VP-10372

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

ENVIRONMENTAL QUALITY COMMITTEE

February 20, 1985

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U.S. Environmental Protection Agency
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Rockville, MD 20852

Gentlemen:

Health and Safety Data Reporting
ITC 15th Report

On behalf of E. I. du Pont de Nemours and Company (Du Pont), I am pleased to submit the enclosed health and safety studies under the Toxic Substances Control Act (TSCA) Section 8(d), 15 U.S.C. 2607(d) and the regulations published at 47 FR 38780 (1982). The determination of reportability was made after consideration of both the requirements of the reporting rule and interpretive guidance Du Pont received from the Agency. Please note that Du Pont has removed certain information that is clearly irrelevant to the assessment of health and safety from this submission (e.g., internal numerical product and laboratory codes, "cc" distribution lists, etc.).

Should you have any questions regarding this submission, please contact Mr. Mark H. Christman at 302/774-6443.

Very truly yours,

J. H. Todd
Director of Environmental Affairs

JHT:mdm
Enc.

1C

from Hueber

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878214989

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E. I. DUPONT de NEMOURS & COMPANY, INC.

DYE WORKS MEDICAL DEPARTMENT

DEEPWATER, NEW JERSEY

MARCH 15, 1940

PRELIMINARY STUDY OF TOXIC EFFECTS WITH CHLOROPRENE

PRELIMINARY STUDY OF TOXIC EFFECTS WITH CHLOROPRENE

Recently two of the operators working in the CD Building have been unable to work because of extreme shortness of breath and sub-sternal and precordial pain on even slight exertion. On questioning it was learned that this complaint was common among the men working in the CD building, so a study was begun to determine if these symptoms were caused by chemicals handled by this group of men. In this building the only probable toxic materials were Chloroprene and Mono Vinyl Acetylene. The fifteen men working in CD were examined and the accompanying chart indicates that of the fifteen men thirteen were suffering from shortness of breath, sub-sternal and precordial pain on examination; nine said they occasionally became dizzy on exposure to the fumes; four were dizzy very frequently; six had fairly severe headaches which were not present before working in CD; three complained of nervousness; eight of weakness and tiredness fairly marked; seven of palpitation on exertion; six of indigestion; two of nausea and vomiting; two of muscle pains. In three of the men a persistently high pulse was noticed. X-ray's were taken unless they had been taken within the last year, and showed nothing remarkable. Cardiograms were taken on five individuals in the group and showed nothing abnormal. Physical examination was normal in most of the men with the exception of one of those who is unable to work at present. This individual showed a deep sighing res-

piration, was dizzy on standing, had marked muscular pains, and the left ankle jerk was absent. Laboratory studies which had been started by Dr. Rankin, were continued in the present series and the accompanying chart indicates some evidence of early liver damage in nearly all cases. Urine analysis were normal with few exceptions. Blood counts were normal except for occasional shift towards premature cells.

The above train of symptoms suggested that the primary trouble with all of these men was referable to the circulatory system and the Crampton test, as recommended by Dr. Polzer and Dr. Fleming, of the Haskell Laboratory, was started. To date nine men in this group have been checked and of these six show very marked circulatory disturbance. One shows slight disturbance with normal limits; four of these men show sufficient circulatory disturbances that they should not be working on any toxic chemical. The Index in two of these men was so low as to indicate the possibility of complete collapse at any time.

A study of the properties of Chloroprene was carried out by Dr. Von Oettingen several years ago and his conclusions were that circulatory disturbances would probably be the first finding in the men handling Chloroprene. Marked blood pressure drops in the animals were observed and explained on the basis of the marked vasodilation particularly involving the abnormal vessels. Secondary changes were experienced in the liver, kidney, and the heart, particularly, and to a larger extent through all tissues of the body due to the passive chronic congestion of these organs, and

to a less extent the direct toxic effect of the Chloroprene. We found by inhalation, one part in a million of Chloroprene exerted a toxic effect upon laboratory animals, and suggested that methods be devised to determine the concentration in the work room of men handling this material and that frequent checks be made. The very close similarity of these symptoms in laboratory animals, to those which are developing in the group of men exposed to Chloroprene ~~with those from the experimenting laboratories is so close that it~~ leaves no doubt that these workers are suffering from the accumulative effects of Chloroprene which is an exceedingly toxic material. We have noticed that even after being away from work for two or three days the Crampton Index still remains low indicating that these cumulative effects are not easily thrown off by the body. The men who are away from work at present on wage disability, complained of gradual increase in the precordial pain and shortness of breath on exertion over a period of many months until they became completely incapacitated. In the Crampton Index, we have an ideal test to indicate when a man should be removed from exposure to this chemical, as the circulatory changes are the first evidence ~~in this chemical~~ of toxic effects from this chemical.
The present study should be continued to include the men working in the Polymerization Building who get considerable exposure to Chloroprene. It is of felt value that all of this group should have a Crampton test at least once every three weeks. An effort should be made to improve the working conditions by improved ventilation and great care in the handling of this chemical. The men should be

told that it is a toxic chemical and should be handled accordingly. An effort should be made to determine the concentration of CD anywhere in the building, whereever desired. It will be interesting to determine whether Vitamin C, which improves tissue respiration will be of value in treating cases of chronic intoxication, but of course the major problem is to improve working conditions, make frequent examinations possible and If, in spite of all improvements toxic symptoms still exist, very frequent checks will have to be made to determine when a man should be removed from this operation.

Submitted by
W. D. Norwood, M.D.

8782149 89

MEN WORKING IN CHLOROPRENE C.D. BUILDING — ROUTINE EXAMINATIONS

CLOCK NUMBER	NAME	AGE	TIME IN COMPANY	SHORTNESS OF BREATH ON EXERTION	SUB-STERNAL OR PRECORDIAL PAIN OR TIGHTNESS ON EXERTION	DIZZY ON EXPOSURE TO FUMES	DIZZY FREQUENTLY	HEADACHE	NERVOUSNESS	WEAKNESS-TIREDNESS	PALPITATION ON EXERTION	INDIGESTION	NAUSEA & VOMITING	MUSCLE PAINS
#301	W.B.Smith	49	3 mo.	N	N	N	N	N	N	N	N	N	N	N
#307	C.A.Ingeman	38	5 yrs.	+	+	N	N	N	N	N	N	N	N	N
#350	H.A.Peterson	32	5 yrs.	++	+	N	N	N	N	N	N	N	N	N
Sal.	R.D.St.George	50	6 yrs. Oper.	+	+	+	N	N	N	N	N	N	N	N
#381	O. Lamont	32	2 yrs.	+++	++	++	++	+	+	++	+	+	N	N
#310	B.D.Brooks	27	5 yrs.	+	+	+	+	+	N	+	+	+	N	N
#308	A.C.Niblock	26	2 yrs.	+	+	+	+	+	N	+	+	+	N	N
#337	H.Stevenson	25	2 yrs.	+	+	+	+	+	N	+	+	+	N	N
#335	H.A.Waterman	33	2 yrs.	+	+	+	+	+	N	+	+	+	N	N
#303	H.Taylor	34	6 yrs.	+	+	+	+	+	N	+	+	+	N	N
#318	Foreman R.O.McCurdy	44	6 yrs. Oper.	N	N	+	+	+	N	+	+	+	N	N
#343	J.Sullivan	42	2 yrs.	++	+	+	+	+	N	+	+	+	N	N
#351	H. Stokely	2	2 yrs.	+	+	+	+	+	N	+	+	+	N	N
#356	J. Byrnes	26	3 mo.	+	+	+	+	+	N	+	+	+	N	N
#353	E.Crispin	1	1 yr.	+	+	+	+	+	N	+	+	+	N	N
15	[illegible]													

Unable to work
Just able to work
Moderate ill

(Had similar attack 11-26-38)

taken off job for time

Worked part time

(Fr)

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CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) • OTS Ø2Ø6752	1	No. of Pages	2				
Doc I.D. 878214990	3	Old Doc I.D. 8DS	4				
Case No.(s) OTS 84ØØ3A II			5				
Date Produced (6) Ø52841	6	Date Rec'd (6) Ø22285	7	Conf. Code • N	8		

Check One: ☐ Publication ☐ Internally Generated ☑ Externally Generated

Pub/Journal Name	9						
	9						
Author(s)	10						
Organ. Name E I DUPONT DENEMOURS & CO INC	11						
Dept/Div	12						
P.O. Box — 13	Street No./Name — 14						
City WILMINGTON 15	State DE 16	Zip 19898 17	Country — 18				
MID No. (7) — 19	D & B NO. (11) — 20						
Contractor — 21							
Doc Type • R.I • U.P • H.E.A.S.D 8D., S.U H.S E.N 22							
Doc Title 23							

TOXICITY OF MONOVINYL ACETYLENE, CHLOROBUTADIENE AND PHOSPHINE

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

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3/8/85 V.S.

