0986

7/1/81
RB

Carcinogenicity Tests of Certain Environmental and Industrial Chemicals [1,2,3]

Elizabeth K. Weisburger,[4] Borge M. Ulland,[5,6] Jun-mo Nam,[7] John J. Gart,[7] *and* John H. Weisburger[7,8,9]

ABSTRACT—Fourteen chemicals of varied uses were tested for carcinogenicity by oral administration in male and female Charles River CD rats. Under the conditions of the tests, propane sultone, propylene imine, and ethylenethiourea, in addition to the positive control *N*-2-fluorenylacetamide, were carcinogenic. Avadex, bis-(2-chloroethyl) ether, the potassium salt of bis(2-hydroxyethyl)dithiocarbamic acid, ethylene carbonate, and semicarbazide hydrochloride were not carcinogenic under the test conditions. Dithiooxamide, glycerol α-monochlorohydrin, and thiosemicarbazide gave somewhat ambiguous results, though administered at high enough dose levels to be toxic. An inadequate number of animals survived treatments with sodium azide, sodium bisulfide, and vinylene carbonate, or the animals may not have received sufficiently high doses of the test chemicals to provide maximum test sensitivity. However, there were no indications that these three chemicals were carcinogenic under the test conditions.—JNCI 1981; 67:75-88.

Long-term toxicity studies in animals, not always standard previously, have become a necessity to determine the safety of environmental materials. An extensive study on various pesticides and other commercial compounds in 2 hybrid strains of mice was reported 10 years ago (*1*). Some of the compounds were found to be carcinogenic in those mice, other compounds were recommended for further tests, while others were not carcinogenic. To confirm and extend the earlier study, several of the carcinogenic or suspect compounds, plus others related structurally to known carcinogens, were tested in rats. These tests were initiated before the promulgation of the NCI guidelines; therefore, the number of animals and length of time on test did not correspond to the guidelines currently recommended. Although all animals received a more thorough histopathologic workup than in most previous studies, the detailed accounting procedures currently required were not followed. In addition to the bioassay results, it was also anticipated that these studies might furnish information on structure-activity relationships among the test chemicals.

MATERIALS AND METHODS

Chemicals.—The structures of the compounds, their physical properties, and some of their environmental uses are shown in table 1. The chemicals were obtained from commercial suppliers as noted in the table. Samples were analyzed, mainly by NMR or GLC to ascertain the purity. Infrared spectra of all compounds were also recorded.

Mixtures prepared as for the animal diets were stored for 10 days at room temperature to determine the possible degradation of the compound added. Generally, the diet mixture was extracted with a suitable solvent (water, methanol, or acetone), and the recovery was calculated after colorimetric, spectrophotometric, or gas chromatographic determination (table 1).

Animal studies.—Charles River CD male and female rats were procured as weanlings from Charles River Breeding Laboratories, North Wilmington, Mass. The animals, after 7-10 days of quarantine and culling of unsuitable individuals, were used for acute, subchronic, or long-term studies. The animals were housed 2 per large plastic cage (12 inches×14 inches×7 inches), on corncob bedding (San-i-cel; Paxton Processing Co., Inc., Paxton, Ill.) and provided with Wayne Lab Blox meal and water ad libitum. Cages, water bottles, and feed cups were changed two to three times weekly. Animal rooms were air-conditioned and were in a facility constructed on the "clean-dirty" corridor principle. All animals receiving a specific compound were kept in the same animal room, but the position of the cages on the racks and the rack positions within the room were changed monthly. A maximum of 480 animals, including the appropriate controls, were maintained in each room.

Acute toxicity: For the acute studies, a single dose of each compound was given by gavage; if no effects were

ABBREVIATIONS USED: GLC=gas-liquid chromatography; MTD=maximum tolerated dose; NCI=National Cancer Institute; NMR=nuclear magnetic resonance; SSV=steroid suspending vehicle.

[1] Received September 28, 1979; revised August 25, 1980; accepted January 15, 1981.

[2] Supported by Public Health Service contract NIH-NCI-69-2085 from the National Cancer Institute with Litton Bionetics, Inc.

[3] The guidelines for the care and use of laboratory animals were followed as set forth by Litton Bionetics, Inc.

[4] Laboratory of Carcinogen Metabolism, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Bethesda, Md. 20205.

[5] Litton-Bionetics, Inc., Kensington, Md. 20795.

[6] *Present address:* Hazleton Laboratories of America, 9200 Leesburg Turnpike, Vienna, Va. 22180.

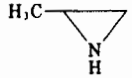
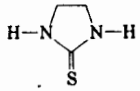
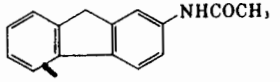
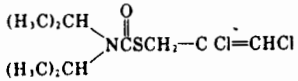
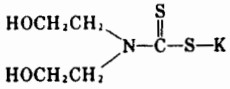
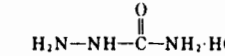
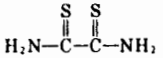
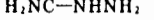
[7] Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Bethesda, Md. 20205.

[8] *Present address:* American Health Foundation, Naylor Dana Institute for Disease Prevention, Valhalla, N.Y. 10595.

[9] The secretarial assistance of Ms. F. M. Williams and the technical assistance of Mr. Robert Cypher are gratefully acknowledged.

9321

TABLE 1.—*Chemical structure and physical properties of test compounds*

Name [synonym]	Structure	Mol wt	Physical constants[a]	Purity, *M* % (method)	Source[b]	Rationale for testing	Required level, ppm[c]	Recovery, %[c]	Stability[c] Days	Stability[c] Loss, %
Propane sultone [3-hydroxy-1-propanesulfonic acid γ-sultone]		122.1	mp: 30–33°C bp: 180°C, 30 mm	91 (NMR)	Aldrich	Suggested as solvent; additive for inks	Ftn *d*	Ftn *d*	Ftn *d*	Ftn *d*
Propylene imine [2-methylaziridine]		57.1	bp: 63–64°C	57 (NMR)	Interchemical	Suggested as adhesive; polymer intermediate	Ftn *d*	Ftn *d*	Ftn *d*	Ftn *d*
Ethylenethiourea [2-imidazolidinethione]		102.2	mp: 197–200°C	90 (NMR)	Aldrich	Decomposition product of various pesticides; rubber-processing chemical	175–350	78.3±4.2	10	2.9
N-2-Fluorenylacetamide		223.3	mp: 193°C	>99	Eastman	Positive control	80–240	—	—	—
Avadex [*S*-2,3-dichloroallyl diisopropylthiocarbamate]		270.2	bp: 150°C, 9 mm	92.5 (NMR) 94.3 (GLC)	Monsanto	Pesticide	150–300	89.3±5.2	13	22.5
Bis(2-chloroethyl) ether	$Cl—CH_2CH_2OCH_2CH_2—Cl$	108.6	bp: 107°C	100 (NMR)	Aldrich	Pesticide; water contaminant	Ftn *d*	Ftn *d*	Ftn *d*	Ftn *d*
Bis(2-hydroxyethyl)dithiocarbamic acid, K salt		219.4		57	Eastman	Pesticide	2,500–5,000	74.6±3.2	7	8.5
Ethylene carbonate [1,3-dioxolane-2-one]		88.1	mp: 35–37°C	99 (NMR)	Aldrich	Solvent	25,000–50,000	94.5±2.7	10	7.8
Semicarbazide hydrochloride [carbamylhydrazine hydrochloride]		111.5	mp: 175–177°C (dec)	96 (NMR)	Aldrich	Laboratory reagent; hydrazine derivative	500–1,000	83.6±3.1	9	None
Dithiooxamide [rubeanic acid]		120.2	mp: 170°C (dec)	97 (NMR)	Aldrich	Analytical reagent; suggested synthetic intermediate	350–700	73.4±6.0	10	None
Glycerol α-monochlorhydrin [3-chloro-1,2-propanediol]	$CH_2OHCHOHCH_2Cl$	110.5	bp: 115–120°C, 15 mm	95	Eastman	Antifertility substance	Ftn *d*	Ftn *d*	Ftn *d*	Ftn *d*
Thiosemicarbazide		91.1	mp: 180–181°C (dec)	80 (NMR)	Aldrich	Reagent; intermediate	100–200	83.1±1.9	7	None
Sodium azide	NaN_3	65.0	700°C (dec)	>99 (NMR)	Fisher	Intermediate; medicinal; air-bag system in automobiles	100–200	83.8±3.8	10	None
Sodium bisulfide [sodium sulfhydrate]	$Na_2S \cdot 9H_2O$	240.2			Fisher	Textile treatment; dye manufacture; chemical intermediate; laboratory reagent	—	—	—	—
Vinylene carbonate [1,3-dioxole-2-one]		86.1	mp: 22°C	98	Aldrich	Solvent	Ftn *d*	Ftn *d*	Ftn *d*	Ftn *d*

[a] bp = boiling point, mp = melting point, dec = decomposes.
[b] Aldrich Chemical Co. (Milwaukee, Wis.); Interchemical Corp. (Carlstadt, N.J.); Eastman Kodak Co. (Rochester, N.Y.); Monsanto Chemical Co. (St. Louis, Mo.); Fisher Scientific Co. (Silver Spring, Md.).
[c] — = no data.
[d] Compound was administered by gavage in SSV; *therefore, dietary stability and recovery studies were not required*

noted, daily doses were administered up to 14 days. Weight gain, food intake, and general toxicologic effects were followed. Gross necropsies were done on animals that died, were moribund, or were killed. The results were used as a guide for doses to be used in the subchronic experiments.

