REPORT ON ETHYLENE DICHLORIDE TO THE SCIENTIFIC REVIEW PANEL

Part B - Health Effects of Ethylene Dichloride

Prepared by

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1. Executive Summary

The health effects for exposure to 1,2-dichloroethane (ethylene dichloride, EDC) in the ambient air are assessed. Adverse health effects, such as systemic and reproductive effects, are not expected to result from exposure to ambient levels of EDC in California urban environments.

EDC, however, is a carcinogen in both sexes of two animal species following oral administration, and could thus be of concern at low levels of exposure. Although the chemical was not shown to be carcinogenic by inhalation, staff of the Department of Health Services (DHS) believes that the negative findings in the inhalation study do not negate the positive findings in the gavage study. DHS staff agrees with the International Agency for Research in Cancer (IARC) that there is sufficient evidence that EDC is carcinogenic in mice and rats and that, in the absence of adequate data in humans, it is reasonable, for practical reasons, to regard EDC as if it presented a carcinogenic risk to humans.

DHS staff performed a risk assessment on hemangiosarcomas of the male rat and hepatocellular carcinomas of the male mouse and derived human risk estimates (Table 1) using five different models for exposure to EDC in air. These values represent the theoretical excess risk of cancer above the background accumulated over a 70-year lifetime with a continuous daily exposure for all 70 years. DHS staff recommends the use of a lifetime excess risk value between 53 and 88 per million for community exposure to 1 ppb EDC. This recommendation is based on the maximum likelihood estimate (MLE) and

the 95% upper confidence limit (UCL) estimate from the Weibull (time-corrected) multistage model for hemangiosarcomas in male rats.

It should be noted that the range between the maximum likelihood estimate and the 95% upper confidence limit represents only the statistical uncertainty introduced by the typically small size of the animal studies of carcinogenic effect. Other perhaps more important uncertainties are introduced by the choice of scaling factor between humans and animals, the choice of extrapolation models, and the additive, synergistic, or antagonistic effects of other chemicals. On the other hand, DNA repair mechanisms, detoxifying enzymes, and other factors might lower the risk below what has been calculated. These uncertainties are particularly to be noted in a case such as that of EDC where the ambient exposures are at the low parts per trillion level while the animal experiments occurred at exposure levels more than a million times higher.

The lifetime risk values given above represent a range of conservative estimates and are unlikely to be exceeded by the actual risk. A lifetime excess risk of 53-88 per million population must be viewed in the context of the overall probability of developing cancer which is in the order of 250,000 cases per million population (25%) over a 70-year lifetime.

Table 1. Human Equivalent Lifetime Excess Cancer Risk for Ambient Exposure to EDC[1].

	0.1 ppb		0.5 ppb	
	MLE	95% UCL	MLE	95% UCL
Male Rat Hemangiosarcoma[2]				
One-Hit Model	3.8×10^{-6}	6.9×10^{-6}	$19. \times 10^{-6}$	34×10^{-6}
Multistage Model	4.3×10^{-6}	6.3×10^{-6}	$21. \times 10^{-6}$	31×10^{-6}
Multistage Model (time corrected)	5.3×10^{-6}	8.8×10^{-6}	$26. \times 10^{-6}$	44×10^{-6}
Probit Model	8.3×10^{-6}	$330. \times 10^{-6}$	$76. \times 10^{-6}$	2300×10^{-6}
Gammma Multihit Model	$1800. \times 10^{-6}$	$22000. \times 10^{-6}$	$3600. \times 10^{-6}$	40000×10^{-6}
Male Mouse Hepatocellular Carcinoma				
One-Hit Model	2.8×10^{-6}	$38. \times 10^{-6}$	$14. \times 10^{-6}$	$190. \times 10^{-6}$
Multistage Model	1.6×10^{-6}	4.6×10^{-6}	6.3×10^{-6}	23×10^{-6}
Multistage Model (time corrected)	2.9×10^{-6}	7.4×10^{-6}	$14. \times 10^{-6}$	$37. \times 10^{-6}$
Probit Model	0.0	1.1×10^{-24}	0.0	1.1×10^{-18}
Gammma Multihit Model	4.6×10^{-6}	$94. \times 10^{-6}$	$21. \times 10^{-6}$	$370. \times 10^{-6}$

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1 The lifetime excess risk for a lifetime TWA exposure to the provided ambient concentrations for a 60-kg person inhaling 18 m^3/day.

2 All hemangiosarcomas.

2. Introduction

This document assesses the health effects from exposure to ethylene dichloride (EDC) in ambient air, with special attention given to analyzing and interpreting findings on the evaluation of EDC as a carcinogen in humans via inhalation exposure. Pertinent information is presented and references are made to relevant reviews where such exist.

3. Chemical Properties

The physical and chemical properties of EDC have been summarized by IARC (1979).

4. Health Effects

The primary adverse health effects of EDC are liver toxicity and central nervous system (CNS) depression. Immediate symptoms such as narcosis following acute inhalation exposure are related to CNS depression. Delayed effects are seen as histopathologic changes of the organs such as congestion and degenerative effects in the liver, spleen, kidneys, lungs, and adrenals. The severity of effects is dependent upon the duration and concentration of exposure. Subchronic and chronic exposure to EDC produced renal and liver damage and affected survival. The dose levels associated with the effects are shown in the following subsections. Information on the mechanism of action is lacking.

For the purpose of the present assessment, all relevant health effects data are evaluated but only findings associated with the lowest levels showing an effect, or studies identifying a no-observed-effect level, are summarized below.

4.1 Animal

The toxic effects of EDC are summarized by IARC (1979) as follows. It has an acute oral LD_{50} of 700 mg/kg and a 4-hr inhalation LD_{50} of 4,000 mg/m^3 (1,000 ppm) for rats. Inhalation exposure of rats to various concentrations of EDC resulted in central nervous system depression, anaesthesia, and coma. Acute exposure caused disseminated hemorrhagic lesions, particularly in the liver. Chronic exposure caused liver and kidney damage.

Following acute inhalation exposure for 5-8 hours, signs of intoxication were seen in rats at 300 ppm but not at 200 ppm (Spencer et al., 1951). Inhalation of EDC to concentrations as high as 12,000 ppm for 0.1 hour had no adverse effects. Subchronic and chronic exposures (6 or 7 hr/day, 5 days/week for 4 to 36 weeks) produced toxic effects in several species at 200 ppm and above but not at 100 ppm (Heppel et al., 1946; Spencer et al., 1951). Death occurred after one to several exposures at concentrations of 200-1000 ppm. Chronic exposure of 14-month-old rats to 50 or 150 ppm EDC showed an indication of renal and liver damage (Spreafico et al., 1980) but no alterations in histology (Maltoni et al., 1980) or clinical chemistry (Spreafico et al., 1980) in rats exposed to EDC at 5-50 ppm. Following administration by gavage, decreased

survival resulted in rats at 47 mg/kg/day (NCI, 1978), and immunosuppression occurred in mice at 4.9 mg/kg/day (Munson et al., 1982). Humoral and cell-mediated immunity was not affected following administration of EDC in drinking water at 3-189 mg/kg-day.

4.2 Human

Reported cases of acute human exposure to EDC involved primarily occupational exposures which were lacking in quantitative data and often included poorly characterized mixtures of EDC and other solvents. Signs and symptoms were often indicative of central nervous system and gastrointestinal disturbances, clinical evidence of liver and kidney dysfunction, and irritation of the respiratory tract and eyes (NIOSH, 1976). Death was usually ascribed to respiratory and circulatory failure. Autopsies frequently revealed pulmonary congestion, cellular degeneration, necrosis, and hemorrhagic lesions of most internal organs. Results of an experimental study with three or four subjects suggested that the threshold of light perception, depth of breathing, and momentary vasoconstriction of the finger increased with exposure to EDC at 1.5 ppm and above but not at 1 ppm (Borisova, 1957).

Available information on long-term exposure to EDC is also limited. Control and exposure data are lacking. The lowest level for which increased morbidity was reported was in the range of 10-15 ppm in a health survey of workers (Kozik, 1957). Typical symptoms and signs of acute poisoning developed with exposure to concentrations in the range of 60 - 200 ppm (Cetnarowicz, 1959). Effects caused by oral exposure were

similar to those by inhalation exposure based on human case reports of accidental or intentional ingestion.

5. Metabolism and Pharmacokinetics

The metabolism and pharmacokinetics of EDC have been thoroughly reviewed by Davidson et al. (1982) and will be briefly described.

EDC was rapidly and virtually completely absorbed after oral administration to rats in either an oil or water carrier with peak blood levels occurring within 15-20 minutes following gavage (Spreafico et al., 1980; Reitz, et al., 1982). The kinetics of the elimination of EDC was dose-related which indicated that the elimination (metabolism) was saturable (nonlinear kinetics) at oral doses above 25 mg/kg.

EDC was also rapidly absorbed by inhalation, and blood levels reached equilibrium by 2-3 hours of continous exposure. Elimination of EDC following inhalation exposure was also rapid and dose-related (e.g.,nonlinear kinetics) in accord with the above oral studies.

Urine was the major route of excretion of inhaled or orally administered EDC, but most of the EDC in urine was in metabolites form. Some EDC was eliminated by exhalation, and the percentage excreted by this route increased when the metabolism was apparently saturated.

The biotransformation of EDC has been studied in vitro and in vivo and a number of metabolites have been identified. These include

chloroethanol, chloroacetic acid, 5-carboxymethyl cysteine, thiodiacetic acid, and thiodiacetic acid sulfoxide. The metabolic pathways include reactions involving microsomal and cytosolic enzymes. Reactive intermediates which can bind to cellular macromolecules (e.g.,DNA) apparently can be formed by both pathways. Possible bioactivation reactions include the formation of the 2-chloroacetaldehyde and S-(2-chloroethyl)-glutathione (and its episulfonium ion).