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Medical Research Project No. MR-17

The Toxicity of Monovinyl Acetylene, Chlorobutadiene and Phosphine

Studies made on animals exposed to relatively low concentrations of the vapors of chlorobutadiene show that concentrations above 50 parts per million, and especially above 100 parts per million, can produce abnormalities of circulation of the type found by the Dye Works Medical Staff in men exposed to chlorobutadiene in the manufacture of "Neoprene".

On the basis of these studies, which are briefly reported below, it is recommended that air analysis be made where men are exposed to chloro-butadiene, and that equipment be provided to prevent the concentrations from rising above 50 parts per million. A concentration not higher than 30 parts per million is desirable. Further, it is recommended that these men be examined at frequent intervals (periods of two weeks to three weeks), by a simple form of examination, which includes the inquiry as to

a few symptoms shown since the last examination, and measurements of blood pressure and pulse rate. It is recommended that any man showing marked abnormality of circulation at this exposure be examined again within a few days, and if the abnormality is still present, arrangements be made either to reduce his exposure by improved conditions at the site of his work, or to give him a period out of exposure so that he may recover from functional disturbance and so avoid the production of definite tissue damage.

HASKELL LABORATORY OF
INDUSTRIAL TOXICOLOGY

John H. Foulger, M. D.
Director

JHF:ass
5/28/41

The Toxicity of Monovinyl Acetylene, Chlorobutadiene and Phosphine

Toxicity of Chlorobutadiene

Experimental and clinical studies made during the last three and one-half years in the Haskell Laboratory have shown that the earliest detectable effects of exposure to concentrations of toxic vapors, gases, or fumes, which might eventually cause serious disturbance of health, are a simple list of complaints made by workers and significant trends in the level of the various blood pressure factors.

The symptoms usually met are headache, ease of fatigue, gastric disturbance (nausea, loss of appetite, frequent belching, distention of the stomach, pain in the epigastrium), dizziness, respiratory distress on exertion, pain around the heart, palpitation, and tingling or pain in the arms. The changes in blood pressure depend upon the atmospheric concentration of the toxic chemical, and are, in general:

For low concentrations:

1. A decrease in pulse pressure (the difference between systolic and diastolic blood pressure);
2. An increase in diastolic pressure.

For high concentrations:

1. Decrease in systolic pressure;
2. Decrease in diastolic pressure;
3. Decrease in pulse pressure.

At the same time, there may be very variable pulse rates.

The blood pressure changes are measurable, and methods have been devised for scoring blood pressure readings so that their trend to and from definitely abnormal levels can be followed.

Routine examination, by the Dye Works Medical Staff, of men exposed to chlorobutadiene and monovinyl acetylene in the manufacture of "Neoprene" showed symptoms and signs of circulatory disturbance. A detailed survey was made of this group by Dr. Norwood The report, entitled: "Clinical Evaluation of Health of Neoprene Workers" was submitted on 8/16/40. In

brief, the significant facts in this report were as follows:

The greatest number of complaints was found in workers with chlorobutadiene, and the least in those working with monovinyl acetylene. In decreasing frequency of occurrence, the complaints were:

Nervousness

Fatigue

Indigestion (heartburn, gas on stomach, or gastric fullness)

Dizziness

Palpitation

Headache

Nausea

Epigastric pain

Precordial pain

Constipation or Diarrhea

The most marked signs were circulatory abnormalities (slow or rapid pulse, moderately elevated or low blood pressure and pulse pressure). There was also frequent abnormality in the response of the circulation to change from a lying to a standing position. These

circulatory abnormalities were most frequent in the men exposed to chlorobutadiene, but occurred also in men exposed to monovinyl acetylene. In both groups, the incidence of these changes was much higher than normal reaching up to about fifty per-cent or more of the workers exposed.

The observations included in Dr. Norwood's report suggested that chlorobutadiene or monovinyl acetylene, or both, behaved in low atmospheric concentrations in the same manner as do many other volatile compounds. The changes observed indicated a functional derangement of the circulation, and not actual, permanent damage to organs of the body.

The atmospheric concentrations to which the men were exposed are not definitely known. Exploratory experiments with a new optical apparatus gave values of 1 to 900 parts per million of chlorobutadiene. Air samples made later, and determined by a chemical method (described below), gave values of 14 to 29 parts per million around the GR reactor, the base of the stripper, and the crude column circulating pump. However, no really sound estimate of the concentration to which the men may be exposed has yet been made.

The problem for this laboratory appeared to be:

(a) To show that chlorobutadiene or monovinyl acetylene, or both, could cause the symptoms and signs of circulatory abnormality observed in the workers, and

(b) To discover an atmospheric concentration which would be without effect, so that a yardstick might be set up for deciding upon the degree of ventilation needed in the operating plant.

The present report deals with studies of the effect of inhaling chlorobutadiene. Experience has shown that information of the required type can be gained by two concurrent experiments:

1. The study of blood pressure and pulse rate, basal metabolism and other functions in dogs exposed to known concentrations of the toxic vapors; and
2. The following of weight changes in guinea pigs exposed to the vapors. This second study is carried out because guinea pigs

appear very sensitive to many toxic vapors, and also, because one can study in them the influence of vitamin "C" metabolism. This is of value because vitamin "C" metabolism appears to be extremely important in protection against certain toxic compounds.

The general mode of procedure, and the results obtained are as follows:

Studies of Circulation, Etc. In Dogs Exposed to Chlorobutadiene

Five male dogs were exposed daily for about six hours a day, on five days of each week, in a gas chamber of about 10 cubic meters capacity. The total number of exposures was between 40 and 50. No exposures were made on Saturdays or Sundays. The concentrations of chlorobutadiene were kept as near as possible to 100 parts per million in the first thirty days of the experiment. They actually varied during this period between 30 and 240 parts per million. This variability was due to the property of polymerization, which chlorobutadiene shows and which interfered with proper

evaporation of known quantities of the material. In the later part of the experiment, the concentrations reached as high as 230 parts per million.

Before the exposure period started, the animals were studied for approximately thirty days in order to establish the normal trend for blood pressure, pulse rate and other factors.

Each day, before and after exposure, measurements were made of pulse rate and blood pressure. From time to time, basal metabolism was measured, blood counts taken, and venous oxygen concentration determined. Electrocardiograms and heart sound tracings were also made. The general results are listed below under the effects of different levels of atmospheric concentration of chlorobutadiene, as far as they could be found by analysis of the whole record:

1. Concentrations below 50 parts per million made no significant changes in either pulse pressure or diastolic blood pressure. The pulse pressure changed within normal limits, sometimes rising; sometimes falling. The diastolic blood pressure tended to rise slightly, but always within normal limits.

2. Concentrations from 50 to 100 parts per million produced a progressive deterioration of circulation in the course of the weekly five-days of exposure. During the non-exposure period, between Saturday and Sunday, the animals appeared to recover. The actual changes consisted of, in general, an increase in both diastolic blood pressure and pulse pressure. Both usually remained within normal limits, but taken together, tended to force the circulation as a whole to abnormal levels.

3. Concentrations of chlorobutadiene above 100 parts per million and less than 150 parts per million produced quite definite increases in diastolic blood pressure up to abnormal levels. The pulse pressure changes were not significant, but the circulation, as a whole, tended to become abnormal.

4. Concentrations above 200 parts per million: Animals not previously exposed to chlorobutadiene could stand one or two six-hour-exposures at concentrations as high as 200 to 220 parts per million without suffering any serious abnormality,

but animals previously exposed to lower concentrations of the order of 50 to 100 parts per million were often so seriously affected by concentrations of 200 parts per million that they suffered circulatory collapse. In one case, a single such exposure caused death in the inhalation chamber. Autopsy of the dead animal showed nothing other than dilatation of all of the blood vessels throughout the body, and all signs of circulatory failure and collapse.

During this experiment, there were no significant changes in pulse rate, basal metabolic rate, blood count or urinalysis. The electrocardiograms, during exposures at concentrations higher than 100 parts per million tended frequently to show a low voltage T wave, or even an isoelectric or dibasic T wave, indications of an acute anoxia of the heart muscle.

Studies on Guinea Pigs Exposed to Chlorobutadiene

Two series, each containing five guinea pigs, were studied; and for each series, there was a control of five guinea pigs of about the same weights. Group A was given the standard diet, plus ample vitamin "C". Group B was given the standard diet plus minimal vitamin "C" in the form of greens. The two groups of animals were exposed at the same time as the dogs, in the same chamber. Neither group of animals grew quite as well as did the corresponding controls, but the growth curves appeared smooth so long as the concentration of chlorobutadiene remained below 100 parts per million. When the concentrations reached values higher than 100 parts per million, there was an almost immediate diminution in the growth rate, producing deflections in the smooth growth curve. This indicates that concentrations of about 100 parts per million may be quite definitely toxic.

Summary

The study briefly reported above, based upon a large number of measurements of blood pressure, pulse rate, basal metabolic rate, et cetera, on dogs, and the following of weight changes in guinea pigs, would indicate that concentrations of chlorobutadiene in the air, when below 50 parts per million, would probably not have any serious effects upon the worker. When above 50 parts per million, there may occur circulatory abnormality, which will be more pronounced if the concentrations reach the level of 100 parts per million or more. Concentrations of 100 parts per million or more, superimposed upon continued exposure to much lower concentrations may produce serious circulatory abnormality, and even lead to collapse.