Subchronic toxicity: The subchronic studies were undertaken to delineate the MTD over a longer time period (*2-5*). On the basis of the results of the acute study, five distinct levels of each compound were mixed in the diet, or given twice weekly by stomach tube, to groups of 3 animals each over 30 days, followed by 30 days of observation for detection of delayed toxicity. Weight gain and clinical signs were observed carefully. Necropsies were again performed on all animals in this phase of the study.

Chronic toxicity: In the chronic studies rats were given the compounds continuously in the diet, or by gavage twice weekly, at the MTD and at one-half that level for 18 months, after which the animals were observed for 6 months before termination of the study. This design permitted the detection of irreversible neoplastic effects while allowing for the regression of any reversible toxic effects without the complications of spontaneous lesions in very aged rats. For diet mixing, a small portion of meal was mixed thoroughly in a mortar with the proper amount of the compound; the resultant concentrate was added to more meal and blended for 15-20 minutes in a twin-shell blender. The diet was prepared in 6-kg lots twice weekly and stored in a refrigerator at 4°C until needed. For gavage, solutions (in water) or suspensions of compounds (in SSV[10]) were freshly prepared on the day of administration, except for sodium bisulfide. A solution of sodium bisulfide was prepared monthly in degassed water and distributed into screw-cap containers which were completely filled and contained sufficient solution for 1 day.

In the long-term studies each chemical was given to groups of 26 male and 26 female rats at the experimentally determined MTD and to similar groups at one-half that level—a total of 104 animals. Usually tests on sets of 4-5 chemicals were begun at the same time. Control groups of animals of each sex, which were selected at the same time as those groups assigned to the testing of a set of chemicals, were initiated into the chronic studies. Also, groups of vehicle controls, administered SSV alone, were simultaneously chosen to be given the compounds in this solvent mixture by gavage (supplemental table 1).[11]

Two groups of positive control animals received the carcinogen *N*-2-fluorenylacetamide in the diet at 80 ppm (32 rats) or 250 ppm (20 rats) to check the response of the strain to a known carcinogen at different dose levels. The data on this test have appeared (*7*).

During the first 4 weeks of the long-term study, any test animals that died were replaced by spare animals from the same shipment. However, except for 5 animals in the group given sodium bisulfide, the number of replacement animals never exceeded 1 per group. Animals that died later than 4 weeks were not replaced.

All animals were examined twice daily, 7 days a week, for toxicologic effects or deaths. The presence of palpable masses was noted during such inspections and during weighing, transfer to clean cages, or gavage. All animals were weighed weekly during the initial month of the chronic study and biweekly thereafter. Food consumption was determined during the initial week and the fourth week of each subsequent 4-week period.

Moribund rats and those with large tissue masses were killed during the course of the chronic study. In addition, at the end of 78 weeks of dosing, 5 animals of each sex from the group on the higher dose were killed and necropsied, and stained sections of the tissues were examined microscopically to detect any changes. These animals were used for calculation of tumor incidence but not survival rate. For any compound if an appreciable number of animals in a group had been necropsied because of early deaths, additional animals in that group were not killed at 78 weeks (supplemental tables 2a, 2b, 3).[11]

Pathology.—All rats were necropsied and the tissues examined histopathologically. The tissues were fixed in 10% neutral buffered Formalin, sectioned, and stained with hematoxylin and eosin. Generally, the following tissues were examined routinely: cerebrum, cerebellum, pituitary gland, spinal cord plus vertebrae, lung, heart, mediastinum, thymus, thyroid gland, parathyroid gland, liver, spleen, pancreas, adrenal gland, kidney, urinary bladder, ovary, uterus or testis, accessory sex organ, esophagus, stomach, intestinal tract, and any abnormal tissue or mass.

Reporting.—Pertinent data on this experiment were recorded in an automatic data processing system—the Carcinogenesis Bioassay Data System (*8*). The data elements included descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by NCI guidelines (*3*) and by the International Union Against Cancer (*9*). Data tables were generated for verification of data transcription and for statistical review.

Statistical methods.—Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (*10*), which is commonly called the "life table method." Animals were statistically censored as of the time they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. For statistical analyses of a possible dose-related effect on survival, we

[10] SSV contains sodium chloride, 9 mg; sodium carboxymethylcellulose, 5 mg; Polysorbate 80, 0.004 ml; benzyl alcohol, 0.009 ml; and water to make 1 liter. The original use of this vehicle was described by Segaloff and Coleman (*6*).

[11] *See* NAPS Document No. 03850 for supplemental tables. Order from ASIS/NAPS, Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, N.Y. 10017. Remit in advance $3.00 for microfiche copy or photocopy; foreign orders, add $3.00 for postage and handling. All orders must be prepaid.

used the method of Cox (*11*) for testing 2 groups of equality and Tarone's (*12*) extensions of Cox's methods for testing for a dose-related trend. One-tailed *P*-values were reported for all tests except the departure from linearity test, which was only reported when its two-tailed *P*-value was less than 0.05.

"Natural" mortality (excluding accidentally and deliberately killed animals) and the mean weights of the test groups, in comparison with the natural mortality and mean weights of the corresponding controls, were used to assess the toxicity of the administered doses. High mortality in a time period could result in fluctuations in the mean weight curve; such fluctuations were due to diminishing group size rather than to actual variation in the weights of the individual animals.

The incidence of neoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals necropsied. The "tissue counts" or number of animals for which particular sites were examined histopathologically were not recorded specifically, since at the time of these experiments those parameters were not part of the protocol. For certain organs an appreciable number of animals may not have had particular sites so examined, though the likelihood of this is minimal because of the care exercised in performing necropsy. However, marginal results for such sites may be considered tentative.

The purpose of the statistical analyses of tumor incidence was to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher's exact probability test (*13*) was used to compare the tumor incidence of a matched negative or vehicle control group with that of a group of dosed animals at each dose level. Inasmuch as the results for 2 dosed groups were compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 could be made. The Bonferroni inequality (*14*) requires that the *P*-value for any comparison be less than or equal to $0.05/2=0.025$. Where this correction was used, a discussion of its application is given in the text. The correction does not appear, however, in the tables when Fisher's exact probability *P*-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (*15*), was also used. Under the assumption of a linear trend, this test determined whether the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

Inasmuch as the high dose was originally twice the amount of the low dose, except for *N*-2-fluorenylacetamide, equally spaced doses were used for the linear dose-response test. For those compounds where the initial doses were altered during the study, the weighted mean doses were additionally considered for testing a linear trend in tumor incidence. The weighted mean dose was computed as: sum of (dose × No. of wk dose received)/total No. of wk in dosing period, where the sum is over that of the different doses used. The statistical methods used are more fully described in (*16*); discussion of the statistical issues in the interpretation of chronic tests can be found in (*17*).

The treated groups were always compared to a "matched" negative or vehicle control, as appropriate. The matched controls, except in the testing of two of the compounds (vinylene carbonate and sodium bisulfide with thyroid extract), were started on test within 2 weeks of the start of the corresponding treated groups. Each treated group and its controls, with one exception (the animals in the study on sodium bisulfide with thyroid extract), were always maintained in the same room. The "pooled" controls, where applicable, included a second control group (in addition to the matched control) that was usually started within 3 weeks of the treated groups but kept in a different room. The details of the experimental design are given in table 2.