Detailed kinetic distribution and binding studies have been conducted with rats using various routes of administration and especially comparing the oral verses inhalation routes (Spreafico et al., 1980: Reitz et al., 1982). The study by Reitz et al. (1982) showed that the administration of ^{14}C-EDC at saturating doses (150 mg/kg orally; 150 ppm by inhalation) resulted in binding to cellular macromolecules (including DNA), and purified DNA. Slightly more macromolecular binding occurred via inhalation exposure, but increased DNA binding occurred following oral administration. There was no correlation between tissue levels of radioactivity or molecular binding and tissue carcinogenic susceptibility.

The DNA binding studies were conducted using ^{14}C-labeled EDC administered to male Osborne-Mendel rats. Rats exposed via inhalation (150 ppm) were terminated immediately following the 6-hour exposure, and those dosed by gavage were terminated 4 hours postdose. Selected tissues were removed and the DNA was isolated and purified. The experiments were conducted using 3 rats per group (inhalation and gavage)

and each exposure was repeated. DNA alkylation was estimated by determining the specific activity of the isolated DNA from the tissue. This method does not differentiate between alkylated DNA and biosynthetic (endogenous) incorporation of radioactivity and thus the results shall be termed "apparent" DNA alkylation to reflect this ambiguity in the results.

DNA binding in rats was greater after gavage exposure than after inhalation exposure for all selected tissues (liver, kidney, spleen, stomach). There was a general agreement in amount of binding between the replicate groups, with the highest levels in the liver and kidney, slightly less in the stomach, and the least in the spleen. Further research needs to be completed in order to understand the relationship between DNA binding and tumor formation. In addition, studies should be completed which differentiate between DNA alkylation and endogeneous incorporation.

6. Reproduction/Teratogenicity

6.1 Animal

EDC did not produce any adverse reproductive effects or abnormalities in mice when given in drinking water in a multigeneration study at doses of 0, 5, 15, or 50 mg/kg/day (Lane et al., 1981). The dose levels used, however, were not high enough to produce any toxic effects, including maternal toxicity. Teratology testing with rabbits and rats exposed to

EDC by inhalation did not produce adverse effects on the developing conceptus (Rao et al., 1980). Maternal toxicity was observed in rabbits exposed to 100 or 300 ppm EDC and in rats at 300 ppm but not 100 ppm. Chronic inhalation exposure of male and female rats to EDC for up to 150 ppm did not result in adverse reproductive effects over one generation (Rao et al., 1980). EDC administered in diet at 250 and 500 ppm (10-35 mg/kg body weight) did not adversely affect the reproductive activity of the male and female rats (Alumot et al., 1976).

Several Russian studies have reported that EDC produced adverse reproductive effects in rats exposed by inhalation (Vozovaya, 1971, 1974, 1976). These studies consistently showed effects at exposure levels lower than those employed by Lane et al. (1981), Rao et al. (1980), and Alumot et al. (1976) but were lacking in some details presented. Based on the study of Vozovaya (1974), Barlow and Sullivan (1982) concluded that exposure of female rats to 14 ppm of EDC by inhalation daily for 6 months produced some lengthening of the estrus cycle and reduction of litter size and postnatal survival but did not affect fertility.

The Russian investigations provide some suggestive but not conclusive evidence of a reproductive effect of EDC. Assuming that the effects reported in these studies were caused by EDC, then the lowest level for an adverse effect (disturbance of estrus cycle) was 0.7876 mg/kg/day (1.25 ppm for 4 hrs/day). When compared to an ambient EDC concentration of 0.5 ppb, the effect level is still greater than one thousand times

the level received by a person exposed to the chemical in the community air.

6.2 Human

There are no published studies on the effects of EDC on human reproduction.

7. Genotoxicity

According to the evaluation of IARC (1979), EDC is mutagenic in Salmonella typhimurium, Drosophila melanogaster, and Hordeum vulgare. The review of Davidson et al. (1982) showed that in addition to the above, EDC elicited DNA reparative synthesis in human lymphocytes in vitro, gene mutation in the Chinese hamster ovary cell/HGPRT culture system, and a mitotic recombinogenic effect in Saccharomyces cerevisiae. The mutagenic activity of EDC appears to be dependent upon the metabolic activity of the test systems, and the mutagenic enhancement appears to occur largely by cytosolic enzymatic reactions. The half-mustard S-(2-chloroethyl)-GSH and chloroacetaldehyde are suggested to be likely mutagenic metabolites of EDC. The review of Rannug (1980) also indicated induction of DNA damage in E. coli. EDC also binds to protein and DNA (Banerjee et al., 1980; Reitz et al., 1982).

8. Carcinogenicity

IARC (1979) has reviewed the available information on EDC and has concluded that there is sufficient evidence of animal carcinogenicity. IARC maintains that in the absence of adequate data in humans, it is reasonable for practical purposes, to regard EDC as if it presented a carcinogenic risk to humans. Staff of DHS agrees with the conclusion of IARC and agrees that EDC should be considered as a potential human carcinogen. The purpose of this section is to present an evaluation of the animal bioassays and provide a basis for a quantitative assessment of potential human risks.

8.1 Animal

The carcinogenic potential of EDC has been investigated in a number of studies in which the chemical was administered to rats and mice by various routes of administration (NCI, 1978; Maltoni et al., 1980; Spencer et al., 1951; Van Duuren et al., 1979; Theiss et al., 1977). However, only two studies can appropriately be used for a quantitative evaluation of risk and this discussion will be limited to these two studies: a gavage study preformed by the National Cancer Institute (NCI) in 1978 and an inhalation bioassy by Maltoni et al. in 1980. Mice and rats of differing strains were exposed to EDC in each of these studies. These studies are described in the following sections.

8.1.1 NCI Bioassay

Rat

EDC (99% pure) in corn oil was administered by oral intubation to Osborne- Mendel rats starting at 8 weeks of age. Fifty rats of each sex were used for each of the two dose levels of 47 and 95 mg/kg/day. Twenty animals of each sex served as untreated controls; an equal number were given the vehicle by gavage. The initial doses administered to the test animals produced early signs of toxicity, which necessitated several changes in the dosages. Weight depression was observed in both groups. By 50 weeks, the weight depression averaged 12% in high-dose rats. Mortality was early and severe in dosed animals, especially those given the highest dose. The mean survival was approximately 55 weeks after the start of treatment for high- dose males and females. The early deaths were usually not due to cancer; rather, the toxic effects of EDC appeared to be responsible for these deaths. Rats dying early had a variety of lesions, including bronchopneumonia and endocardial thrombosis, which may have contributed to early death. The pneumonia may have been the result of a viral, bacterial, or mycoplasmal infection, and the exposure to the chemical may have increased the tendency to develop severe pulmonary lesions, which would lead to death.

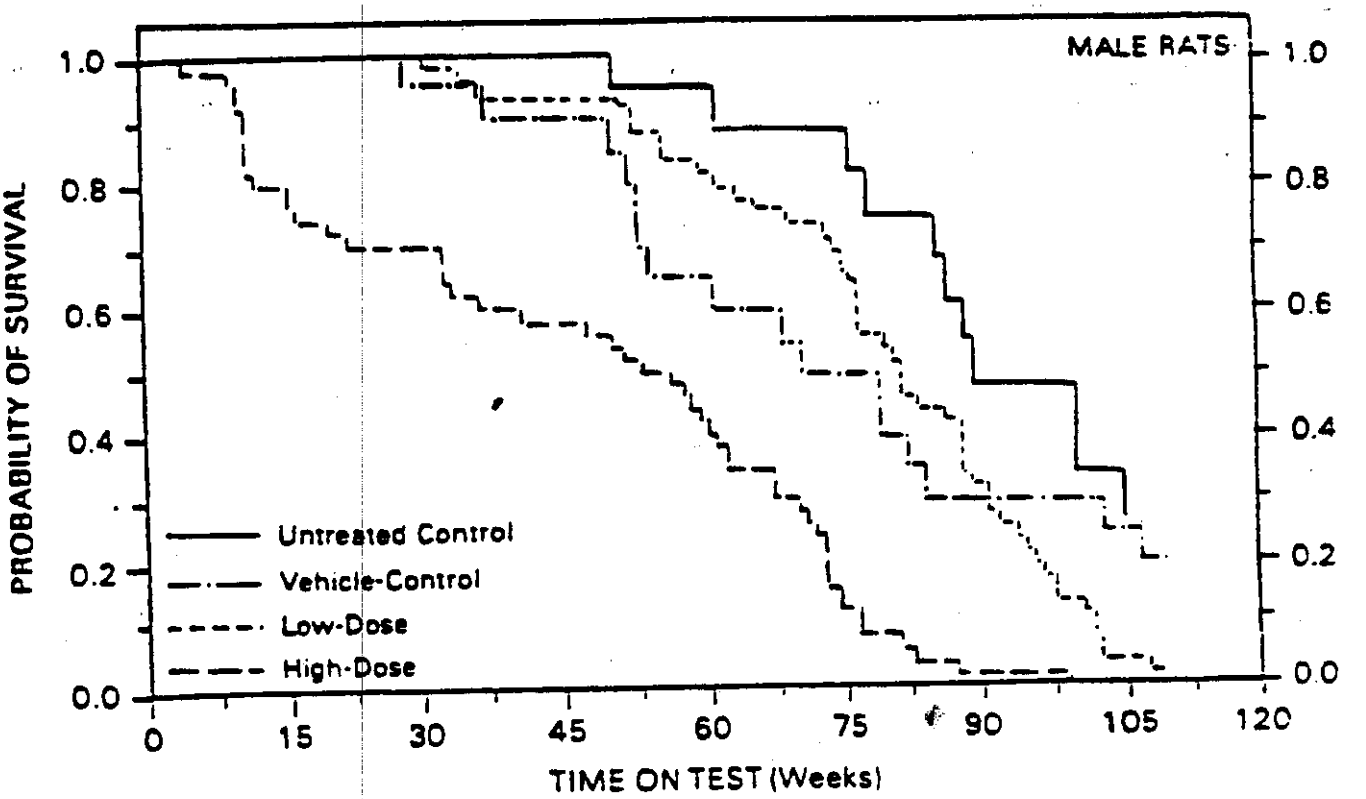
The results obtained on the survival of the animals are shown in Figures 1 and 2. For the high-dose male rats, 50% (25/50) were alive at week 55 and 16% (8/50) were alive at week 75. Survival was higher in the other

