02$; (·) TD_{50} values calculated from summary data (single-dot confidence limits); (:) life-table TD_{50} values (double-dot confidence limits). (*Right*): Numerical value for the TD_{50}; how compatible the data are with a linear model; probability that the dose-response is different from zero. (*) The data are compatible with a linear model; (/) the high-dose group had more tumors than would be predicted by a linear model.

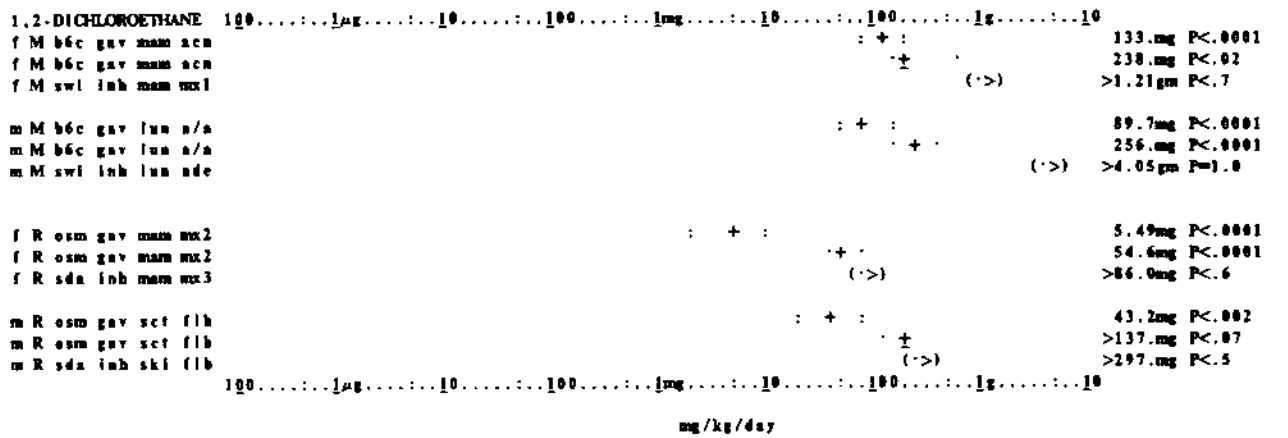

Figure 3

The summary and life-table TD_{50} values from the gavage study are compared with summary TD_{50} values from the inhalation bioassay for simiar tissues. The inhalation data are enclosed in parentheses as they must be considered preliminary until the final data are published by Maltoni. Data are presented as in Fig. 2. Tumor pathology (*left*): (gav) gavage; (inh) inhalation; (mam) mammary gland; (acn) adenocarcinoma; (lun) lung; (car) carcinoma; (ski) skin; (fib) fibroma; (sct) subcutaneous; (a/a) alveolar-bronchiolar adenoma; (ade) adenoma; (mxl) mixture of chiefly carcinomas with carcinosarcomas and possibly fibroma-fibroadenomas; (mx2) mixture of fibroadenomas and adenocarcinomas; (mx3) mixture of fibroadenomas, fibromas, and carcinomas. (*Middle*): (±) The probability is between 0.01 and 0.10 that the dose-response is different from zero—when this probability is greater than 0.025, the upper 95% confidence limit on the TD_{50} is infinite and hence is not plotted; (· >) the experiment did not give a significant carcinogenic response, but if the chemical is a carcinogen, we have 97.5% confidence that the TD_{50} will be greater than the value of the lower confidence limit which is plotted here and listed to the right.

(From the summary incidence data of mammary tumors in female rats [in-chamber control, 36/90; dose 1, 64/90*; dose 2, 43/90; dose 3, 55/90*; dose 4, 52/90*, where * = Fisher exact $p \leq 0.05$], if significant early mortality occurs in higher dose groups, the denominator in these incidences [the number of animals at lifetime risk] effectively becomes smaller, and it is possible that the nonsignificant dose response might become significant. Similar increases in significance might occur at other sites if life-table data were used in the analysis.)