RESULTS

Controls

The 10 negative male and 10 negative female control groups consisted of 184 animals for each sex. The total tumor incidence and variation of incidence among these groups were used in assessing the importance of the differences noted between the treated groups and the matched or pooled negative controls. There were also 4 additional groups of rats that received the vehicle alone or, in one instance, thyroid extract. The tumor incidence in these groups was similar to that of the negative controls, particularly in the female rats.

The rats used in our studies reached a large size, especially the males. By 51 weeks control males had a mean body weight of 709 g (range of mean body wt, 644-760 g); at 77 wk, 766 g (718-854 g); and at 104 wk, 737 g (635-812 g). The corresponding figures for females were 402 g (357-447), 486 g (441-549), and 541 g (487-629) (supplemental table 1).[11] Survival at 52 weeks was 97% for males and 99% for females; at 78 weeks, 86% for males and 84% for females; at 104 weeks, 53% of the males and 46% of the females were still alive. These survival rates compare well with those reported previously (*18*) (table 3; supplemental table 1).[11]

These animals had an appreciable incidence of spontaneous tumors; in some of the control groups all the animals were so affected. Tumor incidence varied considerably among the different control groups. In males the most common tumor was chromophobe adenoma of the pituitary gland, which had an incidence ranging from 17 to 50%. Adrenal tumors were fairly common, followed in rate of incidence by various other neoplasms of the thyroid gland, brain (gliomas), testis, pancreas (islet cell adenomas), lung, and also leukemia or lymphomas, subcutaneous tumors, and

TABLE 2.—*Experimental design: dosage, survival, and room location of animals*

Compound	Dose					Test animals					Control group No.	
	Original	Route of administration	Solvent vehicle	Change[a] At wk:	Change[a] To:	Total wk on drug	Total wk on study	Survival at 52 wk, % Males	Survival at 52 wk, % Females	Room No.	Matched	Pooled[b]
Propane sultone	28 mg/kg	Gavage	H_2O	—	—	60	60	62	39	44	I	I, II
	56 mg/kg	Gavage	H_2O	32	No dose	32	60	15	23			
Propylene imine	10 mg/kg	Gavage	H_2O	8	12.5 mg/kg	58	60	42	12	44	I	I, II
				20	No dose							
				22	10 mg/kg							
	20 mg/kg	Gavage	H_2O	8	25 mg/kg	27	60	12	8			
				20	No dose							
				22	20 mg/kg							
Ethylenethiourea	175 ppm	Diet	None	—	—	78	104	96	100	43	II	II, III
	350 ppm	Diet	None	—	—	78	104	100	100			
N-2-Fluorenylacetamide	80 ppm	Diet	None	—	—	78	104	100	100	28	VI	V, VI
	250 ppm	Diet	None	—	—	52	52	20	40			
Avadex	150 ppm	Diet	None	—	—	78	104	100	96	27	V	V, VI
	300 ppm	Diet	None	—	—	78	104	100	96			
Bis(2-chloroethyl) ether	25 mg/kg	Gavage	SSV	—	—	78	104	96	96	43	IV	IV, V
	50 mg/kg	Gavage	SSV	—	—	78	104	100	65			
Bis(2-hydroxyethyl)dithiocarbamic acid, K salt	2,500 ppm	Diet	None	—	—	78	104	96	96	28	VI	V, VI
	5,000 ppm	Diet	None	—	—	78	104	96	100			
Ethylene carbonate	25,000 ppm	Diet	None	—	—	78	104	92	100	27	V	V, VI
	50,000 ppm	Diet	None	42	♂: No dose	78	104	50	100			
				44	♂: 40,000 ppm							
Semicarbazide hydrochloride	500 ppm	Diet	None	—	—	78	104	100	100	27	V	V, VI
	1,000 ppm	Diet	None	32	No dose	32	104	100	77			
Dithiooxamide	350 ppm	Diet	None	32	300 ppm	78	104	100	92	44	I	I, II
	700 ppm	Diet	None	32	600 ppm	78	104	100	93			
Glycerol α-monochlorohydrin	30 mg/kg	Gavage	H_2O	10	35 mg/kg	72	104	100	100	31	VII	—
				72	No dose							
	60 mg/kg	Gavage	H_2O	10	70 mg/kg	72	104	100	100			
				72	No dose							
Thiosemicarbazide	37.5 ppm	Diet	None	—	—	78	104	100	100	43	II	II, III
	75 ppm	Diet	None	—	—	78	104	100	100			
Sodium azide	100 ppm	Diet	None	—	—	78	104	96	96	44	I	II, III
	200 ppm	Diet	None	—	—	78	104	89	100			
Sodium bisulfide	9 mg/kg	Gavage	H_2O	57	2–3 doses/wk	78	104	85	100	42	III	III, III
	18 mg/kg	Gavage	H_2O	57	2–3 doses/wk	78	104	62	92			
Sodium bisulfide + thyroid extract	9 mg/kg	Gavage	H_2O	57	2–3 doses/wk	78	104	85	73	42	V	—
	18 mg/kg	Gavage	H_2O	57	2–3 doses/wk	78	104	62	85			
Vinylene carbonate	150 mg/kg	Gavage	SSV	—	—	78	104	96	96	41	X	—
	300 mg/kg	Gavage	SSV	—	—	78	104	81	35			

[a] — = dose remained unchanged.
[b] — = no pooled control.

lipomas (table 3; supplemental table 2a).[11] In females the high incidence of pituitary tumors was followed in rate of incidence by benign mammary tumors (fibroadenoma, adenoma, and fibroma), adrenal tumors, mammary adenocarcinomas, and various other tumors (table 3; supplemental table 2b).[11] Tumors appearing in this type of rat were reported in (*18–20*); the present study substantiates those reports, with minor variations.

Carcinogens

Of all the compounds tested, propane sultone, propylene imine, and ethylenethiourea, in addition to the positive control compound *N*-2-fluorenylacetamide, were definitely carcinogenic under the conditions of our tests.

Propane sultone.—Propane sultone was given by gavage as a solution in water. It caused high mortality, and only 4 males and 6 females survived at 52 weeks at the high-dose level (table 2; supplemental table 3).[11] Because of the numerous mammary tumors developing in the females at the 18th week and the high mortality in males, administration of the high dose was discontinued at the 32d week. At the 45th week animals on the low dose began showing signs of central nervous system disturbances. Therefore, the experiment was terminated at the 60th week.

Clearly, propane sultone was tested at a sufficiently high dose to be toxic, but not enough animals survived

to assess its potential for inducing late-appearing tumors. However, the treated animals of each sex had a highly significant incidence of malignant glioma of both the cerebrum and the cerebellum at each dose level (table 4). The gliomas were astrocytomas and were often detected grossly as pea-sized areas of discoloration in the cerebrum or cerebellum. Detailed histopathologic descriptions of these types of tumors have been reported in (*21-23*). In addition, each dose group of female rats had a significantly elevated incidence of mammary adenocarcinoma. A somewhat elevated incidence of granulocytic leukemia was found in each sex. Incidence of adenocarcinomas of the small intestine was 4-12% in the treated animals of each sex, but was zero in the controls. Two squamous cell carcinomas of the ear duct occurred in the male rats and 2 occurred in the female rats, but none developed in the matched or pooled controls. All of these cancers are rarely found in negative control animals. Thus propane sultone induced significant increases in malignant gliomas and mammary adenocarcinomas, and the incidences of leukemia, cancer of the small intestine, and squamous cell carcinoma of the ear duct were increased over those in controls.

Propylene imine.—The association of mortality with dose was highly significant; the deaths among treated animals were so numerous that the few remaining treated animals were killed at week 60. At the 17th week of the study posterior paralysis appeared in high-dose males and later in high-dose females. This flaccid paralysis, which caused the animals to lie prostrate in the bedding, may have led to the high incidence of foreign-body pneumonia (18 at low and 36 at high dose) from inhalation of litter particles. At 51 weeks the mean weights of the surviving high-dose groups were considerably less than those of the matched controls, even though administration of the high dose was stopped at the 28th week. At 52 weeks only 3 males and 2 females of the high-dose groups survived, as did 3 females and 11 males of the low-dose groups.