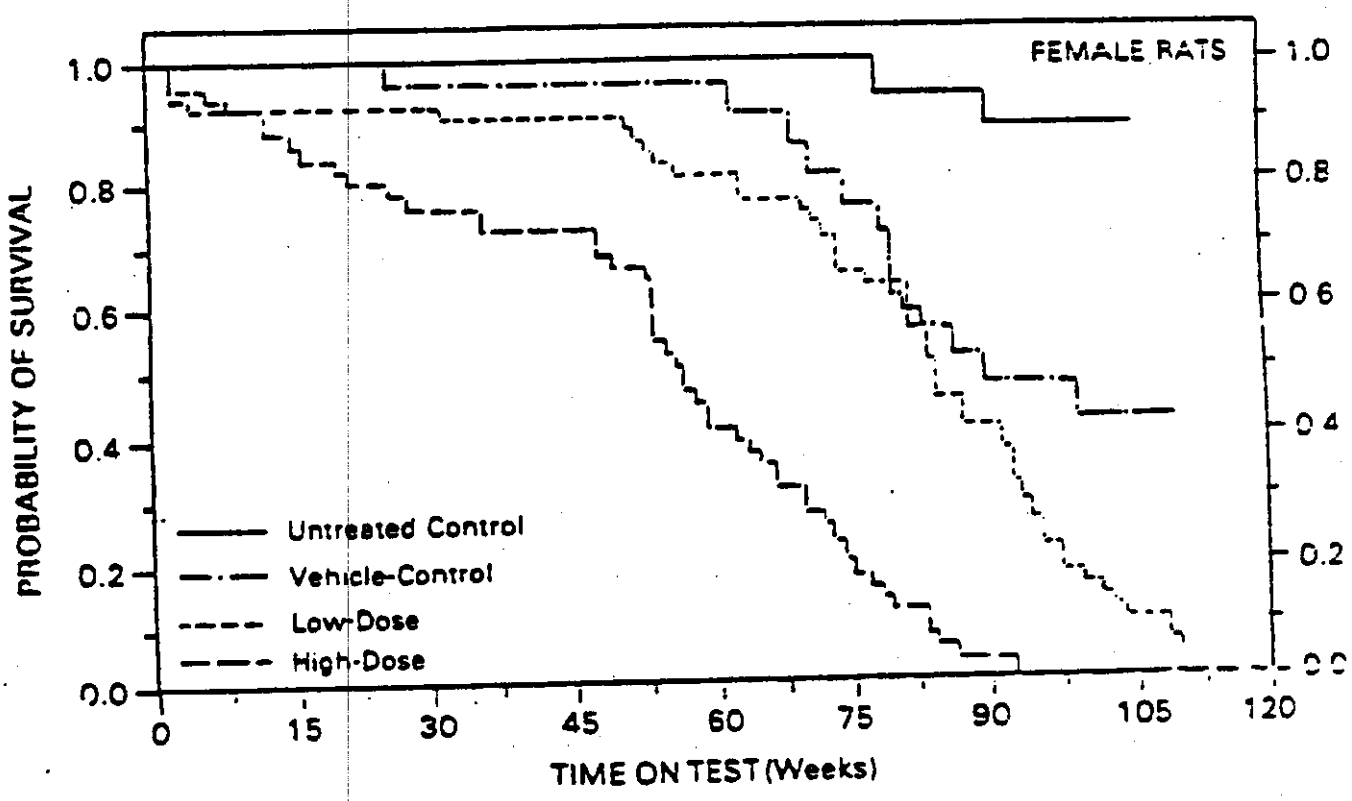
groups: 52% (26/50) of the rats in the low-dose group lived at least 82 weeks, and 50% (10/20) in the vehicle-control group lived at least 72 weeks. In the high-dose female rats, 50% (25/50) were alive at week 57; 20% (10/50) of the rats in the low-dose group were alive at week 85. Despite the sacrifice of five females at week 57, 65% (13/22) of the untreated control group survived until the end of the study. A gross necropsy was performed on each animal that died during the experiment or was killed at the end.

Squamous cell carcinomas of the forestomach were first observed in high-dose male rats at 51 weeks after oral intubation of EDC. Hemangiosarcomas were noted in some treated rats but not in any of the controls. Low-dose males and females showed higher incidences of hemangiosarcoma than high-dose animals. Tumors were observed in several sites, including spleen, liver, adrenal glands, pancreas, large intestine, subcutaneous tissue, and abdominal cavity. In addition to stomach carcinomas and hemangiosarcomas, EDC-treated female rats showed significant increases in the incidence of mammary adenocarcinomas. Tumors were observed in the high-dose group as early as 20 weeks after treatment. An increased incidence of fibromas of the subcutaneous tissue was reported in both high-dose and low-dose male rats when compared with the pooled vehicle-control group.

In summary, a statistically significant increase in the incidence of squamous cell carcinomas of the forestomach, hemangiosarcomas of the circulatory system, and fibromas of the subcutaneous tissue occurred in male rats. There was also a statistically significant increase in the

incidence of adenocarcinomas of the mammary gland and hemangiosarcomas of the circulatory system in female rats. These tumor incidences are shown in Table 2.





Figures 1, 2 Survival comparisons for male and female Osborne-Mendel rats administered 1,2-dichloroethane (EDC) by gavage. (NCI 1978)

Table 2. Tumor Incidences in Osborne-Mendel Rats Treated with EDC

	Squamous cell carcinomas of the forestomach				Hemangiosarcomas				Adenocarcinomas of the mammary gland	
Group	Males	P value[a]	Females	P value[a]	Males	P value[a]	Females	P value[a]	Females	P value[a]
Matched vehicle-control	0/20 (0%)		0/20 (0%)		0/20 (0%)		0/20 (0%)		0/20 (0%)	
Pooled vehicle control	0/60 (0%)		0/59 (0%)		1/60 (2%)		0/59 (0%)		1/59 (2%)	
Low-dose	3/50 (6%)	NS	1/49 (2%)	NS	9/50 (18%)	0.003	4/50 (8%)	0.041	1/50 (2%)	NS
High-dose	9/50 (18%)	0.001	0/50 (0%)	NS	7/50 (14%)	0.016	4/50 (8%)	0.041	18/50 (36%)	0.0001

[a]P values calculated using the Fisher Exact Test. Treated versus pooled vehicle-control.

NS = not significant when P values are greater than 0.05.

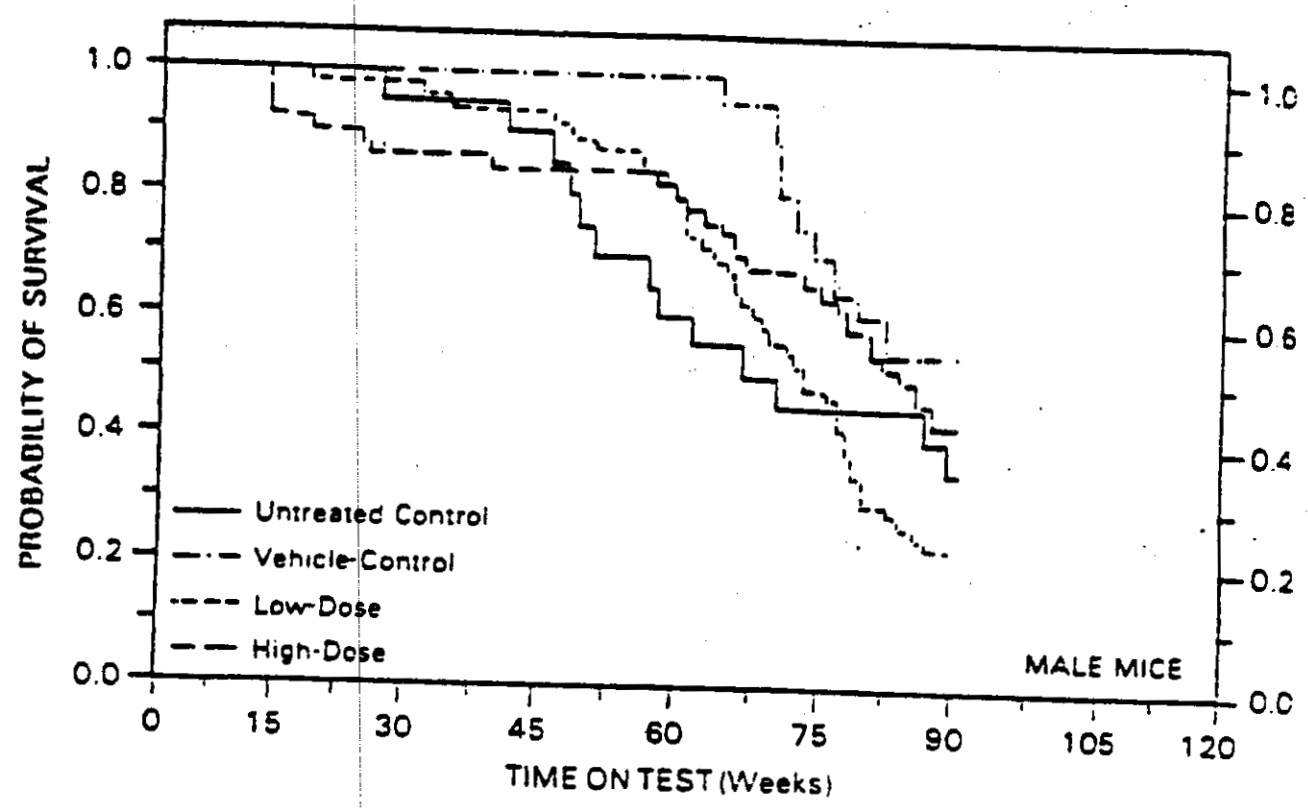
Incidences are based on number of tumor-bearing animals/number of animals examined at site (proportion).