While this experiment was essentially an acute experiment, it has been our general experience with other compounds producing this same type of circulatory abnormality, that the longer a man is exposed to concentrations of toxic chemical capable of producing circulatory abnormality, the less rapidly does he

recover when removed from exposure. This is indicated to some extent by the trend of the results of our animal experiments.

JHF:esg
5/28/41

Determination of Chloroprene in Air

W. R. Halpin

Since there were no methods available for the determination of chloroprene in air, several standard analytical procedures were tried with varying degrees of success.

(1) The compound was burned in air in a combustion tube and the amount of chloride was determined by the Volhard method. Variable results were obtained, probably due to incomplete combustion of the chloroprene. Oxygen was not used in the preliminary experiments with the combustion method because it would be impractical to use in analyzing samples directly from the exposure chamber.

(2) Bromination with pyridine sulfate dibromide reagent (J. Biol. Chem. 94, 401 (1931)) and the estimation of bromine adding to the double bonds gave inconsistent results.

(3) Finally, the most satisfactory results were obtained by brominating the chloroprene in chloroform with bromine obtained from the reaction of

potassium bromate, potassium bromide and dilute hydrochloric acid. The excess bromine was determined by adding potassium iodide and titrating the free iodine with sodium thiosulfate, with starch as an indicator.

Reagents:

Chloroform Merck U.S.P.
0.05N Potassium bromate
Potassium bromide crystals
10% Hydrochloric acid
10% Potassium iodide
0.01N Sodium thiosulfate
1% Soluble starch (Merck)

Procedure:

To a 250 cc. Erlenmeyer flask with a ground glass stopper is added by burette, 30 cc. of the unknown chloroform solution of chloroprene. Ten cubic centimeters of 0.05N potassium bromate is added accurately by burette, plus a few crystals of potassium bromide and 4 cc. of 10% hydrochloric acid. The ground glass stopper, coated with lubriseal, is immediately fitted tightly to the flask and, holding tightly stoppered, the flask is shaken

vigorously for exactly fifteen minutes. The stopper is then carefully removed, 5 cc. of 10% potassium iodide added by pipette plus a little distilled water; 1.0 cc. of freshly made 1% starch solution is added and the mixture titrated rapidly with 0.01N sodium thiosulfate which has been accurately standardized by the iodate method. A blank of 30 cc. of chloroform is titrated similarly.

Calculations:

Under these conditions, analyses of standard solutions indicate that each molecule of chloroprene takes up two atoms of bromine.

(Blank titration - Unknown titration) x Normality of thiosulfate x $\frac{\text{Mol. Wt. of Chloroprene}}{2}$ = Mg. Chloroprene in Unknown

$\frac{\text{Mg. Chloroprene}}{\text{Liters of air}}$ = Mg. Chloroprene per liter

Mg. Chloroprene per liter x 276.5 = PPM by volume

Standard Solutions

Standard solutions of various concentrations of chloroprene in chloroform were prepared by adding an exact amount of chloroprene with a micro-burette to a volumetric flask and diluting to the mark with chloroform. From this solution several dilutions of various concentrations were accurately prepared.

11-6-40

Sample	Mg. Chloroprene Added	Mg. Recovered	% Recovery
1	7.19	8.15	113
2	8.62	9.23	107
3	10.06	11.38	113
4	11.50	12.36	107
5	12.94	13.52	104
6	14.37	15.26	106
11-12-40			
1	7.19	7.07	98
2	8.62	8.93	103
3	10.06	9.55	95
4	11.50	10.43	91
5	12.94	13.71	106

87 8214990

Method of Sampling

The air to be tested was drawn through three absorbers in series, each containing 15 cc. of chloroform, and all immersed in an ice bath. The rate of sampling was not greater than one liter per minute. For concentrations up to 200 parts per million, the volume of the sample taken was 20 liters. With higher concentrations, smaller samples were taken.

WRH:asg
5/28/41

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) •	OTS Ø2Ø6752	1	No. of Pages		2		
Doc I.D.	878214991	3	Old Doc I.D.	8DS	4		
Case No.(s)	OTS 84ØØ3A II				5		
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
111874		Ø22285		N			

Check One: ☐ Publication ☐ Internally Generated ☑ Externally Generated

Pub/Journal Name	9
	9
Author(s)	10
Organ. Name: E I DUPONT DENEMOURS & CO INC	11
Dept/Div	12

P.O. Box		13	Street No./Name		14				
City	WILMIMINGTON	15	State	DE	16	Zip	19898	17	Country	18
MID No. (7)		19	D & B NO. (11)		20				
Contractor		21							

Doc Type	22
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Doc Title	23
ACUTE INHALATION TEST	

Chemical Name (300 per name)	25	CAS No. (10)	24
2- CHLORO-1-3-BUTADIENE		126-99-8	

1A

3/8/85 LS.

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. MR NO. ___

Material Tested	Haskell Nos.	Other Codes	Submitted by
1) Butadiene, 2,3-dichloro-	1) 8798		
2) 1,3 Butadiene, 2-chloro	2) 8849		

ACUTE INHALATION TEST

Procedure: The test material was metered, using a syringe infusion pump, into a ½-inch stainless steel delivery tube. The tube was heated to 40°C for H-8798 and was maintained at room temperature for H-8849. Nitrogen was also metered into the delivery tube to carry the test material to the 20-liter glass exposure chamber. At the port of entry into the chamber, oxygen was added in order to produce a 20% oxygen atmosphere.

Six male albino ChR-CD rats with initial body weights of 248-269 grams were exposed to atmosphere containing the test material for a single four-hour period. All surviving animals were weighed and observed daily for 14 days post-exposure. Selected animals were sacrificed at one, two, seven and fourteen days post-exposure for histopathological examination.

The chamber atmosphere was analyzed quantitatively for the test material. Known volumes of the atmosphere were drawn through two midget impingers in series, each containing 15 milliliters of methanol. The resulting solutions were analyzed directly by gas chromatography. Time-weighted concentrations were calculated.

Results:

Haskell No.	Concentration (mg/liter)	Mortality	Clinical Signs
8798	1.02	0/6	None during exposure. Post exposure: severe initial weight losses and rapid respiration. Deaths occurred from one to nine days. Delayed deaths were preceded by weight gains at three to four days and then continuous losses.
	1.13*	-	
	1.15	1/6	
	1.71	1/6	
	1.84	1/6	
	1.98	3/6	
	3.05	6/6	

Median Lethal Concentration**: 2.08 mg/liter with 95% Confidence Limits of 1.79 and 2.68

* This group was used for serial sacrifice.
** Statistical analysis: Finney, D. J., Probit Analysis (1964).

000002

8798491 (3)

Results: (Continued)

Haskell No.	Concentration (mg/liter)	Mortality	Clinical Signs
8849	1.95	0/6	Labored respiration and pallor at all concentrations above 1.95 mg/liter. Severe initial weight losses. Deaths from two to nine days post-exposure. Delayed deaths were preceded by weight gains at two to four days and then continuous losses.
	6.24	0/6	
	8.42	1/6	
	13.04	2/6	
	13.30	2/6	
	19.58*	-	

Approximate Lethal Concentration: 8.42 mg/liter.

Histopathology:

H-8798 (Pathology Report No. 79-74): Both rats that died one day post-exposure at 3.05 mg/liter demonstrated necrosis of: bronchiolar epithelium, centrilobular to midzonal hepatocytes, renal tubular epithelium and germinal cells of the testes; pulmonary edema and hemorrhage were also observed.

Rats exposed to 1.13 mg/liter showed basically similar lesions as those described above at one day post-exposure. A reparative process was observed in rats sacrificed at two, seven and fourteen days.

H-8849 (Pathology Report No. 80-74): One rat that died two days post exposure demonstrated hemorrhagic infarctions in the lungs and centrilobular and midzonal necrosis in the liver. Rats that were sacrificed at one and two days showed, in addition to the above, desquamation and degeneration of the bronchial and bronchiolar epithelium and pulmonary edema. The tissue changes in the lungs and liver seen at seven and fourteen days were due to regeneration and healing processes of acutely damaged tissue.

Summary:

H-8798: The median lethal concentration (LC_{50}) for young adult male rats exposed to 2,3-Dichlorobutadiene for four hours was 2.03 mg/liter with 95% Confidence Limits of 1.79 and 2.68. This compound caused no obvious clinical signs during exposure, but severe weight losses and rapid respiration were observed during the early recovery period. Deaths, both early and delayed, were preceded only by severe weight losses.

Histopathologically this compound produced necrosis in the bronchiolar epithelium, liver, kidney and testes. The survivors demonstrated partial recovery from these injuries at the end of the 14-day recovery period.

2,3-Dichlorobutadiene should be considered moderately toxic by inhalation exposure.