Propylene imine was clearly administered at toxic doses; the numbers of treated animals that survived those doses beyond 52 weeks were inadequate for an assessment of the compound's potential for inducing late-appearing tumors. Nevertheless, 81% of the low-dose and 38% of the high-dose females had mammary adenocarcinomas, incidences highly significantly different from those in the matched controls. The inversion of incidence and dose appeared to be due to the higher early mortality in the high-dose group. An increased incidence of granulocytic leukemia was found in both high-dose and low-dose males, whereas none was found in the matched and the pooled negative controls. Only 2 cases of granulocytic leukemia and 1 of myelogenous leukemia occurred among the 184 male negative controls in this experiment. Although not statistically significant, 6 malignant gliomas of the cerebrum were found among the 52 treated males, 2 in the 26 low-dose females, 1 in each of the 2 male matched control groups, 2 in the male pooled controls, and 3 in the female pooled controls. Similarly, 3 males in the high-dose group, 1 in the low-dose group, and 2 females in the low-dose group had squamous cell carcinomas of the ear canal, whereas the matched or pooled control groups had none. There were also 4 adenocarcinomas of the small intestine in the treated males and none among the controls.

Under the conditions of this test, propylene imine induced mammary adenocarcinomas in female rats and granulocytic leukemia in male rats. The compound probably was responsible for the increased incidence of the malignant gliomas of the cerebrum and squamous

TABLE 3.—*Summary of survival and tumor incidence in control rats*

Control group	No. of rats: At start of study	Alive at: 52 wk	Alive at: 104 wk	With tumors	Total No. of tumors	Percent (range) of tumor incidence in: Pituitary gland	Adrenal gland	Thyroid gland	Brain	Lymphoid tissue	Respiratory tissue	Ear duct	Bladder	Liver	Pancreas	Subcutaneous tissue	Testis	Other sites	Fatty tissue[a]
Males																			
Negative	184	178	106	110	138	33(17–50)	7(0–16)	4(0–17)	3	3	2	1	1	1	3	7	3	8	3
Vehicle	58	48	27	23	26	21(19–30)	2(0–6)	2(0–10)	3	4	0	0	0	2	2	3	3	0	2
Total	242	226	133	133	164	30	6	4	3	4	1	0.4	0.4	1	2	6	3	6	3

Control group	No. of rats: At start of study	Alive at: 52 wk	Alive at: 101 wk	With tumors	Total No. of tumors	Percent (range) of tumor incidence in: Pituitary gland	Adrenal gland	Thyroid gland	Mammary tissue: Benign	Mammary tissue: Malignant	Brain	Lymphoid tissue	Respiratory tissue	Ear duct	Subcutaneous tissue	Endometrium[b]	Other sites	Fatty tissue[a]
Females																		
Negative	184	182	86	148	237	58(30–78)	4(0–10)	0	43(19–69)	2	2	3	2	0	4	3	5	0.5
Vehicle	58	56	25	44	72	57(31–80)	9(6–13)	2(0–6)	38(13–60)	3	0	2	2	0	5	2	2	0
Total	242	238	111	192	309	57	5	0.4	42	2	1	3	2	0	4	2	4	0.4

[a] Lipomas.
[b] Polyps.

TABLE 4.—*Statistical analysis of tumor incidence*

Compound	Route of administration	Tumor: Organ site	Tumor: Type and parameters	Males: Control groups: Pooled[a]	Males: Control groups: Matched[b]	Males: Treated groups: Low dose	Males: Treated groups: High dose	Females: Control groups: Pooled[a]	Females: Control groups: Matched[b]	Females: Treated groups: Low dose	Females: Treated groups: High dose
				I, II	I	28 mg/kg[c]	56 mg/kg[d]	I, II	I	28 mg/kg[c]	56 mg/kg[d]
Propane sultone	Gavage (in H_2O)	Cerebrum	Malignant glioma	1/26 (4%)	1/16 (6%)	10/26 (38%)	11/26 (42%)	1/26 (4%)	1/16 (6%)	12/26 (46%)	12/26 (46%)
			P-value[e]			0.002 (0.02)	0.001 (0.012)			0.0004 (0.007)	0.0004 (0.0007)
			Equally spaced doses	0.002	0.02			0.0008	0.013		
			Weighted mean doses	0.0002	0.005			0.0000	0.002		
			Wk to first tumors	76	76	33	25	33	33	33	34
		Cerebellum	Malignant glioma	0/26 (0%)	0/16 (0%)	6/26 (23%)	11/26 (42%)	0/26 (0%)	0/16 (0%)	8/26 (31%)	4/26 (15%)
			P-value[e]			0.01 (0.04)	0.001 (0.002)			0.002 (0.013)	0.06 (0.13)
			Equally spaced doses	0.0001	0.001			0.09[f]	0.24[g]		
			Weighted mean doses	0.0000	0.0006			0.005	0.03		
			Wk to first tumors	—	—	38	35	—	—	46	46
		Mammary gland	Adenocarcinoma	—	—	—	—	0/26 (0%)	0/16 (0%)	6/26 (23%)	13/26 (50%)
			P-value[e]							0.011 (0.04)	0.0000 (0.0004)
			Equally spaced doses					0.0000	0.0002		
			Weighted mean doses					0.0000	0.0001		
			Week to first tumors					—	—	41	29
		Blood	Leukemia	0/26 (0%)	0/16 (0%)	0/26 (0%)	4/26 (15%)	0/26 (0%)	0/16 (0%)	2/26 (8%)	3/26 (12%)
			P-value[e]				0.06 (0.13)			0.25 (0.38)	0.12 (0.23)
			Equally spaced doses	0.011	0.02			0.08	0.15		
			Weighted mean doses	0.011[g]	0.02[g]			0.06	0.12		
			Wk to first tumors	—	—	—	21	—	—	24	17
		Small intestine	Adenocarcinoma	0/26 (0%)	0/16 (0%)	3/26 (12%)	3/26 (12%)	0/26 (0%)	0/16 (0%)	2/26 (8%)	1/26 (4%)
			P-value[e]			0.12 (0.23)	0.12 (0.23)			0.25 (0.38)	0.50 (0.62)
			Equally spaced doses	0.10	0.19			0.37	0.49		
			Weighted mean doses	0.053	0.12			0.26	0.39		
			Wk to first tumors	—	—	60	46	—	—	41	47
				I, II	I	10 mg/kg[h]	20 mg/kg[i]	I, II	I	10 mg/kg[h]	20 mg/kg[i]
Propylene imine	Gavage	Mammary gland	Adenocarcinoma	—	—	—	—	0/26 (0%)	0/16 (0%)	21/26 (81%)	10/26 (38%)
			P-value[e]							0.0000 (0.0000)	0.0003 (0.004)
			Equally spaced doses					0.003[f]	0.06[f]		
			Weighted mean doses					0.0000[f]	0.0000[f]		
			Wk to first tumors					—	—	29	24

[a] Pooled controls were matched with the treated groups by time animals were entered in expt.
[b] Matched controls were matched with the treated groups by time animals were entered in expt and by room in which maintained.
[c] The 28 mg/kg was administered by gavage for 60 wk; weighted mean dose was 28 mg/kg.
[d] The 56 mg/kg was administered by gavage for 32 wk; weighted mean dose was 29.9 mg/kg.
[e] Beneath the incidence of tumors in a control group is the value of linear trend test; beneath the incidence of tumors in a treated group is the *P*-value of Fisher's exact probability test for the comparison of the treated group with the pooled controls and the *P*-value (*in parentheses*) for comparison of the treated group with the matched controls.
[f] The data depart significantly from linear trend ($P<0.01$).
[g] The data depart significantly from linear trend ($P<0.05$).
[h] The original dose, 10 mg/kg, was administered by gavage; dose was changed several times during the 58-wk dosing period. Weighted mean dose was 10.17 mg/kg.
[i] The original dose, 20 mg/kg, was administered by gavage; dose was changed several times during the 27-wk dosing period. Weighted mean dose was 20.74 mg/kg.