CONCLUSION

The most likely explanations for the apparently discrepant results are:

1. the strains of test animals differ in responsiveness;
2. the route of exposure does make a difference concerning the carcinogenic action of EDC;
3. an artifact has been introduced by intercurrent mortality that would be corrected by life-table analysis.

We cannot choose among these possibilities on the basis of the information at hand.

ACKNOWLEDGMENTS

We wish to acknowledge Charles B. Sawyer for performing the potency analysis presented here as part of the carcinogenic potency project and also Thomas Haggin, William Havender, and Cesare Maltoni for their help. This work was supported by Department of Energy contract EY-76-S-03-0034 PA156 and a California Policy Seminar grant to B. N. A. and by National Institute of Environmental Health Sciences Center grant ES-01896. (Part of this work was performed while K. H. was employed by the Hazard Alert System, State of California Department of Health Services, Berkeley, California.)

REFERENCES

Maltoni, C. 1980. Results of long-term carcinogenicity bioassays of trichloroethylene: Experiments by oral administration of Sprague-Dawley rats. *Medicina del Lavoro* (Milan). (In press)

McCollister, D. D., R. L. Hollingsworth, F. Oyen, and V. K. Rowe. 1956. Comparative inhalation toxicity of fumigant mixtures. *A.M.A. Archives of Industrial Health* 13:1.

National Cancer Institute. 1978. *Bioassay of 1,2-dichloroethane for possible carcinogenicity*. NCI Carcinogenesis technical report series No. 55, DHEW publication number (NIH) 78-1361, Government Printing Office, Washington, D.C.

Peto, R. 1979. Guidelines on the analysis of tumor rates and death rates in experimental animals. *Br. J. Cancer* 29:101.

Pike, M. C. and F. J. C. Roe. 1963. An actuarial method of analysis of an experiment in two-stage carcinogenesis. *Br. J. Cancer* 17:605.

Sopikof, N. F. and A. I. Gorshunova. 1979. Investigation of the intake, distribution and excretion of ethylene dichloride in rats. *Gig. Tr. Prof. Zabol.* 1SS4:36.

Spencer, H. C., V. K. Rowe, E. M. Adams, D. D. McCollister, and D. D. Irish. 1951. Vapor toxicity of ethylene dichloride determined by experiments on laboratory animals. *A.M.A. Archives of Industrial Hygiene and Occupational Medicine* 4:482.

Withey, J. R. 1976. Pharmacodynamics and uptake of vinyl chloride monomer administered by various routes to rats. *J. Toxicol. Environ. Health* 1:381.

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

_____ . 1980. Chlorinated aliphatic hydrocarbons used in the foods industry: The comparative pharmacokinetics of methylene chloride, 1,2-dichloroethane, chloroform and trichloroethylene after I.V. administration in the rat. *J. Environ. Pathol. and Toxicol.* 3 (in press).

Yllner, S. 1971. Metabolism of 1,2-dichloroethane-^{14}C in the mouse. *Acta Pharmacol. Toxicol.* 30:257.

COMMENTS

WARD: Kim [Hooper], I note that the numbers from the NCI Technical Reports (NCI 1978) for dose calculation are 47 and 95.

HOOPER: Yes. The dose levels (mg/kg · day) given by NCI are calculated for a 78-week exposure period, using a 5-day week. We calculated a daily dose using a 7-day week for an experiment time, which is taken as 90 weeks in mice and 110 weeks in rats (see Hooper et al., this volume). That's why our doses are lower.

AMES: When you calculate an inhalation, what percentage of absorption do you use?

HOOPER: We assume that 100% of the dose is effectively retained. We do not make any adjustments for efficiency of absorption, or attempt to calculate an effective dose. We simply assume at this point that everything given to the animal, by any dose route, stays in the animal.

WARD: Have you ever approximated TD_{50} values in humans to the known human carcinogens to see how they applied to the animal TD_{50} values?

HOOPER: Yes, we are trying to do that using, for example, cigarette smoking data or benzo[*a*]pyrene exposure. And there is agreement, that is, we don't see huge discrepancies in those two cases. But I think we want to just hold off on that. That's another area we could work on—the differences between rats, mice, and people, and how we make that jump in extrapolation.