000003

Summary: (Continued)

H-8849: The Approximate Lethal Concentration (ALC) for young adult male rats exposed to 2-chloro-1,3-butadiene for four hours was 8.42 mg/liter. On the basis of the available data, a median lethal concentration could not be determined for this compound. Labored respiration and pallor were observed during exposures and severe weight losses and deaths, often delayed, were observed in concentrations of 8.42 mg/liter and above.

Histopathologically this compound caused severe damage in the lungs and liver which was partially healed by 14 days post-exposure.

2-chloro-1,3-butadiene is classified as a slightly toxic compound by inhalation exposure.

Report by: Constance E. Barras
Constance E. Barras
Inhalation Toxicology Section

Approved by: Carl S. Hornberger
Carl S. Hornberger
Acting Chief
Inhalation Toxicology Section

CEB:dhg
Date Issued: November 18, 1974

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Microfiche No. (7) •	OTS Ø2Ø6752	1	No. of Pages		2		
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Doc Type	22
• RI • U.P • HEASD 8D . S.U H.S F.N	
Doc Title	23
CLASS B POISON TEST BY SKIN ABSORPTION IN RABBITS	

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

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E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. ___ MR NO. ___

Material Tested: 1,3-Butadiene, 2-chloro[1] Haskell No.:

Material Submitted by: Other Codes:

CLASS B POISON TEST BY SKIN ABSORPTION IN RABBITS[2]

Procedure: The sample, as received, was applied to the clipped trunk of six male albino rabbits at a single dose level of 200 mg/kg of body weight. The rabbits were restrained in stocks, and their trunks were wrapped loosely with aluminum foil and rubber dam for the 24-hour exposure period. After the exposure, the wrappings were removed and the skin washed with water. The animals were observed for 48 hours.

Results:

Dose (mg/kg)	Average Dose (ml)	Average Body Weight (g) Initial	Final	Mortality Ratio	Clinical Signs
200	0.67	3231	3078	0/6	1 Day - Mild to moderate skin erythema with edema 2 Days - Generally mild to moderate skin erythema

Summary: 2-Chloro-1,3-butadiene does not classify as a Class B poison via the skin absorption route when tested according to the ICC Regulations as no deaths occurred when applied at a level of 200 mg/kg of body weight. However, under these semi-occluded conditions, it would be considered a moderate primary irritant and care should be taken to avoid skin contact.

1) Synonym - Chloroprene "C.D."
Impurities: 3,4 Dichlorobutene-1 1.4%; 1,4 dichlorobutene-2 0.1%; plus stabilizers and inhibitors

2) As defined in the Interstate Commerce Commission Regulations, Agent T. C. George's Tariff No. 19, August 5, 1966, page 109, section 73.343.

Report by: Carol W. Colburn
Carol W. Colburn

Approved by: Gordon J. Stopps
Gordon J. Stopps
Assistant Director

CWC:dhg
Date: May 4, 1970

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Doc Title	CLASS B POISON TEST	23					

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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Copies to: P. R. Johnson (6)

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. MR NO.

Material Tested: 1,3-Butadiene, 2-chloro- Haskell No.: ----

Material Submitted by: Other Codes:

CLASS B POISON TEST*

Procedure: The test material, as a solution in corn oil, was administered by intragastric intubation to ten young adult ChR-CD male rats, 249-252 grams in body weight, at a single dose level of 50 mg/kg. Survivors were sacrificed 48 hours later.

Results:

Solution %	Dose mg/kg	Initial Average Body Weight (g)	Average Body Weight at 48 Hours (g)	Mortality Ratio (48 Hours)
1.0	50	250	255	0/10

Summary: 1,3-Butadiene, 2-chloro- does not classify as a Class B Poison when tested via the oral route.

* As defined in Department of Transportation Regulations, Agent T. C. George's Tariff No. 19, November 29, 1968, Page 108, Section 173.343.

Report by: Joan A. Dimeler
Joan A. Dimeler
Oral Toxicology Section

Approved by: Gordon J. Stopps
Gordon J. Stopps
Assistant Director

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JAD/jch
Date: May 13, 1970

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CLASS B POISON TEST	

Chemical Name (300 per name)	25	CAS No. (10)	24
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Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. _____ MR NO. ____

Material Tested: Chloroprene* Haskell No

Material Submitted by: Other Codes: None

CLASS B POISON TEST‡

Procedure: Ten ChR-CD male rats, weighing 248 to 279 grams, were exposed to the test material in a 16-liter bell jar for one hour. The test material was placed in a three-necked flask; nitrogen was passed into the flask through a glass tube. Oxygen, introduced into a side-arm delivery tube, carried the test material into the bell jar. The nominal concentration was calculated on the basis of gas flow and weight of material used. The rats were held 48 hours post-exposure for observation. The nitrogen/oxygen ratio was nominally 4/1 on the basis of relative flow rates.

Results:

Nominal Concentration (mg/L)	Mortality	Clinical Signs: During Exposure	Clinical Signs: Post-Exposure
57.5	0/10	Irregular respiration, red ears, mild lacrimation, and incoordination of front legs	Initial weight loss followed by normal weight gain

Summary: Chloroprene is not classified as a Class B Poison by inhalation administration‡.

* 1,3-butadiene, 2-chloro-.

‡ As defined in the Hazardous Materials Regulations of the Department of Transportation, Agent T. C. George's Tarriff No. 23, September 3, 1969, Subpart G, Section 73.343.

Report by: Susan J. Schlesinger
Dorothy A. Snee

Approved by: John A. Jaffe

SJS:DAS/jch
Date: May 20, 1970

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OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Chemical Name (300 per name)	25	CAS No. (10)	24
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N/A

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Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. _____ MR NO.

Material Tested: 1,3-Butadiene, 2-chloro Haskell No.

Material Submitted by: Other Codes: Chloroprene

CLASS B POISON TEST‡

Purpose: Previously, chloroprene was found not to be a Class B Poison by Haskell Laboratory. This result is not in agreement with the data from literature.‡ Therefore, another Class B Poison Test was conducted, using the same grade material as before.

Procedure: Ten ChR-CD male rats, weighing 250 to 280 grams, were exposed to the test material in a 16-liter bell jar for one hour. A weighed sample of the test compound was put in a midget impinger through which 2.4 L/min. N_2 was passed to carry the vapor into the exposure chamber. Oxygen, to give 20% O_2 (v/v) in the chamber atmosphere, was added to the stream prior to entering the exposure chamber. The oxygen concentration was measured periodically during the exposure. The nominal concentration was calculated on the basis of gas flow and weight of material used. The chamber atmosphere was also analyzed for chloroprene by a gas chromatographic method. The rats were held 14 days post-exposure for observation, 12 days longer than required by the standard Class B Poison protocol.

Results:

Nominal Concentration (mg/L)	Analytical Concentration (mg/L)	Mortality: During Exposure	Mortality: 48 Hours Post-Exposure	Clinical Signs: During Exposure	Clinical Signs: Post-Exposure
150	72.4	0/10	0/10	Irregular respiration, slight lacrimation, head tremors, incoordination of legs	Down to 77% of their initial body weight on the 2nd day post-exposure. All except 2 animals gained weight on the 3rd day; the average body weight approached the pre-exposure value on the 4th day. All animals again lost weight from 5-8 days post-exposure with 2 deaths on the 6th day and 3 deaths on the 7th day. All survivors had started to gain weight by the 14th day post-exposure.

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Haskell Laboratory for Toxicology and Industrial Medicine

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Summary: Chloroprene is not a Class B Poison by inhalation administration.‡ The above test was run at approximately 36 times the concentration required for Class B Poison labelling.

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‡ As defined in the Hazardous Materials Regulations of Transportation, Agent T. C. George's Tariff No. 23, September 3, 1969, Subpart G, Section 73.343

‡ a) Von Oettingen, et al., J. Indus. Hyg. & Toxicol., 18:240 (1936)
b) Chemical Abstracts, Vol. 63, 15438d (1965)
c) Patty, F. A., Ind. Hyg. & Toxicol., Second Revised Edition, Vol. II, pp. 1319-1321, Interscience Publishers, New York (1963)

Report by: Figen O. Tayfun

Figen O. Tayfun
Inhalation Toxicology Section

Approved by: Charles F. Reinhardt

Charles F. Reinhardt
Assistant Director

FOT:pgh
Date: September 13, 1971

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Doc Title RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY AGAINST CHLOROPRENE	23						

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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Doc Title 23

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Chemical Name (300 per name)	25	CAS No. (10)	24
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October 26, 1978

ELASTOMERS DEPARTMENT
LOUISVILLE PLANT

RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY
AGAINST CHLOROPRENE

Results of the evaluation of one Scott chest canister (Part No. 600214-14), two Scott chin canisters (Part No. 272-OV-L) and ten MSA Comfo-Type GMA cartridges (Part No. 459315), three new and seven used sets, against a nominal airborne upstream concentration of 100 ppm (v/v) chloroprene are reported below. The evaluations were conducted at a flow rate of 50 liters per minute approximating a man doing heavy work, at ambient room temperature and at 50% relative humidity. Both airborne upstream and breathing downstream concentrations of chloroprene were monitored by a procedure utilizing a gas chromatograph equipped with a flame ionization detector, a 10% SE 30 column packed with Chromosorb Q and a 1 ml gas sampling loop. The gas sampling loop was attached to the column in the gas chromatograph and connected to the upstream and downstream sampling ports with Teflon® tubing in a way that permitted either a 1 ml upstream or downstream sample to be introduced for analysis by simply switching a two-way sampling valve. The minimum detectable concentration of chloroprene in a 1 ml air sample was 1 ppm (v/v).