TABLE 4 (continued).—*Statistical analysis of tumor incidence*

Compound	Route of administration	Tumor: Organ site	Tumor: Type and parameters	Males: Control groups: Pooled[a]	Males: Control groups: Matched[b]	Males: Treated groups: Low dose	Males: Treated groups: High dose	Females: Control groups: Pooled[a]	Females: Control groups: Matched[b]	Females: Treated groups: Low dose	Females: Treated groups: High dose
				I, II	I	10 mg/kg[h]	20 mg/kg[i]	I, II	I	10 mg/kg[h]	20 mg/kg[i]
Propylene imine	Gavage	Blood	Leukemia	0/26 (0%)	0/16 (0%)	4/26 (15%)	6/26 (23%)	0/26	0/16	0/26	0/26
			P-value[e]			0.06 (0.13)	0.011 (0.04)				
			Equally spaced doses	0.0103	0.35						
			Weighted mean doses	0.011	0.05						
			Wk to first tumors			33	15				
		Cerebrum	Malignant glioma	1/26 (4%)	1/16 (6%)	3/26 (12%)	3/26 (12%)	1/26 (4%)	1/16 (6%)	2/26 (8%)	0/26 (0%)
			P-value[e]			0.30 (0.51)	0.30 (0.51)			0.50 (0.68)	0.50[j] (0.38)[j]
			Wk to first tumors	76	76	51	39	33	33	40	—
				II, III	II	175 ppm	350 ppm	II, III	II	175 ppm	350 ppm
Ethylenethiourea	Diet	Thyroid gland	Follicular carcinoma	0/20 (0%)	0/10 (0%)	2/26 (8%)	15/26 (58%)	0/20 (0%)	0/10 (0%)	2/26(8%)	6/26 (23%)
			P-value[e]	0.0000[g]	0.0000	0.31 (0.52)	0.0000 (0.001)	0.009	0.03	0.31 (0.52)	0.02 (0.12)
			Wk to first tumors	—	—	104	78	—	—	91	68
			Papillary carcinoma	0/20 (0%)	0/10 (0%)	1/26 (4%)	2/26 (8%)	0/20 (0%)	0/10 (0%)	1/26 (4%)	2/26 (8%)
			Wk to first tumors	—	—	104	104	—	—	104	104
				V, VI	VI	80 ppm[k]	250 ppm[l]	V, VI	VI	80 ppm[k]	250 ppm[l]
N-2-Fluorenylacetamide (positive control)	Diet	Liver	Hepatocellular carcinoma	1/64 (2%)	1/32 (3%)	3/16 (19%)	10/10 (100%)	0/64 (0%)	0/32 (0%)	2/16 (13%)	2/10 (20%)
			P-value[e]			0.02 (0.10)	0.0000 (0.0000)			0.04 (0.11)	0.02 (0.052)
			Equally spaced doses	0.0000	0.0000			0.006	0.03		
			Weighted mean doses	0.0000[f]	0.0000[g]			0.006	0.03		
			Wk to first tumors	104	104	77	35	—	—	89	39
		Mammary gland	Adenocarcinoma-carcinoma	—	—	—	—	1/64 (2%)	0/32 (0%)	8/16 (50%)	7/10 (70%)
			P-value[e]							0.0000 (0.0000)	0.0000 (0.0000)
			Equally spaced doses					0.0000	0.0000		
			Weighted mean doses					0.0000[g]	0.0000[g]		
			Wk to first tumors					44	—	70	32

[a] Pooled controls were matched with the treated groups by time animals were entered in expt.
[b] Matched controls were matched with the treated groups by time animals were entered in expt and by room in which maintained.
[e] Beneath the incidence of tumors in a control group is the value of linear trend test; beneath the incidence of tumors in a treated group is the *P*-value of Fisher's exact probability test for the comparison of the treated group with the pooled controls and the *P*-value (*in parentheses*) for comparison of the treated group with the matched controls.
[f] The data depart significantly from linear trend ($P<0.01$).
[g] The data depart significantly from linear trend ($P<0.05$).
[h] The original dose, 10 mg/kg, was administered by gavage; dose was changed several times during the 58-wk dosing period. Weighted mean dose was 10.17 mg/kg.
[i] The original dose, 20 mg/kg, was administered by gavage; dose was changed several times during the 27-wk dosing period. Weighted mean dose was 20.74 mg/kg.
[j] *P*-value is given in the direction of a negative linear trend.
[k] The dosing period was 78 wk.
[l] The dosing period was 52 wk.

TABLE 4 (continued).—*Statistical analysis of tumor incidence*

Compound	Route of administration	Tumor: Organ site	Tumor: Type and parameters	Males: Control groups: Pooled[a]	Males: Control groups: Matched[b]	Males: Treated groups: Low dose	Males: Treated groups: High dose	Females: Control groups: Pooled[a]	Females: Control groups: Matched[b]	Females: Treated groups: Low dose	Females: Treated groups: High dose
								I, II	I	100 ppm	200 ppm
Dithiooxamide	Diet	Mammary gland	Mammary tumor	—	—	—	—	5/26 (19%)	3/16 (19%)	15/26 (58%)	9/27 (33%)
			P-value[c]					0.19[f]	0.35[g]	0.005 (0.014)	0.20 (0.25)
			Wk to first tumors					75	75	42	77
					VII	30 mg/kg[m]	60 mg/kg[n]		VII	30 mg/kg[m]	60 mg/kg[n]
Glycerol α-monochlorohydrin	Gavage	Parathyroid gland	Adenoma		0/20	0/26	3/26 (12%)		0/20 (0%)	0/26 (0%)	0/26 (0%)
			P-value		0.06	—	0.18				
			Wk to first tumors				72				
								II, III	II	37.5 ppm	75 ppm
Thiosemicarbazide	Diet	Mammary gland	Mammary tumors	—	—	—	—	7/20 (35%)	3/10 (30%)	19/26 (73%)	16/26 (62%)
			P-value[c]					0.07[g]	0.17[g]	0.011 (0.02)	0.07 (0.09)
			Wk to first tumors					83	99	71	78
				I, II	I	100 ppm	200 ppm	I, II	I	100 ppm	200 ppm
Sodium azide	Diet	Pituitary gland	Chromophobe adenoma	7/26 (27%)	3/16 (19%)	6/26 (23%)	3/26 (12%)	10/26 (38%)	6/16 (38%)	19/26 (73%)	14/26 (54%)
			P-value[c]	0.12[f]	0.30[f]	0.50[j] (0.53)	0.54[j] (0.26)[j]	0.17[g]	0.29[g]	0.012 (0.03)	0.20 (0.24)
			Wk to first tumors	94	104	84	98	62	62	69	97
		Mammary gland	Mammary tumor	—	—	—	—	5/26 (19%)	3/16 (19%)	17/26 (65%)	10/26 (38%)
			P-value[c]					0.10[f]	0.26[f]	0.0009 (0.004)	0.11 (0.16)
			Wk to first tumors					75	75	69	80

[a] Pooled controls were matched with the treated groups by time animals were entered in expt.
[b] Matched controls were matched with the treated groups by time animals were entered in expt and by room in which maintained.
[c] Beneath the incidence of tumors in a control group is the value of linear trend test; beneath the incidence of tumors in a treated group is the *P*-value of Fisher's exact probability test for the comparison of the treated group with the pooled controls and the *P*-value (*in parentheses*) for comparison of the treated group with the matched controls.
[f] The data depart significantly from linear trend ($P<0.01$).
[g] The data depart significantly from linear trend ($P<0.05$).
[j] *P*-value is given in the direction of a negative linear trend.
[m] The original dose, 30 mg/kg, was administered by gavage for 10 wk and at 35 mg/kg for next 62 wk.
[n] The original dose, 60 mg/kg, was administered by gavage for 10 wk and at 70 mg/kg for next 62 wk.

carcinomas of the ear canal in each sex, as well as for adenocarcinomas of the small intestine in the male rats.

Ethylenethiourea.—Ethylenethiourea was much less toxic than propane sultone or propylene imine. At 78 weeks the survival of test animals was comparable to that of the controls: 89% or higher in each of the 4 treated groups. At 104 weeks the survival of test animals was 60%. We found no association of mortality with dose for the males, but we did find a significantly positive association for the female matched controls ($P=0.03$). Conversely, the mean weights of the high-dose males were less than those of the matched controls during the treatment period, but the mean weights of the treated female groups differed little from the mean weights of the negative controls.

Follicular cell carcinomas of the thyroid gland were found in both the treated male and female rats, i.e., in 58% of the high-dose males and in 23% of the high-dose females. No such tumors were detected in the matched or pooled controls. Only 1 follicular cell and 1 C-cell carcinoma of the thyroid gland occurred among the 184 male negative controls in the experiment, and none was found among the 184 female controls.

Three papillary carcinomas of the thyroid gland also were found among the 52 treated animals of each sex. Diffuse hyperplasia of the thyroid gland occurred among the animals of all the treated groups. At the

lower dose, 2 neoplastic (hyperplastic) nodules occurred in the livers of males and 1 in a female; at the higher dose, 1 such lesion was found in a male. Under the experimental conditions of this study, ethylenethiourea clearly was carcinogenic to the thyroid gland.

N-2-Fluorenylacetamide (positive control compound).—The mortality of the high-dose groups was so great that the male and female survivors were killed at 52 weeks. Although the low-dose groups had somewhat more mortality than did the untreated control groups of each sex, the low-dose groups differed little in mean weight from that in the control groups, and survival at the low dose was good (75% of the males and 88% of the females survived to 78 wk).