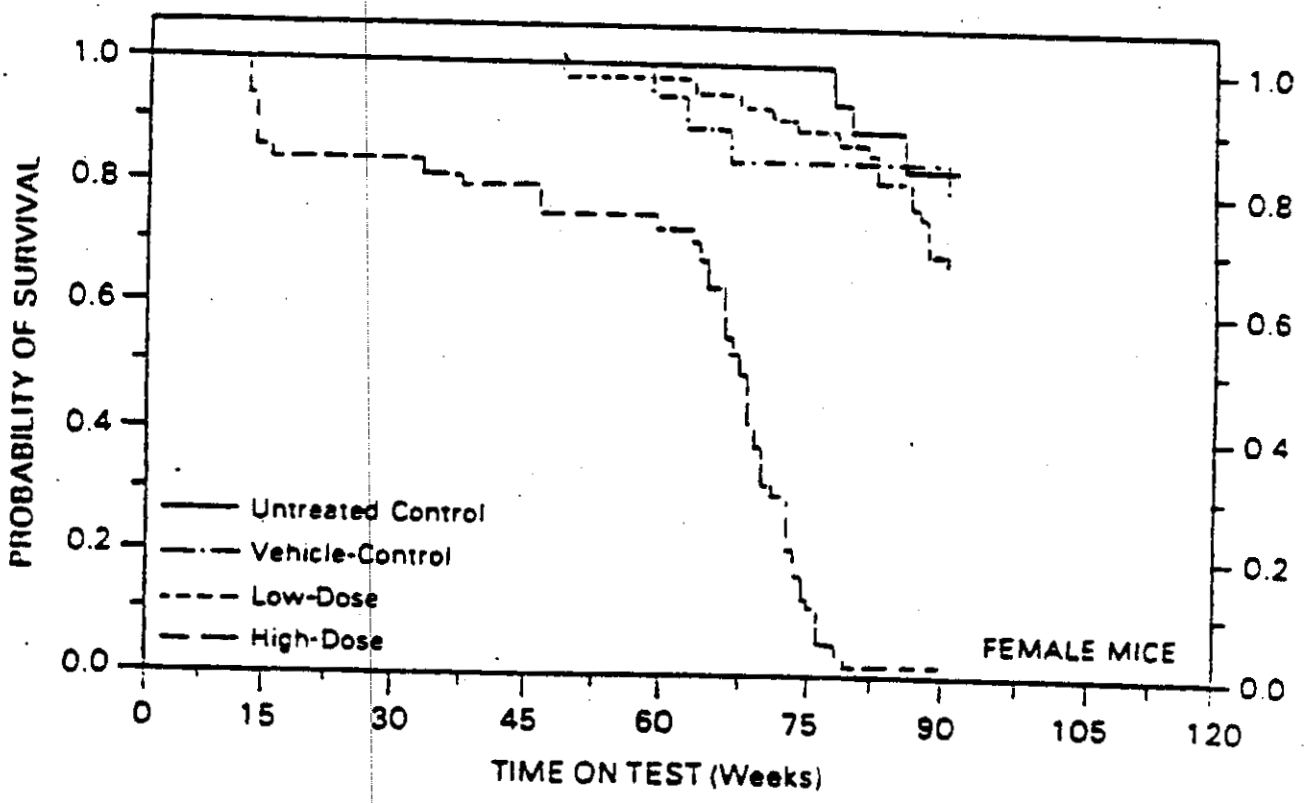
Source: NCI, 1978 (modified).

Mouse

EDC was administered by gavage to 200 B6C3F1 mice starting at 5 weeks of age. Fifty mice of each sex were used at each of the two dose levels for the chronic study: 97 and 195 mg/kg/day for male mice and 149 and 299 mg/kg/day for female mice. Twenty mice of each sex served as untreated controls, and an equal number were given the vehicle (corn oil). The initial doses administered to the test animals produced signs of toxicity in these animals early in the study, which led to changes of the dosages several times. No dose-related mean body weight depression was observed in male mice or low-dose female mice. A depression in the mean body weight of the high-dose female mice was apparent as early as week 15. Survival data are shown in Figures 3 and 4. For male mice, no statistically significant association between dosage and mortality was observed. In the high-dose group, 50% (25/50) of the mice were alive at 84 weeks and 42% (21/50) survived until the end of the study. In a low-dose group, however, survival was low. By 24 weeks, 52% (26/50) of the low-dose group and 55% (11/20) of the untreated control group had died. In the high-dose group, 72% (36/50) of the animals died between weeks 60 and 80. These deaths may have been tumor-related since 69% (25/36) had one or more tumors. Survival was high in the other groups: 68% (35/50) of the low-dose and 80% (16/20) of the untreated control groups survived until the end of the study. A gross necropsy was performed on each animal that died during the experiment or was killed at the end.

Results of the NCI study in B6C3F1 mice demonstrated a statistically significant increase in incidences of hepatocellular carcinomas and alveolar/bronchiolar adenomas in male mice, and a statistically significant increase in incidences of alveolar/bronchiolar adenomas, mammary carcinomas, and endometrial tumors in female mice. The tumor incidences are shown in Table 3.





Figures 3, 4 Survival comparisons for male and female B6C3F1 mice given 1,2-dichloroethane (EDC) by gavage. (NCI 1978)

Table 3. Tumor Incidences in B6C3F1 Mice Treated with EDC

Group	Hepatocellular carcinomas				Alveolar/bronchiolar adenomas				Adenocarcinomas of the mammary gland		Endometrial polyp or endometrial stromal sarcomas	
	Males	P value[a]	Females	P value[a]	Males	P value[a]	Females	P value[a]	Females	P value[a]	Females	P value[a]
Matched vehicle controls	1/19 (5%)		1/20 (5%)		0/19 (0%)		1/20 (5%)		0/20 (0%)		0/20 (0.0%)	
Pooled vehicle controls	4/59 (7%)				0/59 (3%)		2/60 (3%)		0/60 (0%)		0/60 (0.0%)	
Low-dose	6/47 (13%)	NS	0/50 (0%)	NS	1/47 (2%)	NS	7/50 (14%)	0.046	9/50 (18%)	0.0001	5/49 (10.0%)	0.016
High-dose	12/48 (25%)	0.009	1/47 (2%)	NS	15/48 (31%)	0.0025	15/48 (31%)	0.0001	7/48 (15%)	0.0003	5/47 (11.0%)	0.014

[a]P values calculated using the Fisher Exact Test (one-tailed). Treated versus pooled vehicle-control.

NS = not significant.

Incidences are based on number of tumor-bearing animals/number of animals examined at site (proportion).

Source: NCI, 1978 (modified)

8.1.2 Maltoni et al. study

Rat

Maltoni et al. exposed four groups of 12-week-old Sprague-Dawley rats (each group consisting of 180 rats of both sexes) to EDC concentations of 250-150 ppm, 50 ppm, 10 ppm, and 5 ppm, respectively, 7 hours per day, 5 days per week, for 78 weeks. After several days of 250 ppm exposure, the rats began to exhibit severe toxic effects, and the concentration was reduced to 150 ppm. Two groups, composed of 180 rats per group, served as controls. One of the two control groups was kept in an exposure chamber under the same conditions and for the same length of time as the exposed rats. At the end of the treatment period, the animals were allowed to live until spontaneous death. All detectable gross pathologic changes were recorded. A complete autopsy was performed on each animal.

Figures 5 and 6 shows the survival data for the animals. The extent of mortality varied with the different groups, but there appears to be no direct relationship between mortality and exposure to EDC. The highest survival rate was observed in both males and females of the group exposed to EDC at 5 ppm. In females the highest mortality rate was observed in the control group in the chamber and in the group exposed to EDC at 250-150 ppm. The overall survival rates at 52 and 104 weeks of age were 93.9% and 27.3% respectively. The overall survival rates after 52 weeks from the start of the experiment was 83.7%.

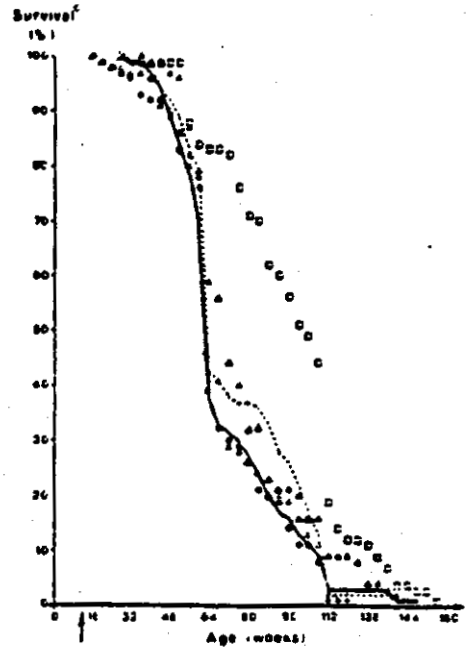


Figure 5

Survival of male Sprague-Dawley rats in experiment BT 501. (•) Group treated with 250-150 ppm EDC; (▲) 50 ppm; (△) 10 ppm; (□) 5 ppm; (———) untreated control group in chambers; (– – – –) untreated control group out of chambers.