The Scott chest canister was initially evaluated at the test conditions mentioned above with no breakthrough (less than 1 ppm chloroprene downstream) after 67 hours of continuous exposure. This evaluation was terminated after it was decided to evaluate two Scott chin canisters. Both of these canisters were evaluated separately at similar test conditions until 10% breakthrough. At 6½ and 5½ hours respectively, breakthrough was detected downstream on the breathing side of each chin canister.

ES-3606

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October 26, 1978

ELASTOMERS DEPARTMENT
LOUISVILLE PLANT

RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY AGAINST CHLOROPRENE

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Canister Results:

	Mean Analytical Upstream Concentration ppm (v/v) Chloroprene	Relative Humidity %	Flow Rate Liters/ Min.	10% Breakthrough Time
Scott Chest	139	47	44	< 1 ppm after 67 hrs.
Scott Chin	175	35	42	[illegible] hrs.
	137	38	42	[illegible] hrs.

The cartridge evaluations were conducted at similar test conditions as described for the canisters. Ten sets of cartridges (two cartridges per set) three new sets and seven used by individuals at the Louisville plant were evaluated. First air was passed over the seven sets of used cartridges to see if any chloroprene vapors could be detected downstream without an upstream concentration. Less than 1 ppm chloroprene was detected downstream for each of the cartridge sets. However, when a challenge concentration of approximately 100 ppm chloroprene was introduced upstream 10% breakthrough occurred within one minute for each of the seven used sets of cartridges. The three new or unused cartridge sets had an average 10% breakthrough time of 34 minutes.

Cartridge Results:

	Mean Analytical Upstream Concentration ppm (v/v) Chloroprene	Relative Humidity %	Flow Rate Liters/ Min.	10% Breakthrough Time
MSA Comfo-Type GMA New (unused)				
1	48	44	44	42 min.
2	72	60	44	30 min.
3	191	48	44	30 min.
Used				
#2	107*	30	44	1 min.**
#3	110*	57	44	1 min.
G. Irwin	100*	60	44	1 min.
R. Kincaid	483*	58	44	1 min.
B. Mueller	129*	40	44	1 min.
L. Benford	190*	35	44	1 min.
S. Martin	71*	42	44	1 min.

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S. Martin	71*	42	44	1 min.

Davtyan, R.M. 1972. Toxicological characteristics of the action of chloroprene on the reproductive function of male rats. Toksikol. Gig. Prod. Neftekhim. Neftekhim. Proizvod Vses. Konf. (Dokl.) 2nd 1971, p. 95.

Davtyan, R.M., V.N. Fomenko and G.P. Andreyva. 1973. Question of the effect of chloroprene on the generative function of mammals (males). USSR Academy of Medical Sciences. The toxicology of new industrial chemical substances, Issue No. 13:53.

Ducatman, A., K. Hirschhorn and I.J. Selikoff. 1975. Vinyl chloride exposure and human chromosome aberrations. Mutat. Res. 31:163.

Funes-Cravioto, F., B. Lambert, J. Lindsten, L. Ehrenberg, A.T. Natarajan and S. Osterman-Golkar. 1975. Chromosome aberrations in workers exposed to vinyl chloride. Lancet. i:459.

Garro, A.J., J.B. Guttenpaln and P. Milvy. 1976. Vinyl chloride dependent mutagenesis:effects of liver extracts and free radicals. Mutat. Res. 38(2):81.

Greim, H., G. Bonse, Z. Radwan, D. Reichert and D. Henschler. 1975. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes and a function of metabolic oxirane formation. Biochem. Pharmacol. 24:2013.

Herbert, J.T., April 12, 1976. Angiosarcoma of the liver in a rubber worker. CDC Memorandum.

Huberman, E., H. Bartsch and L. Sachs. 1975. Mutation induction in Chinese Hamster V79 cells by two vinyl chloride metabolites chloroethylene oxide and 2-chloroacetaldehyde. Int. J. Cancer. 16:639.

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Date Produced (6) 022879	6	Date Rec'd (6) 022285	7	Conf. Code • N	8		

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Doc Title 23

RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY WITH CHLOROPRENE (CD) PART II

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

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Doc Type 22

• RI • U.P • H.E.A.S.D 8D. , S.U H.S E.N

Doc Title 23

RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY WITH CHLOROPRENE (CD) PART II.

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

VA

3/8/85 U.S.

ES-3606

(9)

878214997

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY

February 26, 1979

ELASTOMER CHEMICALS DEPT.
LOUISVILLE, KY.

RESPIRATOR CARTRIDGE AND CANISTER BREAKTHROUGH STUDY WITH CHLOROPRENE (CD) PART II

Results of additional respirator cartridge and canister evaluations for breakthrough against chloroprene for the Pontchartrain plant in Louisiana, the Louisville plant in Kentucky and Maydown plant in Ireland are reported below. The evaluations were conducted at a flow rate of 50 liters per minute approximating a man doing heavy work, ambient room temperature, and measured relative humidity. The cartridges from the Pontchartrain plant were exposed to CD at the plant and analyzed to determine if saturation had occurred. All other cartridges and canisters were tested as received from the supplier.

Both airborne upstream and breakthrough downstream concentrations of chloroprene were monitored by a procedure utilizing a gas chromatograph equipped with a flame ionization detector, a 10% SE 30 column packed with Chromosorb Q and a 1 ml gas sampling loop. The gas sampling loop was attached to the column in the gas chromatograph and connected to the upstream and downstream sampling ports with Teflon® tubing in a way that permitted either a 1 ml upstream or downstream sample to be introduced for analysis by simply switching a two-way sampling valve. The minimum detectable concentration of chloroprene in a 1 ml air sample was 1 ppm (V/V).

Results:

1. Pontchartrain Plant - Louisiana

   a. Exposed cartridges - MSA, GMA organic vapors #459315
      (Evaluated at 80% R.H. per request from W. T. Dove)

Sample I.D.		Mean Upstream Concentration-ppm	Downstream Concentration at Breakthrough-ppm (V/V)	R.H.%
2nd floor LPKS	1 week	411	5 in 1 min.	80
2nd floor LPK	5 days	104	3 in 2 min.	70
1st floor Poly	1 week	0	37 in 5 min.	76
Special 3 months		0	25 in 5 min.	78

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ES-3606

(9)

878214997

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY

February 28, 1979

ELASTOMER CHEMICALS DEPT.
LOUISVILLE, KY.

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Results of additional respirator cartridge and canister evaluations for breakthrough against chloroprene for the Pontchartrain plant in Louisiana, the Louisville plant in Kentucky and Maydown plant in Ireland are reported below. The evaluations were conducted at a flow rate of 50 liters per minute approximating a man doing heavy work, ambient room temperature, and measured relative humidity. The cartridges from the Pontchartrain plant were exposed to CD at the plant and analyzed to determine if saturation had occurred. All other cartridges and canisters were tested as received from the supplier.

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1st floor Poly	1 week	0	37 in 5 min.	76
Special 3 months		0	25 in 5 min.	78

000002

b. MSA chin canister, GMA organic vapors #448974

Sample I.D.	Mean Upstream Concentration-ppm	10% Breakthrough Time	R.H.%
1	141 ± 87	96 hrs.	60
2	128 ± 38	52 hrs.	59

2. Maydown Plant - Ireland

a. Cartridge - MSA, GMA organic vapors #459315 (8710)

Sample I.D. Cartridge Pair	Mean Upstream Concentration-ppm	10% Breakthrough Time	R.H.%
	131 ± 53	8.3 hrs.	59

b. Auer® 2730A Cartridge

1	127 ± 104	25 hrs.	63
2	147 ± 90	29 hrs.	60

3. Louisville Plant - Kentucky
(Breakthrough Cross check)

a. Cartridge MSA, GMA Part #459315 (8790)

Sample I.D.	Mean Upstream Concentration-ppm	Flow Rate LPM	10% Breakthrough Time	Calculated Grams CD Adsorbed at Breakthrough	Analyzed Grams CD Adsorbed at Breakthrough
1 (pair)	555	53	3.9 hrs.	24.9	-
2	674	55	6.1 hrs.	49.0	-
3	500	50	4.5 hrs.	24.8	16.3

b. Weight of two unexposed cartridges supplied

Number	Gms. Charcoal
1 (single)	43.6
2	42.1

c. Breakthrough curves plotted as ppm CD vs. time (see attached graph).

b. MSA chin canister, GMA organic vapors #448974

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2	42.1

c. Breakthrough curves plotted as ppm CD vs. time (see attached graph).