Significant numbers of hepatocellular carcinomas, neoplastic (hyperplastic) nodules and foci, or areas of cellular alteration were found in each sex of the treated animals at each dose, as already reported in detail (7). A highly significant increase in mammary carcinomas occurred in the treated female rats at each dose level. Clearly, these rats were susceptible to this known carcinogen (7).

Other Compounds Producing Negative Results

Under the conditions of this experiment, on the basis of the comparison of tumors in controls and experimental animals, five compounds were considered negative. They all appeared, however, to have been tested at a sufficiently high dose in at least one sex, and all were associated with adequate survival in at least one sex, though the sizes of the groups were smaller than currently recommended.

Avadex.—The mean weights of all the treated groups were less than those of their matched controls. However, at least 77% of the animals in each treated group survived to 78 weeks, indicating that Avadex was tested at sufficiently high dose levels to be toxic but with adequate rat survival. No increase in the incidence of tumors was noted in the treated rats of either sex.

Bis(2-chloroethyl) ether.—Although the mortality in male rats was not associated with treatment, we found a significant association of mortality with treatment among the high-dose females, as compared to the dose-mortality association for either the matched or the pooled vehicle control groups. There was a substantial difference between the mean weights of the treated females and the mean weights of the corresponding controls, and a reduction in mean weight in the high-dose males. Except for the high-dose female group, at least 81% of the animals were alive at 78 weeks.

Although there is some question whether the dose levels for males were sufficiently high to provide maximum test sensitivity, under the conditions of this experiment bis(2-chloroethyl) ether was not carcinogenic in male or female rats.

Bis(2-hydroxyethyl)dithiocarbamic acid, K salt.—At least 88% of the animals in each treated group survived to 78 weeks, but all treatment groups had lower mean weights than did the matched controls. The high-dose animals, especially the males, showed a megalocytosis of epithelial cells in the convoluted tubules of the kidney. This was characterized by marked enlargement of the tubular lining cells with a concommitant increase in nuclear size, but we found no specific degenerative lesion of the kidney.

This chemical appears to have been tested at adequately high levels to be toxic but with satisfactory animal survival. Among the low-dose males, 3 had hepatocellular carcinoma and 2 had neoplastic nodules of the liver. No hepatocellular carcinomas were found in the high-dose males, though that group had 2 rats with liver nodules and 1 with benign hepatoma. However, a single rat in the matched controls had both hepatocellular carcinoma and a neoplastic nodule. Thus we found no statistically significant association of treatment with tumor induction in the males. Among the high-dose females, only a single rat had neoplastic nodules of the liver, whereas 2 of the female matched controls had such nodules. Under the conditions of this experiment, the potassium salt of bis(2-hydroxyethyl)dithiocarbamic acid was not found to be carcinogenic in rats.

Ethylene carbonate.—The mortality of the high-dose males was so great that treatment was stopped at week 42 and resumed at a lower dose level at week 44. Despite this, only 3 of the high-dose males survived to 52 weeks, and all died by week 60. While 20 in the low-dose group survived to 52 weeks, only 10 survived to 78 weeks. The survivors in each of the female groups numbered 26 at 52 weeks; at 78 weeks, they numbered 23 for the low-dose group and 20 for the high-dose group. Treatment was significantly associated with the mortality in male rats ($P<0.0001$), but we found no significant association between mortality and treatment for female rats. Both male treatment groups had substantially lower mean weights than did the matched controls, but the treated female groups had mean weights similar to those of the control groups. The treatment caused severe nephrotoxicity in high-dose males, evidenced by diffuse severe chronic nephritis. Strongly birefringent crystals, probably oxalic acid, were found in the convoluted tubules of the kidney, the collecting tubules, and sometimes in the renal pelvis and urinary bladder. Low-dose males were not affected appreciably until the 60th week. The females remained unaffected clinically, even though they received the same dose levels.

Perhaps ethylene carbonate could have been administered at higher dose levels to females, with a possible increase in sensitivity of the test. However, since the compound was already administered at 5% of the diet, physical effects of higher dose levels might have complicated the situation. In males the number of survivors was so small that induction of any late-occurring tumor could have gone undetected. Under these conditions the treated groups did not show any increase in the incidence of tumors over that of the controls. The difference in toxicity and survival between males and females may relate to differences in metabolism as a function of sex, similar to that of the parent compound ethylene glycol (*24*).

Semicarbazide hydrochloride.—The number of early deaths in each of the high-dose groups was so large that the treatment was discontinued at week 32. A trend test for mortality yielded a significant association for males but not for females. Survival was adequate in all the groups (at least 20 animals) at 78 weeks except for the high-dose males; of these only 13 survived to 52 weeks. For each sex the mean weights of the treated animals were less than those of the matched controls. The chemical also induced osteolathyrism which could be diagnosed grossly commencing with the 10th week in all 4 treated groups. Signs of this disorder were rough coat, protrusion of the sternum and bowing of the legs, and stiffness of the joints with bony growths (revealed by cleaning the bones). Histologically, osteoporosis was noted in the long bones. Various histopathologic aspects of osteolathyrism have been reported (*25*), and the subject has been reviewed (*26*).

Thus semicarbazide hydrochloride was tested at sufficiently high dose levels to cause toxic effects in both sexes. Except for the high-dose males, survival was adequate in the treated groups. However, there was no indication of tumor induction from the treatment.

Compounds Producing Ambiguous Results

Three compounds gave ambiguous results but apparently were tested at sufficiently high doses to be toxic and with an adequate number of survivors.

Dithiooxamide.—No association of mortality with treatment was found in either sex; at least 22 animals survived to 78 weeks in each of the 4 treated groups. The mean weights of the high-dose groups during the treatment period were less than those of the matched negative controls for each sex. Dithiooxamide thus appeared to have been administered at a sufficiently high level to show a toxic effect in each sex, and adequate numbers of treated animals survived.

In the low-dose female group 1 rat had a mammary adenocarcinoma and 1 had a papillary carcinoma. We found no trend with dose for all the mammary tumors, inasmuch as the incidence (58%) in the low-dose group exceeded the incidence (33%) in the high-dose group. However, the incidence in the low-dose group differed significantly from the incidence in both the matched negative controls (19%, $P=0.014$) and the pooled negative controls (19%, $P=0.005$).

Among the 10 female negative control groups in this experiment, totaling 184 animals, 4 (or 2%) had mammary carcinomas (supplemental table 2b).[11] It was not unusual to find 2 animals in 26 with the carcinoma (8%). Of 10 groups, 3 had mammary tumor incidences exceeding the 58% found in the low-dose group, and 7 of 10 groups had incidences exceeding the 33% in the high-dose group. The average incidence among the 184 negative controls was 45%. Thus the significant elevation at the low dose appeared to be due to the exceptionally low incidence (19%) in the low-dose negative control groups.

Glycerol α-monochlorohydrin.—The mortality in the male rats was significantly associated with treatment but not significantly in the females. At least 18 animals survived to 78 weeks in each of the male and female groups. The high-dose groups of each sex had mean weights less than those of the matched control groups, but the low-dose groups exhibited no such relative depression. All the male rats at both dose levels showed severe testicular degeneration and atrophy, with concomitant polyarteritis in the testes in almost one-half of these rats. The question whether the testicular degeneration was reversible was not pursued.

Glycerol α-monochlorohydrin apparently was tested at sufficiently high dose levels with adequate rat survival. Three parathyroid adenomas were found in the high-dose males. The trend test for proportions [control (0/20) vs. low-dose (0/26) vs. high dose (3/26)] yielded a marginal result, $P=0.044$. However, the incidence in the high-dose group was not significantly different from that in the control group. We discovered no parathyroid tumors in any of the female groups and only a single parathyroid adenoma among the 184 negative control males. We have no assurance, however, that the parathyroid gland was examined in all the animals. Consequently, the evidence is not conclusive for an indictment of glycerol α-monochlorohydrin as a parathyroid tumorigen.

Thiosemicarbazide.—We found no significant association of mortality with treatment in either sex, as measured by the trend test, but the low-dose females had greater mortality than did the matched negative controls. At least 22 animals of each treated group survived to 78 weeks. The survival in each matched control group was good, but at 78 weeks the survivors numbered only 9 in the male group and 10 in the female group. The high-dose male and female groups had mean weights less than those of either the matched or pooled controls during treatment, but no such difference was noted for either low-dose group. Therefore, thiosemicarbazide apparently was tested at sufficiently high dose levels to show some toxicity. Furthermore, adequate numbers of animals in the treated groups survived.