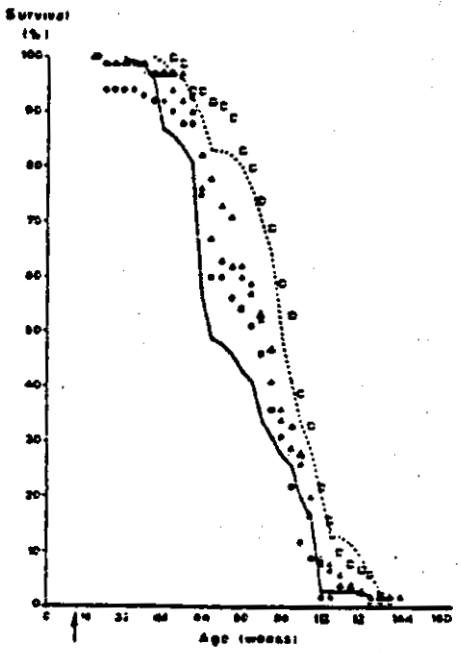


Figure 6

Survival of female Sprague-Dawley rats in experiment BT 501. (•) Group treated with 250-150 ppm EDC; (▲) 50 ppm; (△) 10 ppm; (□) 5 ppm; (———) untreated control group in chambers; (– – – –) untreated control group out of chambers.

The results of histopathologic analysis showed no statistically significant increase in the incidence of any specific type of tumor in the treated rats when compared with controls except for an increased incidence of mammary fibromas and fibroadenomas in groups exposed to EDC at 250-150 ppm, 50 ppm, and 5 ppm, when compared to controls in the chamber, but not significant when compared to controls outside of the chamber. The greatest difference in incidence was found between the control groups in the chamber, which had low survival rates, and the groups treated with EDC at 5 ppm, which had high survival rates. The difference in survival rates thus appears to be related to the difference in incidence in the two control groups.

Mouse

Four groups of Swiss mice (180 mice of both sexes per group) were exposed to EDC in concentrations of 250-150 ppm, 50 ppm, 10 ppm, and 5 ppm, respectively, 7 hours per day, 5 days per week, for 78 weeks. After several days of 250 ppm exposure, the mice began to exhibit severe toxic effects, and the concentration was reduced to 150 ppm. One group of 249 mice served as controls.

At the end of the treatment period, the animals were allowed to live until spontaneous death. The same procedures used for the rat study were followed. Survival data are shown in Figures 7 and 8. The survival rates for female mice were slightly lower in the group treated with EDC at 250-150 ppm. The overall survival rates for mice at 52 and

78 weeks of age were 82.4% and 45.9% respectively. The overall survival rates after 52 weeks from the start of the experiment was 67.8%.

The results of histopathologic analysis of various tumors do not indicate a statistically significant increase in the incidence of any specific type of tumor in the treated mice as compared with controls.

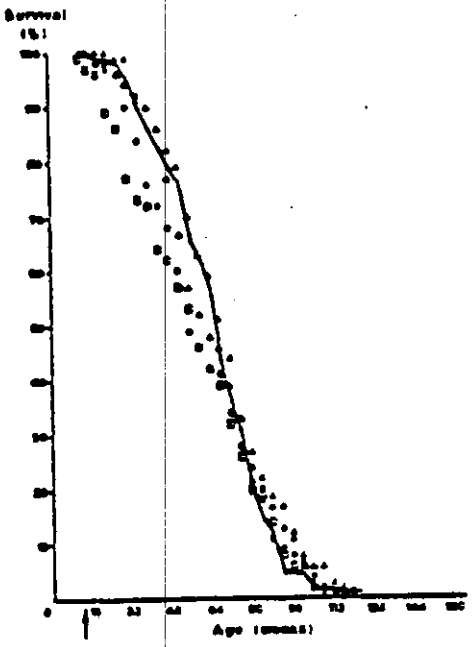


Figure 7
Survival of male Swiss mice in experiment BT 502. (●) Group treated with 250–150 ppm EDC; (▲) 50 ppm; (△) 10 ppm; (□) 5 ppm; (———) untreated control group.

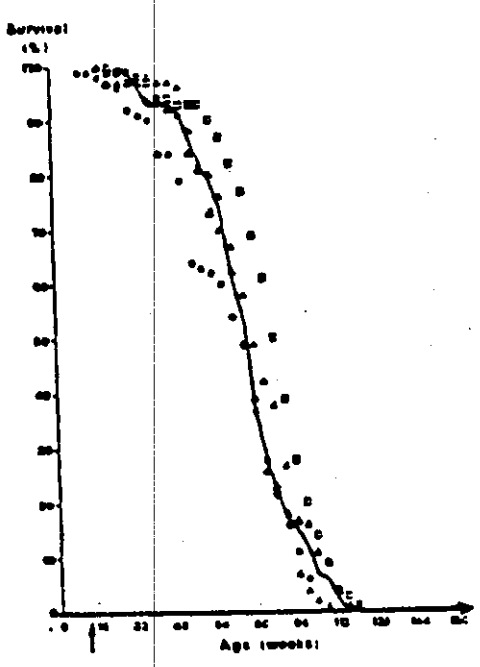


Figure 8
Survival of female Swiss mice in experiment 502. (●) Group treated with 250–150 ppm EDC; (▲) 50 ppm; (△) 10 ppm; (□) 5 ppm; (———) untreated control group.

8.1.3 Discussion of Bioassays

The NCI and Maltoni et al. studies suffered from some short-comings many of which have been discussed in the literature (Hooper et al., 1980; Maltoni et al., 1980; Davidson et al., 1982). A major problem with these studies was the early and high mortality rates. Early indications of toxicity in the NCI study necessitated several changes in the dosage levels and the dosing schedule. Even after resorting to a cyclical dosage regime in both the high-and low-dose treatment groups in order to lower the dosage levels, the mortality rate remained especially high in the high-dose groups for male and female rats, and for female mice. The early deaths, with the exception of male mice, were dose-related. These deaths were attributed not to carcinogenicity but to toxicity, which in many cases may have increased the animals susceptibility to viral, bacterial, or mycoplasmal infection (Ward, 1980). Although the high mortality was due in part to an overestimate of the maximum tolerated dosage (MTD) in the high-dose group, early mortality was also observed in the vehicle-control groups for male rats and female mice, and in the untreated control groups for male rats and mice.

Similarly, initial indications of toxicity required Maltoni et al. to reduce their high dosage level very early in the study. High mortality also occurred in the treatment chamber-control groups. The mortality was especially significant in rats and female mice. The mortality rate for the chamber-control group was greater than for all of the treated

groups of female rats throughout the study period and for the treated male rats approximately halfway through the study period.

The high mortality seen in these two studies is evident from the Kaplan-Meier survival curves (Figures 1 to 8). The cause of the high mortality in the control groups in both two studies has not been addressed by the original investigators nor has it been discussed in the literature. These results suggest significant "unexplained problems" with both studies. Additionally, the NCI study was criticized for allowing possible exposure to other chemicals because the animals were housed in the same room while bioassays with other halogenated hydrocarbons were conducted concurrently.

9. Threshold

For toxicologic purposes, a threshold dose is one below which a specified outcome does not occur. The self-propagating, clonal nature of tumor growth and development from a single damaged cell however suggests that the effective dose for carcinogenesis may be so low as to be indistinguishable from zero. While threshold models (based on detoxification enzyme saturation, the existence of DNA repair mechanisms, recurrent cytotoxicity) have been proposed, none has been convincingly demonstrated.

An "epigenetic mechanism" that could theoretically embody threshold doses has been invoked to explain the carcinogenic action of substances that do not directly produce genetic damage in short-term tests.

However, neither short-term tests nor non-linearities in dose-response curves from animal bioassay can reliably distinguish between "genetic" versus "epigenetic" carcinogenesis primarily because of the limited sensitivities of the experimental methodologies. DHS staff agrees with the conclusion of the IARC (1983) that there is insufficient evidence at present to justify creating separate classes of carcinogens (based on mechanism) for which different risk assessment methods would be used. In any case, in view of the strong evidence for EDC's genotoxicity, it would be inappropriate to suggest that this substance's carcinogenicity is due to an epigenetic mechanism.

Thus, in the absence of compelling evidence to the contrary, DHS treats carcinogenesis a non-threshold phenomenon.

10. Risks Estimates Based on the Oral Bioassay (NCI)

The NCI study, demonstrated statistically significant carcinogenic responses to EDC for both sexes of rats and mice (Tables 2,3). Due to the high mortality rate, only those animals that survived at least 50 weeks are used as the denominator of the incidence rate for the dichotomous risk assessment models. The time-to-death with tumor multistage ("Weibullized" or time-corrected multistage) model directly corrects for the observed intercurrent mortality.

The results of the the human risk estimates based on low-dose extrapolation for both the male rat hemangiosarcomas and the male mouse hepatocellular carcinomas are shown in Table A. Five models are used

for these risk estimates. The calculations for this table are provided in Appendix IA.

Choice of data set

The tumor data chosen for risk assessment is based on the findings on the most sensitive site, sex, and species. The selection of the most sensitive site, sex, and species for use in quantitative risk assessment often can not be made directly from the crude incidence data. For example, the NTP tables and crude data only provide the responses for the generic dose levels (e.g., high, intermediate, and low) for each sex and species. The actual dose levels (mg/kg-day) and the lifetime daily, surface area corrected doses (mg/S.A.-day) will differ significantly by sex and species. Even using the surface area corrected dose levels it is still not possible to determine the most sensitive site, sex, and species by visual inspection. The dose-response curve is statistically fit to the experimental data by maximum likelihood, thus its shape cannot be predicted a priori. This is further complicated for a temporal model. Although the crude tumor incidence rates for two different sites may be identical , the time-to-death with and without tumor will strongly influence the shape of the dose-response curve.

In selecting the most sensitive site, the staff of DHS is guided by prior risk assessments and especially those from the Carcinogen Assessment Group of the Environmental Protection Agency. Additionally, the staff often use the data from different tumor sites to verify

others' selections of the most sensitive site or to develop this information when it is not available in the literature. Considerations for selection include the demonstration of a statistically significant increase in tumor incidence in the treated animals and a dose-response relationship. The final selection is based on the results showing the highest risk obtained after assessment of the data using the model(s).