Conclusions:

Results of additional testing of cartridges and canisters against chloroprene at varying concentrations, relative humidities and amounts of activated carbon demonstrate the variability of breakthrough times that can be encountered in the field. Selection of appropriate air purifying devices should be based on the reported data along with recommendations from an industrial hygienist.

Results of the breakthrough crosscheck conducted with the Louisville plant averaged about 1.6 times the predicted 10% breakthrough time of three hours for an upstream concentration of 500 ppm chloroprene, as calculated from G. O. Nelson's empirical formula[1]. The cartridge capacity for chloroprene at this level was about 16 grams, or about 0.27 grams of chloroprene per gram of charcoal[2].

Report by: _____
Norman W. Henry, III
Research Chemist

Approved by: Ronald E. Hemingway
Ronald E. Hemingway
Chief, Analytical & Metabolism Section

[1]Respirator cartridge efficiency studies: VIII. Summary and Conclusions. Gary O. Nelson and A. Nicholas Correia. American Industrial Hygiene Association Journal, September 1976.

[2]Calculated as a ratio of analyzed grams of CD to weight of activated carbon removed from cartridge after exposure.

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Doc I.D.	878214998	3	Old Doc I.D.	8DS	4		
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Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
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Doc Type	22
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Doc Title	23
INHALATION SUBACUTE AND REPRODUCTION STUDIES	

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

3/5/85 U.S.

(10)

878214998

2 CHLORO-1,3-BUTADIENE (β-CHLOROPRENE)

Report by: David P Kelly
David P. Kelly
Assistant Toxicologist

Approved by: Henry J. Trochimowicz
Henry J. Trochimowicz
Chief, Inhalation Toxicology Section

DPK/jhm
Date: October 6, 1975

Material Tested - 2 Chloro-1,3-butadiene (β-chloroprene)

Date Sample Submitted - January 1975

Submitted by - R. S. Barrows

Department - Elastomer Chemicals

Inhalation Subacute and Reproduction Studies

Medical Research Project Nos. 2074 and 2131

Haskell Laboratory Report No. 580-75

Introduction

These tests were conducted as part of a program to determine the inhalation toxicity of 2-chloro-1,3-butadiene (β-chloroprene). Soviet authors[1,2] have reported effects on the male rat reproductive system and function from repeated inhalation of these vapors at low concentrations (< one ppm). To evaluate these findings, tests were conducted which approximate the highest dosage used in the Soviet experiments. Davtyan et al.[2] exposed male rats for four hrs./day, 48 days, at 10.8 ppm [CxT = (4x48) hrs. x 10.8 ppm = 2074 ppm hrs.]. We exposed males for four hrs./day, 22 days, at 25 ppm [CxT = (4x22)hrs. x 25 ppm = 2200 ppm hrs.].

Methods

Subacute and reproduction studies were conducted on young male ChR-CD rats weighing initially between 150 and 200 grams each. Fifteen test and ten control rats were used in the subacute study, five test and five control rats were used in the reproduction study. In both studies, test animals were exposed four hours daily for 22 consecutive days to vapors of β-chloroprene at a 25 ppm (v/v) level in a 1.4 m^3 stainless steel chamber. Control rats were treated similarly except for exposure to the test material. When rats were not in the exposure chambers, they were supplied with food and water ad libitum. Weight records were maintained and the animals observed for signs of toxicity during and post-exposure. Autopsy was performed on the following numbers of animals at the indicated times post-exposure: subacute study - eight test and five controls at one day, seven test and five controls at 14 days; reproduction study - five test and five controls at 60 days.

Pathology*

At autopsy the following organs were weighed: lung, heart, liver, kidney, testis, and thymus. Histologic examination was performed on only the testis and epididymis of the males used for the reproduction study; in the rats used for the subacute study, the trachea, lung, thyroid, parathyroid, heart, esophagus, stomach, intestine, pancreas, liver, kidney, testis,

* Pathology performed by Taisan Chiu, D.V.M., Pathology Report Nos. 25-75 and 28-75.

epididymis, adrenal, lymph node, spleen, bone marrow, thymus, brain, eye, and skin were histologically evaluated.

Vapor Generation and Analysis

Liquid β-chloroprene was kept refrigerated (max. temp. = -20°C) until used for vapor generation, at which time it was allowed to come to room temperature. The liquid was then delivered at a constant rate via syringe drive into a room temperature air stream which carried the resulting vapors into the test chamber.

Samples of the chamber atmosphere were taken every half-hour by trapping β-chloroprene in toluene. The resulting mixture was analyzed by using gas chromatography (flame ionization detector).

Sample Purity

β-Chloroprene used in this study was from the same analyzed batch. To determine if sample purity was being affected by the handling procedure, a sample of the test liquid which had been at room temperature in the delivery syringe for about 5 hours was analyzed by gas chromatography* and found to contain <50 ppm of dimer and 0.03% α-chloroprene, not a significant change from the original composition.

Reproduction Study

Soviet experimenters[1,2] report embryomortality (exhibited mainly by pre-implantation losses) in 30-40% of the offspring of male rats exposed to β-chloroprene vapors for various periods of time and levels of exposure. Offspring of control animals in the same studies showed embryomortalities from 10-13%.

To test for results of this magnitude a small scale reproduction study was performed at Haskell Laboratory. A single comparison of numbers of pups per litter sired by test males to numbers sired by controls would show whethe. such losses occurred using our materials and conditions.

Starting the day after the last inhalation exposure, eight sequential mating trials were conducted on five test and five control male rats. In each mating trial, a male was housed with three untreated virgin female ChR-CD rats for seven days. After mating, the females were housed separately and allowed to deliver and raise their pups to weaning. The number of pups per litter and their average body weight at weaning were calculated along with the following indices of reproduction: (1) the male mating index (the percentage of successful matings), (2) the viability index (the percentage of pups that survive 4 days or longer), and (3) the lactation index (the percentage of pups alive at four days and surviving for the 21 day lactation period).

After the eighth mating trial, the males were autopsied and the reproductive organs examined histologically.

---

* A. L. Barney, Elas. Chem. Department.

Results

Observations and Weights

No clinical signs of toxicity were observed during the test. Weight gain patterns in test animals were similar to controls.

Analytical

In the subacute study the average chamber concentration ± s.d. was 22.4 ppm ± 5.6. (This includes an excursion to 195 ppm for < 1/2 hr.)

In the reproduction study the average chamber concentration ± s.d. was 21.5 ppm ± 2.7.

Pathology

Gross and histopathologic examination revealed no changes attributable to the test compound. The mean testes-to-body weight ratios were calculated for the 15 test and 10 control rats autopsied within 2 weeks of exposure. The results show no significant difference between test and control groups.

$$\frac{\text{Testes}}{\text{Body Wt.}} \pm \text{s.d.} = \begin{cases} 0.96\% \pm 0.10 \text{ control group} \\ 0.90\% \pm 0.10 \text{ test group} \end{cases}$$

Reproduction Study

The results of this eight week study showed no significant differences between test and control groups for any of the calculated reproduction parameters:

Parameter	Test Group	Control Group
Mating Index	66.7	70.8
Pups/Litter	11.65	11.75
Viability Index	95.9	96.7
Lactation Index	99.5	99.0
Weanling Body Wt.	44.1	43.9

Discussion

The results of our reproduction study appear to be at variance with those published by Soviet authors for experiments utilizing similar dosages (CxT) of β-chloroprene. Since the handling and purity of the test materials used in the Russian studies[1,2] are not reported, it is impossible to say whether the materials tested were significantly different from those used in our studies. However, in light of the instability of β-chloroprene, it is a possibility and could explain differences in test results.

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Summary

In a subacute inhalation test consisting of 22 consecutive 4-hour exposures, vapors of β-chloroprene at the 25 ppm level produced no apparent toxic effects in male rats.

A reproduction study of male rats similarly exposed showed no effects on reproductive organs or function.

DPK/jhm

Date issued 10/6/75

References

1. Davtyan, R. M., Toksikol. Gig. Prod. Neftekhim. Proizvod., Vses. Konf., (Dokl.), 1971: 62-4.

2. Davtyan, R. M., Fomenko, V. N., and Andreyeva, G. P., Toksikol. Nov. Prom. Khim. Veshchestv. 1973: No. 13:58-62.

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Microfiche No. (7) •	OTS 0206752	1	No. of Pages		2		
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Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
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Pub/Journal Name	—	9					
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Author(s)		10					
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MID No. (7)	— 19	D & B NO. (11)	—	20			
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Doc Type	• RI • U.P • H.E.A.S.D 8D. , SU H.S F.N	22					
Doc Title	EMBRYOTOXICITY AND TERATOGENICITY STUDIES ON INHALED BETA CHLOROPRENE	23					

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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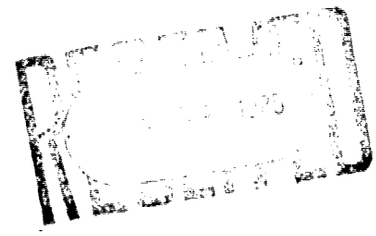
2 CHLOROBUTADIENE-1,3



Report by: David P. Kelly
David P. Kelly
Assistant Toxicologist

Approved by: Henry J. Trochimowicz
Henry J. Trochimowicz
Chief
Inhalation Toxicology Section

DPK:dhg
Date Issued: October 10, 1975

Material Tested - 2 Chlorobutadiene-1,3

Date Samples Submitted - January 1975

Department - Elastomer Chemicals

Embryotoxicity and Teratogenicity Studies on Inhaled β-Chloroprene

Introduction

A teratogeni ity and an embryotoxicity study were conducted on pregnant rats exposed to vapors of β-chloroprene. The test protocols for both studies are similar, differing mainly in the number of days of exposure and the time interval during gestation when pregnant animals are exposed to the test material.