In the high-dose group, 2 female rats had mammary adenocarcinomas, whereas the low-dose group or the matched or pooled controls had none. However, the difference was not statistically significant (table 4).

In the 10 negative control groups in this experiment, we found 4 (2%) mammary carcinomas among 184 female rats, and our finding 2 (8%) in 26 animals at the high dose was not particularly unusual. In the 184 control animals, the incidence of mammary tumors was 45%, which is less than the rate in either the low- or high-dose groups but considerably above the 30 and 35% found in the matched and pooled negative controls, respectively. One negative control group had an incidence exceeding the 73% in the low-dose group, and 2 other groups had incidences approaching the 62% in the high-dose groups. The increase in tumors of the mammary gland in females given thiosemicarbazide at low doses may be due to the variability in response at this site.

Other Compounds

Experiments with sodium azide, sodium bisulfide, and vinylene carbonate may have had inadequate survival in the treated groups, or the animals may not have received sufficiently high doses of the test chemicals, to determine carcinogenicity.

Sodium azide.—We found no association of treatment with mortality in either sex and little indication of a difference between the mean weights of the treated group and those of the matched controls for each sex. At least 22 animals survived to 78 weeks in each of the 4 treated groups.

Although there were adequate numbers of survivors in the groups, the dose levels administered did not appear to affect the mortality or weights in either sex. We found pituitary chromophobe adenoma in 19 of 26 (73%) female rats in the low-dose group but only 54% in the high-dose and 38% in the control groups (table 4). The low-dose incidence was significantly elevated beyond that for both the matched ($P=0.03$) and pooled ($P=0.012$) control groups. The experiment had 10 female control groups totaling 184 animals; 3 of these groups had pituitary adenoma incidences exceeding 73%, and 5 of the groups had incidences exceeding 54%. The incidence in the total pooled negative control group was 106 of 184, or 58%. Thus the significant increase in the female low-dose group, compared to that in its negative controls, appeared to be due to the exceptionally low incidence (38%) in its negative controls. No increase occurred among the male test groups; the high-dose males had only 12% chromophobe adenomas as compared to 27% in the pooled controls. We found no convincing evidence that sodium azide caused pituitary chromophobe adenomas under the conditions of this experiment.

In female rats, 17 of 26 (65%) of the low-dose group had mammary tumors, one of which was an adenocarcinoma. The high-dose group had 38% mammary tumors, whereas the control groups had 19%. The incidence in low-dose females was significantly higher than that in either the matched ($P=0.004$) or the pooled ($P=0.0009$) negative controls.

Of the 10 historical control groups, 3 groups had incidences of mammary tumors that approached or surpassed 60%, and 6 groups had incidences that surpassed 38%. The total incidence for the pooled negative controls was 83 (45%) of 184 animals. Thus the significant increase in mammary tumors in the low-dose females may have been due to the exceptionally low incidence (19%) in its negative control groups.

The suggestion of an increase in pituitary and mammary tumors in the low-dose females may relate to the variability in response of these tumors. However, it was not clear that sodium azide was administered at high enough levels in this experiment.

Sodium bisulfide.—Sodium bisulfide was administered by itself and with thyroid extract to separate negative and thyroid control groups.

Without thyroid extract: A significant association of mortality with dose occurred for the male groups, as compared to the dose-mortality association for the pooled controls ($P<0.05$). However, the females in the treated groups had significantly lower mortality than did their pooled controls ($P<0.05$). At least 19 animals survived to 78 weeks in all the treated groups except the high-dose male group in which only 13 animals survived. For each sex the mean weights of the treated animals did not differ appreciably from those of their matched controls. Therefore, sodium bisulfide may have been tested at high enough dose levels to be toxic in the male rats but not in the females.

Of the low-dose females, 5 of 26 had adenomatous polyps of the endometrium. The pooled controls had none, and the high-dose females had 1. That all 6 instances of polyps were not found until terminal kill (104 wk) may be due to the better survival rate of the treated female groups as compared to that of the controls. The statistical test comparing the rates in the low-dose and pooled controls among 104-week survivors (5/18 vs. 0/16) yields an exact one-tailed $P=0.031$, a result not significant at the 0.05 level with application of the Bonferroni correction. Note that adenomatous polyps of the endometrium also occurred among animals in the other control groups.

With thyroid extract: No association of mortality with dose was found for the male groups. The treated female groups, however, exhibited a higher mortality, but the trend test did not quite reach significance. The mean weights of both the male high- and low-dose groups were lower than those of the control group given thyroid extract alone near the end of the treatment (77 wk). However, this comparison was not conclusive inasmuch as too few animals survived to 78 weeks. The mean weights of the treated female groups were greater than those of the controls at 77 weeks, but again too few females survived to 78 weeks. At least 16 of 26 from each of the 4 treated groups survived to 52 weeks.

Because of the poor survival in all the groups, including the controls, it was difficult to establish whether sodium bisulfide with 1% thyroid extract was tested at high enough levels in this experiment. The numbers of animals surviving to 78 weeks were also not adequate to test for a possible carcinogenic effect. Further difficulties in the interpretation of this test arose because the so-called "matched controls" on 1% thyroid extract were entered in the experiment more than 3 months after the treated groups and were maintained in a different room.

Vinylene carbonate.—The interpretation of the results was complicated by numerous deaths in the 71st and 72d weeks from heat exhaustion in low- and high-dose males and in low-dose females. These deaths apparently resulted from the need to transfer the animals from one animal facility to another. In the analysis of the survival curves, these deaths were treated as if they were accidental, rather than natural, inasmuch as they do not appear to be solely attributable to the treatment. Under these circumstances the trend in mortality with dose was significantly associated with treatment in both the male ($P=0.002$) and female

($P<0.0001$) rats. In the first year the mean weights of the treated groups of each sex were less than the mean weights of the vehicle controls. The percentage of treated animals surviving at 1 year was better than 80% in all but the high-dose females; their survival rate was only 23%. However, only 6 animals of each of the 4 treated groups survived to 78 weeks.

It appeared that vinylene carbonate was tested at high enough levels to be toxic. However, the survival of the treated animals was so poor, particularly beyond 72 weeks, that the test was not adequate to evaluate its carcinogenic potential over the entire 2-year period. Under these less than adequate conditions, no statistically significant increase in tumors was found in the treated groups. The lack of tumors after 1 year may indicate that vinylene carbonate carries no potent effect.

DISCUSSION

The immediate purpose of the present series of tests was to determine the responses in rats to compounds deemed as causing positive or suspicious responses in mice (*1*). Furthermore, to correlate the results of these tests to eventual possible risks for exposed humans, the materials tested were those that were available commercially. Therefore, levels of the compounds in commercial material were determined, but purification or separation from accompanying substances was not attempted. Although it is possible that the impurities might have influenced the outcome, the purpose of the study was to determine the carcinogenicity of commercial, environmentally disseminated materials. Determination of the effects of each impurity would require a separate research project.

Except for ethylenethiourea, the effects of most compounds in rats did not correspond to those in mice. Thus responses to Avadex, the potassium salt of bis(2-hydroxyethyl)dithiocarbamic acid, and bis(2-chloroethyl) ether were negative in the rats, whereas in mice the administration of these compounds led to an elevation in tumor incidence (*1*). From other studies as well as the present one, it is evident that compounds that affect the liver in mice may not always do so in rats. In addition, although ethylenethiourea led mostly to hepatomas in mice, the thyroid gland was the primary target organ in rats.

Although the compounds selected were mostly of environmental concern, structure-activity correlations were also sought among the compounds. Of particular interest were the effects of the C=S and C=O groups on the activity of the specific chemicals. For this reason the C=S and C=O analogs, such as semicarbazide hydrochloride and thiosemicarbazide; ethylenethiourea, ethylene carbonate, and vinylene carbonate; Avadex and bis(2-hydroxyethyl)dithiocarbamic acid; and bis(2-chloroethyl) ether and glycerol α-monochlorhydrin were tested to obtain some information in this area. However, only ethylenethiourea had appreciable carcinogenicity, affecting the thyroid gland as do other thioureas (*27*). The results of these tests, reported in an early communication (*28*), have been substantiated (*29-31*). In addition, the National Institute for Occupational Safety and Health reviewed ethylenethiourea as an occupational hazard and has recommended control measures (*32*). There is a report that semicarbazide hydrochloride caused lung and blood vessel tumors in mice (*33*), which is in contrast to evidence of the lack of carcinogenicity in rats. The amount of semicarbazide hydrochloride administered to the mice in the drinking water in the test reported in (*33*) was approximately 200 mg all totaled, whereas in our study the rats consumed a total dose of approximately 7-14 g. Although the mice were reported to show a carcinogenic response, no mention, however, was made of osteolathyrism in the mice. Conversely, despite a thirtyfold to seventyfold greater dose, rats showed no carcinogenic effects but were susceptible to the lathyrogenic action of semicarbazide hydrochloride, as reported previously (*34*). The lack of carcinogenicity in rats also is comparable to that of several other hydrazine derivatives (*35*). The underlying biochemical mechanism requires elucidation.