In the case of EDC data in mice, the EPA identified the male hepatocellular carcinomas as a more sensitive indicator of tumor response than the female mammary tumors. DHS concurs with EPA's conclusions based on the following analysis.

Although the crude (unadjusted) dosage and tumor incidences suggest that the female mouse mammary gland is a more sensitive site than male livers, this is true only before but not after the correction factors and the model are applied to the data. Before the dosages are corrected for surface area, the response data are corrected for intercurrent mortality, and the model is used for risk assessment, the observed adenocarcinoma incidence in female mammary glands appears to be greater than the hepatocellar carcinoma incidence in male mice (Table 3). The highest crude incidence rate of 21% (adjusted for background tumor incidence) for the male hepatocellular carcinomas occurred in the high-dose group administered EDC at 195 mg/kg-day, whereas the highest incidence rate of 18% for the female mammary tumors occured in the low-dose group administered EDC at 149 mg/kg-day. Therefore, the crude data showed that for comparable tumor rates, the female tumors occurred at approximately 75% of the dosage required for the male liver

tumors. The ratio of the crude incidence rates per unit dose for the male to female tumors is 0.89.

A Fisher exact test comparing the mammary tumor incidence at a specified dose level to that of the control was statistically significant for each dose level; however, the trend test, with a P-value of only 0.128, was not significant. This trend test is especially important in establishing a dose-response relationship with only three points (a control and two dose levels). Regardless of the absence of a dose-response relationship, the female mammary tumor data were modeled using the simple (non time-dependent) multistage model. The estimated low-dose risks based on this tumor site using the model for low-dose extrapolation were from 2-to-2.5 fold lower than those risks calculated using the incidences of male hepatocellular carcinoma. Thus, DHS concurs with EPA's conclusion that the male liver showing hepatocellular carcinomas is the most sensitive site for mice in the study.

Choice of model

Of the models in general use today, the DHS agrees with the EPA (1984) in the use of the multistage model. The underlying principle behind the use of the multistage model is the biologic plausibility of the theory that carcinogenesis is a multistage process. It is a flexible model in that the form of the model is not determined a priori, and thus it may take on some of the characteristics of the other models

depending on the number of stages used and the corresponding parameter values.

When time-to-death with tumor data are available, DHS recommends the use of the time-dependent form of the multistage model (Weibullized-multistage model) and will calculate risks using the Crump and Howe program Global 82. The Weibullized-multistage model has the same properties as the simple multistage model and, in addition, it uses time-to-death with tumor data and corrects for intercurrent mortality. In the absence of adequate survival data, the DHS will preferentially use either the quantal multistage model as calculated by the Watson and Howe computer program Global 79 or the Howe and Crump program Global 82. DHS will provide both the maximum likelihood and the 95% upper confidence limit, using the linearized multistage confidence limits from the above programs.

In the case of EDC, the staff of DHS assesses risks using models other than the simple or time-dependent multistage only for comparison. The values derived from these other models are not suggested for use for regulatory purposes, and they only demonstrate the dependence of the low-dose risk extrapolation on the mathematical model. It will be noted that the low-dose risk estimated by the gamma multihit model for the male hemangiosarcoma is more than two orders of magnitude greater than that estimated by the simple or time-dependent multistage model. One reason that the staff of DHS has refrained from using the gamma multihit model for risk assessment is that when the number of hits, k, is less than one, the model will yield unrealistically high risks for

low doses. This is because the slope becomes extremely steep at low doses. Under these conditions the shape of the dose-response curve at low doses becomes concave upward. In the case of male rat hemangiosarcomas this indeed is evident: the slope (shape) parameter, k, is 0.44. Additionally, the model suggests a background tumor rate whereas the actual rate entered was zero. This is also one reason why the gamma multihit model is not recommended for use.

11. Discussion of The Inhalation Bioassay (Maltoni et al.)

The negative results of the (Maltoni et al.) inhalation study are in apparent conflict with the positive findings of the (NCI) gavage study. These divergent results appear especially significant considering the facts that the inhalation study used (Maltoni et al.) four dose levels as compared with the two used in the gavage bioassay, and that the inhalation bioassay (Maltoni et al., 1978) used more animals per dose level (180 versus 100 animals) per dose level. This section examines possible explanations for these conflicting results.

The highest dose level for the male and female rats in the two studies differed by approximately two fold. The highest dose level for the male mice in the two studies were comparable (Table B). No statistically significant increased maligancies were detected in the Maltoni et al. study in either sex of treated rats or mice as compared with the controls whereas the NCI bioassay results demonstrated positive carcinogenicity for both sexes of mice and rats. This discrepancy between the two studies has been discussed in the literature. Hooper et al.

(1980) suggested that these conflicting results may be the result of the following:

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.

These authors concluded, "We cannot choose among these possibilities on the basis of the information at hand." DHS staff believes that all of the possibilities listed by Hooper et al. may be responsible for the negative results. The following discussion addresses each of these points in turn.

Strain Differences - Maltoni et al. used the same strains of rats and mice in the EDC bioassay as were used in the bioassays of several halogenated two-carbon compounds (alkanes and alkenes). It is possible, however, that these strains may have quantitative differences in their carcinogenic susceptibility since they were different from the strains used in the NCI bioassay. Comparison with other carcinogenicity studies conducted by Maltoni suggest that if there is a strain-related difference in response it is probably less than one order of magnitude.

Route of Exposure - The route of exposure affects the pharmacokinetics of a compound in a variety of ways and may influence the carcinogenicity of a compound. Gavage dosing resulted in a rapid increase in the EDC blood concentration, a bolus effect, and inhalation caused much lower levels. The total areas under the blood concentration curves, however, were similar (i.e., approximately equal doses).

Reitz et al. (1982) observed that after single exposures of EDC by gavage and inhalation at the same dose level, that in vivo binding (apparent alkylation, See Appendix II A3) of radioactively labeled EDC to rat DNA following gavage was 2 to 5 times higher than after inhalation. This binding, however, was not directly related to carcinogenic susceptibility of the target organs. It is believed that DNA damage is a common denominator in both mutagenesis and carcinogenesis, and that the presence of an increased level of DNA damage in somatic cells may result in an increased risk of cancer. This may suggest that the effective "DNA damaging" dose by inhalation as compared to gavage may be only one-half to one-fifth as great and therefore may have decreased the expected number of tumors. This issue is discussed in Appendix II A3.

Intercurrent Mortality - Hooper et al. (1980) also suggested that the negative results of Maltoni et al. could be an introduction of an artifact due to intercurrent mortality. Life table analysis of this study, as suggested by these authors, could not correct for the intercurrent mortality because no cancers were observed.

An additional point implied by Hooper et al. (1980) is that the two lower dose levels used in the Maltoni et al. study are too low to provide a carcinogenic response for the number of animals at each dose.

A further discussion of the inhalation bioassay is contained in Appendix II. Several conclusions can be reached from these discussions and will be stated here (Note - These conclusions are independent from the "unexplained problems" connected with this bioassay):

1. It is concluded that no tumors were expected at the two lower dose levels and that statistically significant tumor rates would be expected only at the highest dose. These conclusions are based on the following assumptions:

   a. There is 100% absorption of the inhaled dose.

   b. The exposure dose is equal to the effective dose.

   c. The potency of EDC by inhalation is the same as by gavage and that the response in the inhalation study can be predicted from the most sensitive site in each sex and species (hemangiosarcoma in male rats and heptatocellular carcinoma in male mice) from the gavage study. It is demonstrated that due to the low concentrations employed in the bioassay no tumors are projected for the lower two doses levels for either the mice or rats (Table E). Even if the projected number of tumors occurred at the two higher dose levels of the male rat or male mouse bioassay, only the number of tumors

at the highest dose level would be statistically significant. It is concluded, therefore, that the numbers of animals in the inhalation study were too low at the lower dosages to provide for a positive carcinogenic repsponse.

2. If the assumption is made that DNA binding provides a surrogate measure of the effective carcinogenic dose (See Appendix II A2) and tumor latency is a function of dose, that the time-to-death with tumor is also a function of dosage (Druckery relationship), then calculations suggest that the expected number of tumors in the high-dose rats will range from 0 to 1, with the expected mean number of tumors as 0. These calculations indicate that all the dosages were too low or that there were insufficient numbers of animals at each dose level to obtain a positive response.

3. Differences in carcinogenic susceptibility between the strains of animals used in the two studies may also explain why the inhalation study was negative. If the Osborne-Mendel rat strain is only 2 fold less sensitive than the Sprague-Dawley (NCI bioassay) then the expected number of tumors at the highest dose level would range from 0 to 1, with the expected number of tumor as 0.

It is not possible to determine the influence, if any, that the differences in route of exposure and animal strain had on the inhalation bioassay. The above calculations do demonstrate, however, that either of these factors could resolve the differences between the bioassays, without the need to conclude that EDC was not carcinogenic by the inhalation route.

4. As with most negative studies, this bioassay at best can only provide an upper bound of the risk as determined by the statistical power of the study. This study does not demonstrate that EDC is not a carcinogen via inhalation. Calculations suggest that the one-sided upper confidence bound of the Maltoni et al (1980) study is consistent with $q^* = 2.4 \times 10^{-3}$ [mg/(kg)(day)]$^{-1}$ (the lifetime excess carcinogenic risk of 2.9×10^{-6}/ ppb of EDC in the atmosphere).

It must be stressed that this upper bound is only valid in the absence of methodological problems, otherwise it could be significantly higher. The high mortality rate of the Maltoni et al chamber-controls suggests such a problem in the study, and therefore DHS does not recommend using the upper-bound estimate from the inhalation studies to establish a dose-response relationship for exposure to EDC in the ambient atmosphere.