I think Dr. Ames' point was that we wanted to take monkey data as an approximation and see how that compares. For example, if we have a compound that's been tested in rats, mice, hamsters, and monkeys and the potency in all those four species appears to be the same, then I think we can state that we have a higher probability, or more confidence, that the human point would be somewhere near that. If there's a great spread—a thousandfold difference between all of them—then I am not sure what we would do. But that's the approach we're taking at present.

WARD: The discrepancy between the interpretation of your statistics and Dr. Maltoni's is based on mammary tumors in the rat—the fibroadenomas. I wonder if Dr. Maltoni can add any data on the incidence of fibroadenomas in other Sprague-Dawley controls in his laboratory. Are they compatible with most of his other controls, or are they groups of controls where you might have 50% incidence under the same conditions? Besides the life-table methods, these data might give you more weight of the evidence one way or the other.

HOOPER: Right. So that the one control used might be abnormally low.

WARD: Well, he had two controls, 25–30%, I believe.

MALTONI: We have an average of 50% of our female rats with spontaneous mammary tumors; fibroadenomas in males range from 7–11%. We will soon publish all spontaneous pathology in these 6000 animals and give data on the year of birth, because it may change from 5 to 6 years; their weights; the survival at this time; those neoplastic pathologies found out at this time; and those neoplastic pathologies found out at invervals of 4 weeks. With this data I think that one can get a sort of meter to coequate our data within the spontaneous group.

Since we also are discussing scientific methodology here, a good new model for bioassays would be to give data on incidence of the tumor in a particular situation, in treated animals as well as in the proper controls, plus the incidence of historical control (within the laboratory) with the range of the population.

I would also like to make an observation. It is surprising that in the NCI project for EDC you have a greater sensitivity for rats than for mice, because in our laboratory studies mice always have been far more sensitive to things, in line with their enzymatic profile.

HOOPER: In the NCI study, the rats seem more sensitive than mice. They cannot tolerate such a high dose as mice (about half that of mice), and their mortality curve is much steeper, even at these lower doses. Part of this may be due to the significant amount of murine pneumonia present in the rat colony and virtually absent from mice.

Just to add to Dr. Maltoni's comments, we feel we cannot stress too much the importance of people reporting life-table data for their cancer bioassays so that the effects of intercurrent mortality can be corrected for.

REITZ: Although epidemiological studies, as you pointed out, Kim, don't have the statistical power that carefully controlled animal studies do, there are cases where you can take human data and use it to put perspective on risk analysis. One of these is the ethylene dibromide (EDB; 1,2-dibromoethane) case.

I think, if you recall, that the threshold limit value (TLV) for EDB allowed workers to be exposed to many times higher concentrations than your calculated TD_{50}. But there is data in the literature by Drs. Ott and Gehring, which indicate that the actual incidence of cancer in these workers is much less than predicted, in fact, not distinguishable from background. So this points out the need to check our predictions against actual human data wherever possible.

INFANTE: If you're going to try to assess the risk epidemiologically, then you need to have adequate sample size, latency, and follow-up period. Now, I haven't seen the study that you're speaking of with EDB, but tomorrow I'm going to present some of the data that's been published on some of the structurally related compounds (see Infante and Marlow, this volume). In every instance, there are so many study deficiencies that these are all no-decision studies. Right now I don't think that you can be saying that there is something different between animals and humans unless you have the adequate population to follow for an adequate period to make that evaluation.

TER HAAR: There may be a chance of risk in humans compared to animals, but I think if you look at the EDB data on its surface there is a clear-cut difference in both between humans.

References

NCI. 1978. *Bioassay of 1,2-dichloroethane for possible carcinogenicity*. Technical Report Series No. 55. DHEW publication number (NIH) 78-1361. Government Printing Office, Washington, D.C.

Banbury Report

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ETHYLENE DICHLORIDE: A Potential Health Risk?

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