The purpose of the teratology study was to evaluate the potential of inhaled β-chloroprene to induce growth abnormalities or mortality in the fetus during its period of organogenesis (days 6-15 of the rat's 21-day gestation period). The embryotoxicity study was conducted to determine whether exposure to β-chloroprene could induce pre-implantation losses (ova released minus ova implanted in the uterine wall) which usually occur in rats during days one through six of the gestation period.

Methods

Adult ChR-CD pregnant female rats were used in both studies and were exposed to vapors of β-chloroprene at levels of 0, 1, 10, and 25 ppm (v/v) for four hours a day. The concentration of β-chloroprene was analytically monitored at frequent intervals during exposure. Methods for vapor generation and analysis, as well as procedures for sample handling, have been previously discussed in detail in Haskell Laboratory Report No. 580-75. Except during exposure, animals were allowed food and water ad libitum.

A. Teratology Study

The 100 rats (25/level) used in this study were mated at Charles River Laboratories and shipped to Haskell Laboratory. One-half of the rats were two days pregnant and the other half three days pregnant at the time of delivery. All animals were exposed from day of delivery to day 20 of gestation, one-half receiving 18 days and the other half 19 days of exposure.

Methods (Continued)

A. Teratology Study (Continued)

After exposure (day 20 of gestation), all rats were anesthetized with chloroform and gross autopsies performed on the mothers. The uterine horns were opened, fetuses removed, and the following observations made:

1. Number of corpora lutea in each ovary.
2. Number of implantation sites in each horn.
3. Number and location of live and dead fetuses.
4. Number and location of late and early resorptions.
5. Weights and crown-to-rump lengths of all live fetuses.
6. Gross examination of all fetuses for external anomalies.

In addition, at the time of sacrifice, about two-thirds of the fetuses from each litter were preserved in 95% alcohol for subsequent maceration in 1% aqueous KOH, cleaning and staining with Alizarin Red, and examination to detect skeletal abnormalities. The remaining third of the fetuses were fixed in Bouin's fluid for free-hand razor blade sectioning (Wilson's Method*) and examined for visceral and neural anomalies under the dissecting microscope.

B. Embryotoxicity Study

Two hundred (50/level) ChR-CD rats, mated at Haskell Laboratory, were used in this study. The morning that sperm was observed in a vaginal smear was considered day one of gestation.

All rats were exposed to β-chloroprene vapors on days one through 12 of gestation. After the last exposure, animals were held for five days and sacrificed on day 17 of gestation.

At the time of sacrifice, all pregnant rats were chloroformed and gross autopsies were performed on the mothers. The uterine horns were opened, fetuses removed, and the following observations made:

1. Number of corpora lutea in each ovary.
2. Number of implantation sites in each horn.
3. Number and location of live and dead fetuses.
4. Number and location of late and early resorptions.

Results

A. Analytical Determinations

The nominal and average analytical concentrations for each entire exposure period for each study are shown below:

---

* Wilson, J. G. (1965) Methods for administering agents and detecting malformations in experimental animals. In: Teratology Principles and Techniques. J. G. Wilson and J. Warkany, eds., Univ. of Chicago Press, pp. 262-277.

Results (Continued)

A. Analytical Determinations (Continued)

	Nominal Conc. (ppm, v/v)	Analytical Conc. ± 1 s.d. (ppm, v/v)
Teratology Study	1	0.8 ± 0.3
	10	8.6 ± 2.7
	25	22.7 ± 6.4
Embryotoxicity Study	1	1.3 ± 0.2
	10	9.9 ± 1.4
	25	24.7 ± 2.8

B. Clinical Observations

No adverse clinical signs were observed in the pregnant rats during or post-exposure at any inhaled level of β-chloroprene.

C. Gross Pathology - Pregnant Rats

No gross pathological changes were observed in the uterine horns, ovaries, or other organ systems at any of the test levels.

D. Embryotoxicity and Teratogenic Parameters**

The results of the embryotoxicity study are given in Table I. Teratogenicity test results are shown in Tables II and III.

In both studies, implantation sites per litter, litter size, pre-implantation losses of fertilized ova, fetal resorptions, and number of females with partial resorptions did not differ significantly between test and control animals.

In the teratology study (Table II), there were no significant differences between control and test animals relative to fetal length or crown-to-rump length. Table III shows the incidence and type of fetal anomalies and the number of fetuses examined. The number of minor gross anomalies, minute subcutaneous hematomas and petechial hemorrhages observed by external examination of fetuses from rats exposed to β-chloroprene was not significantly greater than in those from control litters.

Likewise, no major skeletal or soft tissue abnormalities were found in fetuses from mothers exposed to β-chloroprene. The number of unossified sternebrae and thoracic centra was comparable in all treated groups and was somewhat lower than in the control group in which the body measurements of the fetuses were also lower. Ossification was not affected by the exposure.

Supernumerary and wavy ribs and hydronephrosis are spontaneous congenital variants in this strain of rats and were not treatment-related.

** Teratogenic and embryotoxicity parameters were evaluated by Rudolph Culik, Ph.D., and are reported in Pathology Report Nos. 24-75 and 49-75.

Results (Continued)

D. Embryotoxicity and Teratogenic Parameters** (Continued)

The data were compared with available standard historical values established and quoted for Charles River-CD rats. Under the conditions of this test, the data are in good agreement. The standard values are listed in the last column of the attached tables.

Summary

Teratogenicity and embryotoxicity studies were conducted on pregnant female rats exposed to vapors of β-chloroprene (2-chlorobutadiene-1,3) for four hours a day at 0, 1, 10 or 25 ppm (v/v), respectively. In the teratology study, rats were exposed from day two or three to day 20 of gestation. In the embryotoxicity study, rats were exposed from day one to day twelve of gestation. Results are summarized:

1. The test compound was not toxic to mothers and did not affect their body and gravid uterus weights.
2. The pregnancy outcome, measured by pre- and post-implantation losses of fertilized ova and the number of live fetuses per litter, was not significantly different from that of the control animals.
3. The embryonal development measured by the weight and size of the fetuses was not affected.
4. No major external, skeletal, or soft tissue malformations were found.

β-chloroprene was not teratogenic or embryotoxic under the conditions of this experiment.

DPK:ljm:dhg
Date Issued:

TABLE I - EMBR OXICITY STUDY

EFFECT OF CHLOROPRENE ON THE OUTCOME OF PREGNANCY
OF THE RAT AND ON VARIOUS PARAMETERS OF FETAL DEVELOPMENT

	Inhalation Exposure to Chloroprene at Levels				Standard Values (Range) fc
	I 0 (Control)	1 PPM	10 PPM	25 PPM	Charles River (CD) Rats (1
Number Females Bred	50	50	49	51	
Number Females Pregnant (%)	45 (90.0%)	44 (88.0%)	48 (87.8%)	48 (94.1%)	90% (62-100)
Number Corpora Lutea	653	615	604	637	
No. Corpora Lutea/Pregnant Female	14.5	14.0	14.0	13.3	12.9 (10.8-15.7)
Number Implantation Sites	485	464	454	493	
Number Implanatation Sites/ Pregnant Female	10.8	10.5	10.6	10.3	10.9 (8.6-14.1)
Pre-implantation Loss (%)	168 (25.7%)	151 (24.6%)	150 (24.8%)	144 (22.6%)	11.9% (3.8-26.0)
Number Live Fetuses	446	417	419	459	
Number Live Fetuses/Litter	9.9	9.5	9.7	9.6	10.2 (8.2-12.7)
Number Dead Fetuses	0	0	0	0	
Late Resorptions (%)	0	0	2 (0.4%)	0	
Early Resorptions (%)	39 (8.0%)	47 (10.1%)	35 (7.7%)	34 (6.9%)	
Post-implantation (fetal) Loss (%)	39 (8.0%)	47 (10.1%)	37 (8.1%)	34 (6.9%)	6.0% (1.2-12.4)
No. Females Showing Complete Resorption (%)	0	2 (4.5%)	0	0	0.3% (0-5.9)
No. Females Showing Partial Resorption (Excluding Complete Resorptions) (%)	23 (51.1%)	22 (50.0%)	23 (53.5%)	24 (50.0%)	40.6% (10.5-77.8)

(1) Personal communication; unpublished data obtained from Charles River Breeding Laboratories, Wilmington, Mass., for period 1964-1969.