DHS staff does not believe that the inhalation bioassay was scientifically sufficient to negate the use of the NCI bioassay in quantiatative risk estimation for the reasons outlined above and in Appendix II. The DHS staff recommends, therefore, that the NCI gavage bioassay of EDC be used in the quantitative risk estimation of inhalation exposure to EDC. The staff also recognizes that the the data contained in the NCI bioassay are not strong (especially in light of the high mortality rates) and caution that the uncertainty in this evaluation may be quite large.

The staff of DHS believes that the calculations given here demonstrate that the Maltoni et al (1980) study did not employ sufficiently high

concentrations of EDC to induce the expected tumor in either mice or rats. The negative finding of the expected tumor incidence at the highest dose level can be explained by a relatively small difference in response (two-fold) between the strains used in the NCI study and those in the Maltoni study. For these reason, DHS does not see the need to modify the gavage doses for projected inhalation doses.

12. Conclusions

1) On the basis of the findings discussed in the preceeding sections, it can be concluded that adverse health effects, such as systemic toxicity or reproductive effects, are not expected to result from exposure to EDC in community air.

2) DHS concurs with the IARC conclusion that there is sufficient evidence that EDC is carcinogenic in mice and rats and that, in the absence of adequate data in humans, it is reasonable, for practical purposes, to regard EDC as if it presents a carcinogenic risk to humans.

3) It is also the opinion of the staff of DHS that EDC is potentially carcinogenic via inhalation.

4) There is no evidence to demonstrate that the carcinogenicity of EDC has a threshold and EDC's carcinogenic activity should be treated as having no threshold.

5) The staff of DHS recommend the use of the low-dose extrapolation value as provided from the "Weibellized" multistage model as providing the best estimate of risk for ambient concentrations of EDC. This model suggests a lifetime excess cancer risk of between 53 and 88 per million for lifetime exposures to EDB at a concentration of 1 ppb.

APPENDIX I

A. Calculation of Gavage Dosage

The animals in the NCI gavage study were dosed for five consecutive days per week on a mg/kg basis. The time-weighted average (TWA) doses, as provided by NCI, are 47 and 95 mg/kg-day for rats, and 97 and 195 mg/kg-day for male mice, for the treatment period. The male rat hemangiosarcomas and the male mouse hepatocellular carcinomas provided the highest incidence rates and are used to calculate the equivalent human risk. The time-weighted average body weights of the mice and a body weight of 0.5 kg for the rats are used to calculate the risk. These dosages were converted to equivalent human dosages for a 60-kg person incorporating a surface area correction according to the following calculations.

Male Rat Hemangiosarcomas

Human-equivalent dose, surface area corrected for a 60-kg person:

$$\text{Low dose} = (47 \text{ mg/kg-day}) \times (5/7) \times (78/104) \times (0.5/60)^{1/3}$$
$$= 5.10 \text{ mg/kg-day}$$

$$\text{High dose} = (95 \text{ mg/kg-day}) \times (5/7) \times (78/104) \times (0.5/60)^{1/3}$$
$$= 10.3 \text{ mg/kg-day}$$

Male Mouse Hepatocellular Carcinoma

Human-equivalent dose, surface area corrected for a 60-kg person:

$$\text{Low dose} = (97 \text{ mg/kg-day}) \times (5/7) \times (78/90) \times (.0305/60)^{1/3}$$
$$= 4.79 \text{ mg/kg-day}$$

$$\text{High dose} = (195 \text{ mg/kg-day}) \times (5/7) \times (78/90) \times (0.0292/60)^{1/3}$$
$$= 9.50 \text{ mg/kg-day}$$

B. Calculation of Human Inhaled Dose from Ambient Exposure

The assumption is made that the average person weights 60 kg and inhales air at 18.05 m^3/day. The lifetime average daily dose from a continuous exposure is calculated as follows:

$$\text{dose} = \text{ppb} \times (4.047 \times 10^{-3} \text{mg/m}^3) \times (18.05 \text{ m}^3\text{/day}) \times (1/60 \text{ kg})$$

The lifetime average daily doses in air are:

$$0.1 \text{ ppb} = 1.22 \times 10^{-4} \text{ mg/(kg)(day)}$$

$$0.5 \text{ ppb} = 6.09 \times 10^{-4} \text{ mg/(kg)(day)}$$

C. Low-Dose Extrapolation Models

Dichotomous Models - The data for the quantal models are shown in Table C. The Crump and Watson program "GLOBAL 79" is used to calculate the quantal form of the multistage model. The Kovar and Krewski program "RISK 81" is used to calculate the probit and gamma multihit models. The Howe program "GLOBAL 82" is used to calculate the upper bound for the multistage model for the inhalation studies. The one-hit model is calculated by linearizing the data and fitting the transformed data to a linear regression. The 95% UCL for the one-hit model is calculated by deriving the 95% UCL with the binomial expansion for each data point and then linearizing and fitting the transformed data to a linear regression.

Time-Dependent Model - The Howe and Crump program "Weibull 82" is used to fit the time-to-death with tumor data. The 95% UCL was calculated by maximizing both the dose and time terms of the expression. The maximum likelihood estimate of risk and the 95% UCI are calculated at 90 weeks. The model fits the data very well up to 90 weeks but poorly beyond this period. This seems reasonable because the median lifespan for control animals is also less than 90 weeks (Approximately 70% of the control animals died before 90 weeks).

APPENDIX II

A. Inhalation Bioassay

A1. Risk Estimates

Statistical Power of Study

The 95% UCL for the linear and multistage models are calculated for the mice and rat inhalation study. These confidence limits provide an upper bound of the risk consistent with the negative findings. The upper bounds for these risk estimates assume that the Maltoni bioassay is unflawed. The calculations are made independent of pharmacokinetics, effective dosage and dependence of time-to-tumor on the dose, as discussed later.

95% UCL from the Multistage Model:

Male rats: slope $q^* = 1.4 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

Male mice: slope $q^* = 2.4 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

The 95% UCL for the one-hit model is derived by considering each dosage point individually and calculating the risk that is consisted with the

observed response for a one-sided 95% UCL using the binomial function. A surface area corrected dosage for a 60-kg person is used.

95% UCL from the One-Hit model:

Male rats: slope $q^* = 7.3 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

Male mice: slope $q^* = 3.8 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

The Maltoni study can only demonstrate the upper bound of carcinogenic potency, if EDC is carcinogenic by inhalation. The Maltoni results can not demonstrate that EDC is not carcinogenic by this route of exposure. The calculated ambient concentration corresponding to a human risk of 10^{-6}, based on the one-sided 95% upper confidence limit for the male rat inhalation study using the multistage model, is 0.01 ppm.

A2. Expected Tumor Rate, Uncorrected for Effective Dosage and Time-to-Tumor

The calculated tumor rate for the Maltoni et al. study assumes that 100% of the inhaled dose is effectively retained. No adjustment is made for mortality, and the administered dose is assumed equal to the effective dosage. The calculations assume that the average weight of

the rats is 0.5 kg and that they inhale 0.1 liter/min of air. The average weight of mice is assumed to be 0.035 kg with a respiratory rate of 0.03 liter/min. The MLE from the multistage model is used to estimate the tumor rate. The MLE coefficients from the multistage model for both the rat and mouse bioassays are used as follows:

Rat bioassay - $q_0 = q_2 = 0.0$

$q_1 = 3.507 \times 10^{-2}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

Mouse bioassay - $q_0 = 5.4067 \times 10^{-2}$

$q_1 = 9.7340 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

$q_2 = 1.5639 \times 10^{-3}$ $[mg/(kg\text{-}bw)(day)]^{-1}$

The projected number of cancers for each dose level is shown in Table E. These calculations confirm that in the absence of any additional factors (nonequivalency of inhaled and gavage dosages and strain differences in carcinogenic response) the two lower dose levels in both the rat and mouse study would result in a negative carcinogenic response. Although the penultimate dose level is projected to result in approximately 4 tumors for rats and 2 tumors for mice, these results are not statistically significant. Thus, the observed negative response is consistent with the calculated projections for all but the highest dosage.

A3. Effect of Decreased Dosage on Projected Tumor Incidence (Corrected for Time-to-Tumor)

Only the highest dose level in the inhalation study for mice and rats is projected to result in a statistically significat number of tumors.

This section considers the projected differences in carcinogenic response due either to small differences in strain sensitivity to EDC or to the nonequivalency of administered dose verses effective dose.

Stain difference between the animals used in the inhalation and gavage studies could account for the observed carcinogenic response differences in the gavage and inhalation studies. A useful surrogate estimate of lower interspecies carcinogenic response is the administered dose level. If there is a 2-to-4 fold lower carcinogenic sensitivity in the strains used in the inhalation study, this would be equivalent to a decrease in the administered dosage by this amount.

Although Rietz's DNA binding data suggest that the effective dose may be 2-to-5 fold lower than the administered dose, it will be assumed that the effective inhalation dosage is only one third as great as the administered dosage due to either strain differences or the difference effective versus administered dose.

Time-to-tumor is a function of the dosage. The latency period for tumor increases with decreasing dose. In order to estimate the effect of decreased dosage, it is necessary to correct for the tumor latency period. The relationship of the tumor latency period to dosage is quantified by the Druckery (1967) relationship:

$$\text{Constant} = C = dt^n$$

The highest observed potency by gavage for EDC is with the male rat hemangiosarcomas; this most sensitive site, sex and species is used for the calculations to provide an upper estimate of projected carcinogenic response. The NCI data for time-to-death with tumor for the male rat hemangiosarcoma are used to estimate the constant, C, and the time factor, n.