Two other compounds, propane sultone and propylene imine, exhibited appreciable carcinogenicity. The activity of propane sultone in mice after its use in skin painting and in rats after subcutaneous injection was reported previously (*36-38*). The present series of tests was the first to show appreciable activity in various organs after oral administration of the compound (*39*).

The effectiveness of propylene imine in causing mammary adenocarcinoma and small numbers of other types of tumors is not surprising since it is the next higher homolog of ethylene imine. Ethylene imine caused pulmonary tumors and hepatomas in mice after oral administration (*1*), had mutagenic and teratogenic activity, and was included on the original Occupational Safety and Health Administration list of carcinogens. However, ethylene imine has not been tested in rats by the oral route in comparable extent to our study of propylene imine.

From a comparison of the results of the present study with those of other studies, one can draw the following conclusion: Compounds that cause hepatomas in mice may not always be carcinogenic in rats, or if carcinogenic in both species, the compounds may have different primary target sites. The extrapolation of such results to define any risk to humans remains a troublesome problem.

REFERENCES

(*1*) Innes JR, Ulland BM, Valerio MG, et al. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J Natl Cancer Inst 1969; 42:1101-1114.

(*2*) Weisburger JH, Weisburger EK. Tests for chemical carcinogens. Methods Cancer Res 1967; 1:307-398.

(*3*) Sontag JM, Page NP, Saffiotti U. Guidelines for carcinogen bioassay in small rodents. Washington, D.C.: U.S. Govt Print Off, 1976 [DHEW publication No. (NIH)76-801].

(*4*) Weisburger JH. Bioassays and tests for chemical carcinogens. In: Searle CE, ed. Chemical carcinogens. Am Chem Soc Monogr 173. Washington, D.C.: Am Chem Soc, 1976:1-23.

(*5*) Page NP. Concepts of a bioassay program in environmental carcinogenesis. In: Kraybill HF, Mehlman MA, eds. Environmental cancer, advances in modern toxicology. Vol 3. Wash-

ington, D.C.: Hemisphere, 1977:87-171.

(6) SEGALOFF A, COLEMAN RH. Failure to show carcinogenicity of the hydrocarbons 5α- and 5β-androstane. Steroids 1964; 3:215-217.

(7) ULLAND BM, PAGE NP, SQUIRE RA, et al. A carcinogenicity assay of Mirex in Charles River CD rats. J Natl Cancer Inst 1977; 58:133-140.

(8) LINHART MS, COOPER JA, MARTIN RL, et al. Carcinogenesis bioassay data system. Comput Biomed Res 1974; 7:230-248.

(9) BERENBLUM I, ed. Carcinogenicity testing: A report of the panel of carcinogenicity of the Cancer Research Commission of UICC. Vol 2. Geneva: UICC, 1969.

(10) KAPLAN EL, MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-481.

(11) COX DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187-220.

(12) TARONE RE. Tests for trend in life table analysis. Biometrika 1975; 62:679-682.

(13) COX DR. Analysis of binary data. London: Methuen, 1970:48-52.

(14) MILLER RG JR. Simultaneous statistical inference. New York: McGraw-Hill, 1966:6-10.

(15) ARMITAGE P. Statistical methods in medical research. New York: Wiley, 1971:362-365.

(16) THOMAS DG, BRESLOW N, GART JJ. Trend and homogeneity analyses of proportions and life table data. Comput Biomed Res 1977; 10:373-381.

(17) GART JJ, CHU KC, TARONE RE. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. JNCI 1979; 62:957-974.

(18) PREJEAN JD, PECKHAM JC, CASEY AE, et al. Spontaneous tumors in Sprague-Dawley rats and Swiss mice. Cancer Res 1973; 33:2768-2773.

(19) WEISBURGER EK, RUSSFIELD AB, HOMBURGER F, et al. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol 1978; 2:235-356.

(20) SHER SP. Mammary tumors in control rats: Literature tabulation. Toxicol Appl Pharmacol 1972; 22:562-588.

(21) MAWDESLEY-THOMAS LE, NEWMAN AJ. Some observations on spontaneously occurring tumors of the central nervous system of Sprague-Dawley derived rats. J Pathol 1974; 112:107-116.

(22) OYASU R, BATTIFORA HA, CLASEN RA, et al. Induction of cerebral gliomas in rats with dietary lead subacetate and 2-acetylaminofluorene. Cancer Res 1970; 30:1248-1261.

(23) WECHSLER W, KLEIHUES P, MATSUMOTO S, et al. Pathology of experimental neurogenic tumors chemically induced during prenatal and postnatal life. Ann NY Acad Sci 1969; 159:360-408.

(24) MORRIS HJ, NELSON AA, CALVERY HO. Observations on the chronic toxicities of propylene glycol, ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether, and diethylene glycol mono-ethyl-ether. J Pharmacol Exp Ther 1942; 74:266-273.

(25) PONSETI IV, SHEPARD RS. Lesions of the skeleton and other mesodermal tissues in rats fed sweet pea (*Lathyrus odoratus*) seeds. J Bone Joint Surg 1954; 36A:1031-1058.

(26) BARROW MV, SIMPSON CG, MILLER EJ. Lathyrism: A review. Q Rev Biol 1974; 49:101-128.

(27) International Agency for Research on Cancer. Some anti-thyroid and related substances, nitrofurans and industrial chemicals. IARC Monogr Eval Carcinog Risk Chem Man 1974; 7:53-109.

(28) ULLAND BM, WEISBURGER JH, WEISBURGER EK, et al. Thyroid cancer in rats from ethylene thiourea intake. J Natl Cancer Inst 1972; 49:583-584.

(29) GRAHAM SL, HANSEN WH. Effects of short-term administration of ethylenethiourea upon thyroid function of the rat. Bull Environ Contam Toxicol 1972; 7:19-25.

(30) GRAHAM SL, HANSEN WH, DAVIS KJ, et al . Effects of one-year administration of ethylenethiourea upon the thyroid of the rat. J Agric Food Chem 1973; 21:324-329.

(31) GAK J-C, GRAILLOT C, TRUHAUT R. Difference de sensibilité du hamster et du rat vis-a-vis des effects de l'administration a long terme de l'ethylene thiourea. Eur J Toxicol Environ Hyg 1976; 9:303-312.

(32) GREGORY AR. NIOSH special occupational hazard review with control recommendations for ethylene thiourea. [Washington, D.C.: U.S. Govt Print Off, 1978 DHEW Publication No. (NIOSH)79-109].

(33) TOTH B, SHIMIZU H, ERICKSON J. Carbamylhydrazine hydrochloride as a lung and blood vessel tumour inducer in Swiss mice. Eur J Cancer 1975; 11:17-22.

(34) LEVINE CI. Collagen in experimental osteolathyrism. Fed Proc 1963; 22:1386-1388.

(35) International Agency for Research on Cancer. Some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents. IARC Monogr Eval Carcinog Risk Chem Man 1974; 4:159-179.

(36) DRUCKREY H, KRUSE H, PREUSSMANN R, et al. Cancerogene alkylierende Substanzen. IV. 1,3-Propansulton und 1,4-Butansulton. Z Krebsforsch 1970; 75:69-84.

(37) VAN DUUREN BL, MELCHIONNE S, BLAIR R, et al. Carcinogenicity of isosters of epoxides and lactones: Aziridine ethanol, propane sultone, and related compounds. J Natl Cancer Inst 1971; 46:143-149.

(38) DOAK SM, SIMPSON BJ, HUNT PF, et al. The carcinogenic response in mice to the topical application of propane sultone to the skin. Toxicology 1976; 6:139-154.

(39) ULLAND BM, FINKELSTEIN M, WEISBURGER EK, et al. Carcinogenicity of industrial chemicals: Propylene imine and propane sultone. Nature 1971; 230:460-461.