Let:

t = mean time-to-death with tumor, in weeks

d = dosage in mg/kg-day (surface area corrected for a 60-kg person)

Then:

For the low dose group: $C = 5.109(88.8)^n$

For the high dose group: $C = 10.32(72.6)^n$

Thus:

$C = 1.59189 \times 10^8$ and $n = 3.49028$

The binding of labeled EDC to DNA resulting from gauage exposure as comparred with an equivalent administered inhalation dose was 2 - to 5-fold greater. If the DNA binding data are used as a surrogate measure of the effective carcinogenic dose, it may be conservatively estimated that the effective dosage by inhalation is only one half of the dosage by gavage. The calculated effective dosage therefore, for the high-dose group for the Maltoni et al., (1980) study was 2.62 mg/kg-day (Note - For consistency, throughout this document this dose is in the surface over corrected format). The mean time-to-death with tumor for this dosage, from the Druckery

relationship, is 107.6 weeks. Similarly, the time for the first appearing tumor was calculated to be 88.6 weeks, as follows:

Calculated time-to-death for first appearing tumor - 87 weeks

Calculated " " " " mean tumor appearance - 108 weeks

The observed standard error of the mean for the NCI low-dose male rats gavage group for time-to-death with tumor - 12.5 weeks

The presentation of the Kaplan-Meier survival curves (Figures 5-8, Section 8.1.2) by Maltoni et al. is somewhat misleading because the authors used the time of birth for t = 0, as opposed to using the time from dosing. It is necessary to correct the time axis for the 12 weeks of life for the animals before the dosing in order to establish the expected appearance of tumors. The survival of the rats in high-dose group is comparable, if not worse, with that in the high dose group of the NCI study if the above correction is made.

The percentage and number of surviving animals for several time intervals using the time notation of Maltoni et al. and also by defining t = 0 as the start of the dosing period are shown in Table D. The expected number of cancer cases for the high dose group of the Maltoni et al. study is calculated using the potency derived from the multistage model for male rat hemangiosarcoma in the gavage study and by assuming that the effective dose for the Maltoni high dose group is 2.62 mg/kg-day. The maximum likelihood estimate from the multistage model for male rat hemangiosarcomas is used for

the projected cancer incidence. The MLE for this data has only one value of q, $q_1 = 0.0351$. The projected hemangiosarcoma rate for the rat inhalation study is:

$$P_t(d) = 1 - e^{-[(3.51 \times 10^{-2})\,(mg/kg\text{-}day)^{-1} \times (2.62\ mg/kg\text{-}day)]}$$

$$P_t(d) = 0.0879 \text{ or } 8.8\%$$

The calculated mean time-to-death with tumor is 108 weeks. At this time, approximately 1 animal was still alive in the Maltoni et al. study. Thus if all of the cancers occurred at 108 weeks, no cancers would have been observed. Assuming that time-to-tumor is a Poisson distribution with a mean of 108 weeks, the standard error of the mean (SEM) is approximately 10 weeks. The 95% UCL for tumor appearance is then the mean value for the time-to-death with tumor $\pm$1.96 SEM, or 86 to 128 weeks. The earliest projected time-to-tumor from the calculation falls within this calculated interval. The mean expected cancers from the Maltoni et al. bioassay is 0, and the range is 0-1. This calculation suggests that the results from the Maltoni et al. study are not inconsistent with those of the NCI bioassay when the data are adjusted for the effective dosage by inhalation versus the dosage for gavage and when the survival of animals is considered. Essentially, the Maltoni et al. study does not have the statistical power to detect the low carcinogenic potency of EDC given the above assumptions that

between the two studies there is either a difference in sensitivity of strains or that the effective dose by inhalation is less than that administered dose by gavage.

Although the difference observed in DNA binding has been suggested as a possible explanation for the disparate bioassay results (a hypothesis consistent with the suggestion that the gavage verses inhalation exposures were not equivalent), DHS staff believes that these results have academic value and that they are useful in discussions of these bioassays, but DHS staff does not believe that the DNA binding data are sufficiently complete to be used in a quantitative risk assessment for the reasons outlined below.

The DNA binding experiments as described above (Reitz et al., 1982) were adequately conducted and the data appear valid. DHS staff does not question the validity of the data. The experiments, however, are limited by the following:

1) Only one dose was employed for the inhalation (150 ppm) and gavage (150 mg/kg) routes of exposure. It is unknown whether the binding is dose-related.

2) Only one sampling time was used for the tissues from which the DNA was isolated. Thus there is no information, as to the time-dependence (if any) of the DNA binding.

3) The study was conducted in only one species, rats. Moreover, the strain (Osborne-Mendel) used in the DNA binding assay was not the

same as in the inhalation bioassay (Sprague-Dawley). It is unknown, therefore, whether the route of exposure-dependent DNA binding seen in Osborne-Mendel rats would be similar (e.g.,inhalation vs. gavage) in Sprague-Dawley rats, or Swiss-Webster mice, or other animal species or strains.

4) The DNA binding assay only detected "apparent" DNA alkylation as discussed in a prior section on pharmacokinetics (Section 5). The assay could not resolve between alkylated DNA and DNA radioactivity labeled through biosynthetic incorporation of radioactive label (i.e. via single carbon pool). The differences observed in binding may not have been due to differences in alkylation but due to increased endogenous incorporation in gavage dosed animals. This issue is critical and needs experimental clarification before the data are acceptable as an indication of DNA alkylation.

DHS staff does not recommend use of the DNA binding data cited above in the formal quantitative risk estimation of EDC. The staff believes the data may be used for academic and speculative purposes but conclude that they are not sufficiently complete for further use.

Table A. Comparison of Human Risks for Five Dose-Response Models[a] from an Oral Dose of 1.0 ug/kg-day of EDC

	Risk/million	
	MLE	95% UCL
Male Rat Hemangiosarcoma		
One-hit Model	34	56
Multistage	35	68
Multistage ("Weibullized")	32	72
Gamma Multihit	4,600	48,000
Probit	140	3,900
Male Mice Hepatocellular Carcinoma		
One-hit	23	36
Multistage	9.7	38
Multistage (Weibullized")	23	61
Gamma Multihit	34	56
Probit	0.0	5.1×10^{-17}

[a]Based on the NCI gavage bioassay, surface area corrected dosage for a 60-kg person. Lifetime risk from a lifetime averaged daily dose. Oral dose of 1 µg/kg-day is equivalent to 0.82 ppb /EDB in air assuming a respiratory rate of 18.05 m^3/day for a 60 kg person and 100% absorption and retention.

Table B. Average lifetime daily doses in EDC experiments.

	mg/(kg-bw)(day)[a]	
	gavage (NCI)[b]	Inhalation (Maltoni)[c]
Rats	50.9	25.8
	25.2	8.60
		1.72
		0.860
Mice	121[d]	110.6
	60[d]	36.0
	185[e]	7.37
	92.2[e]	3.69

[a]Dosage not surface area corrected.

[b]Animals dosed 5 days a week for 78 weeks. Lifetime daily dose assumed to be 104 weeks for rats and 90 weeks of the experimental period for mice.

[c]Animals exposed for 7 hr/day for 78 week period; observed for lifetime assumed to be 110 weeks for both rats and mice. It is assumed that rats inhale 0.1 l/min and weight 0.5 kg, and that mice inhalation 0.03 l/min and the average weight of mice assumed to be 0.035 kg (Gold, L.S., 1984).

[d]Males

[e]Females

Table C. Data for Quantal Models

Male Rat Hemangiosarcoma

Dose Level	Response	P value[a]
Matched-Vehicle Control	0/38	---
Pooled-Vehicle Controls	1/60	---
Low Dose	9/47	0.003
High Dose	7/27	0.001

Linear Trend test P = 0.001

Male Mice Hepatocellular Carcinoma

Vehicle Control	1/19	---
Pooled-Vehicle Control	4/59	---
Low Dose	6/47	0.34 NS
High Dose	12/48	0.009

Linear Trend test P = 0.004

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[a]P values calculated using the Fisher Exact Test (one-tailed). Treated versus pooled-control. Cochran-Armitage Trend test.

TABLE D. Survival of Male Rats in Maltoni High Dose Group

Age from Birth (in weeks)	Time from First Dosing (in weeks)	% Survival	Number Surviving
52[a]	40	87.8	79
64	52[a]	74	67
72	60	34	31
80	68	32	29
88	76	20	18
100	87[b]	12	12
104[a]	92	11.1	10
120	108[c]	1	0-1

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[a]Values provided by authors, all others estimated from survival curves.
[b]Time for earliest calculated time-to-death with tumor.
[c]Calculated mean time-to-death with tumor.

Table E. Projected tumor incidence rates for inhalation studies.[a]

Male Sprague-Dawley Rats - Hemangiosarcomas in the Circulatory System.

Concentration	Surface Area Corrected[b] Dosage (mg/kg-day)	Survivors after 52 weeks from start	Projected Number[c] Number of Tumors	P value[d]
150 ppm	5.23	67	11.2	<0.001
50 "	1.74	70	4.2	0.071 NS
10 "	0.349	70	0.85	0.52 NS
5 "	0.174	75	0.46	NS
0 (chamber controls)	0.0	64	----	----

Male Swiss Mice - Hepatocellular Carcinomas.

150 ppm	9.24	39	9.7	<0.001
50 "	3.08	46	2.0	0.15 NS
10 "	0.616	59	0.39	NS
5 "	0.308	42	0.13	NS
0	0.0	72	---	---

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[a]Based on MLE slope for the multistage model from the NCI gavage studies; projected tumor incidence calculated for each dose group separately from the rat and mouse studies for the observed end point.

[b]Assuming: rats weigh 0.5 kg, inhale 0.10 l/min and have a 110 week lifetime; mice: weigh 0.035 kg, inhale 0.03 l/min and have a 110 week lifetime (Gold, L.S., et al., 1984). Dosage corrected for a 60-kg person.

[c]No allowance for animal life time or time-to-death with tumor.

[d]P values calculated using the Fisher Exact Test (one-tailed). Treated versus combined controls.

NS = not significant.

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