TABLE II - TERATOLOGY STUDY

EFFECT OF CHLOROPRENE ON THE OUTCOME OF PREGNANCY OF THE RAT AND ON VARIOUS PARAMETERS OF FETAL DEVELOPMENT

	Inhalation Exposure to Chloroprene at Levels				Standard Values (Range) for Charles River (CD) Rats (1)
	0 (Control)	1 ppm	10 ppm	25 ppm	
Number Females Bred	25	25	25	25	
Number Females Pregnant (%)	21 (84.0)	24 (96.0)	21 (84.0)	19 (76.0)	90% (62-100)
Number Corpora Lutea	140	290	252	241	
No. Corpora Lutea/Pregnant Female	12.7*	12.1	12.0	12.7	12.9 (10.8-15.7)
Number Implantation Sites	200	205	197	201	
Number Implantation Sites/ Pregnant Female	9.5	8.5	9.4	10.6	10.9 (8.6-14.1)
Pre-implantation Loss (%)	30 (21.4)*	85 (29.3)	55 (21.8)	40 (16.6)	11.9% (3.8-26-0)
Number Live Fetuses	192	191	172	184	
Number Live Fetuses/Litter	9.1	8.0	8.2	9.7	10.2 (8.2-12.7)
Number Dead Fetuses	0	0	0	0	
Late Resorptions (%)	0	1	1	0	
Early Resorptions (%)	8	13	24	17	
Post-implantation (fetal) Loss (%)	8 (3.9)	14 (6.8)	25 (12.7)	17 (8.5)	6.0% (1.2-12.4)
No. Females Showing Complete Resorption (%)	0	0	0	0	0.3% (0-5.9)
No. Females Showing Partial Resorption (Excluding Complete Resorptions) (%)	7 (33.3)	7 (29.2)	13 (61.9)	11 (57.9)	40.6% (10.5-77.8)
Mean Fetus Weight (g)	3.78	3.92	3.92	4.07	3.69 (2.90-4.22)
Mean Fetus Crown-Rump Length (cm)	3.28	3.30	3.27	3.41	

* Values from 11 females only.

(1) Personal communication; unpublished data obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts, for period 1964-1969.

TABLE III - TERATOLOGY STUDY

GROSS EXTERNAL, SKELETAL AND SOFT TISSUE ABNORMALITIES
FOUND IN FETUSES FROM MOTHERS EXPOSED TO CHLOROPRENE DURING FETAL DEVELOPMENT

Abnormalities Found	Inhalation Exposure to Chloroprene at Levels				Standard Values (Range) for Charles River (CD) Rats (1)
	0 (Control)	1 ppm	10 ppm	25 ppm	
1. Gross External-Number Examined	192	191	172	184	32915
a. Subcutaneous hematomas	4 (2.1%)	9 (4.7%)	4 (2.3%)	10 (5.4%)	
b. Small, stunted	2 (1.0%)	0	1 (0.6%)	1 (0.5%)	
c. Hemorrhage	5 (2.6%)	6 (3.1%)	3 (1.7%)	2 (0.1%)	0.3% (0-3.5)
d. Edema	0	1 (0.5%)	0	0	
2. Skeletal-Number Examined	126	122	112	122	7127
a. One or more sternebrae unossified	58 (46.0%)	39 (32.0%)	33 (29.5%)	45 (36.9%)	11% (5-30)
b. 14 Rib(s) or remnants	91 (72.2%)	76 (62.3%)	67 (59.8%)	77 (63.1%)	21% (6-36)
c. Wavy ribs	4 (3.2%)	5 (4.1%)	3 (2.7%)	4 (3.3%)	
d. One or more thoracic centra unossified or bipartite	2 (1.6%)	3 (2.5%)	2 (1.7%)	8 (6.6%)	
3. Soft Tissue-Number Examined	66	69	60	62	2979
a. Hydronephrosis	9 (13.6%)	6 (8.7%)	1 (1.7%)	7 (11.3%)	7.89%

(1) Personal communication; unpublished data obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts for period 1964-1969.

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Pub./Journal Name —	9
—	9
Author(s) —	10
Organ. Name E I DUPONT DENEMOURS & CO INC	11
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P.O. Box — 13 Street No./Name —	14
City WILMINGTON 15 State DE 16 Zip 19898 17 Country —	18
MID No. (7) — 19 D & B NO. (11) —	20
Contractor LITTON BIONETICS	21
Doc Type • RI • U.P • H.E.A.S.D 8.D. , S.U H.S F.N	22
Doc Title	23

MUTAGENIC EVALUATION

FINAL REPORT ON

COMPOUND 9159

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

N/A

3/8/85 V.S.

(12)

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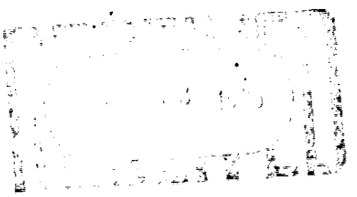

MUTAGENIC EVALUATION

FINAL REPORT

B Chloroprene ON 1st report

COMPOUND 9159

SUBMITTED TO

HASKELL LABORATORY FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE 19711

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

NOVEMBER 11, 1974

Litton BIONETICS

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DATE: November 8, 1974

SPONSOR: Haskell Laboratory

SUBJECT: Mutagenic Evaluation of Compound 9159 Received 10-9-74

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

A sample of what appeared to be greater than 10 ml of clear liquid packed in dry ice labeled 9159. A bottle labeled inhibitor was also received with this compound. This test chemical was stored at -80°C at all times.

B. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

C. Indicator Organisms

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4: $\frac{\alpha}{a}$, $\frac{\text{ade 2-2}}{\text{ade 2-1}}$, $\frac{\text{try 5-12}}{\text{try 5-27}}$

- Salmonella typhimurium, strains:

  TA-1535; hisG, uvrB, rfa (missense mutation)
  TA-1537; hisC, uvrB, rfa (- frameshift mutation)
  TA-1538; hisD, uvrB, rfa (+ frameshift mutation)

D. Reaction Mixture

The following reaction mixture was employed in the activation tests:

	Component	Final Concentration/ml
1.	TPN (sodium salt)	6 µM
2.	Isocitric acid	49 µM
3.	Tris buffer, pH 7.4	28 µM
4.	$MgCl_2$	1.7 µM
5.	Isocitric dehydrogenase	1.0 Unit
6.	Tissue homogenate or cell fraction	72 mg

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

ASSAY	CHEMICAL[a]	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY[b]
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrosofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine or Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

[a] Concentrations given in the Results Section.

[b] BPS = base-pair substitution; FS = frameshift.

[c] Previously shown to be non-mutagenic.

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately 10^9 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and

control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

2. Rats

The same procedures as described for mice was used for this mammal.

3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (M. mulatta) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set was recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant micro-organisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.

Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.

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SUMMARY OF TEST RESULTS

COMPOUND 9159

A. Suspension Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}): TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}): Ade+	Try+
NA-PC	-	-	303.78	218.97	73.04	126.84	149.92
NA-NC	-	-	2.34	11.84	8.48	6.72	6.72
NA-H	-	-	3.15	8.65*	12.69	5.50	5.74
NA-L	-	-	2.56	14.09	5.54	8.00	5.28
A-NC (-C)	-	-	1.36	9.68	8.57	5.87	2.61
A-NC (+C)	-	-	2.25	8.56	10.20	2.15	3.00
A-PC	M	Li	165.96	22.44	47.43	9.45	8.62
A-PC	M	Lu	1.31	5.97	11.11	4.32	4.17
A-PC	M	T	2.06	10.04	8.09	3.35	4.59
A-H	M	Li	1.85	3.83*	10.69	Toxic	Toxic
A-L	M		7.70	2.02*	8.77*	6.93	4.78
A-H	M	Lu	3.92	2.09*	8.45	2.01	1.21
A-L	M		2.22	1.91*	9.84	5.89	5.89
A-H	M	T	1.90	4.60*	14.15	6.51	4.39
A-L	M		1.21	9.69	8.28	5.86	5.27

a NA = non activation; NC = negative control; PC = positive control; A = activation; H = high dose; L = low dose

b M = mouse; Mo = monkey; R = rat

c Li = liver; Lu = lung; T = testes

(-C) = solvent control; (+C) = chemical control

* Data from retest experiment (separate negative controls included).

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Litton BIONETICS

COMPOUND 9159

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-	+	+	+
NA-NC	-	-	-	-	-
NA-H	-	-	-	-	-
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	M	Li	+	+	+
A-PC	M	Lu	-	-	-
A-PC	M	T	-	-	-
A-H	M	Li	-	-	-
A-H	M	Lu	-	-	-
A-H	M	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 02498

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COMPOUND 9159

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	R	Li	+	+	+
A-PC	R	Lu	-	-	-
A-PC	R	T	-	-	-
A-H	R	Li	-	-	-
A-H	R	Lu	-	-	-
A-H	R	T	-	-	-

[a] NA = non activation; NC = negative control; PC = positive control; A = activation; H = high dose; L = low dose

[b] M = mouse; Mo = monkey; R = rat

[c] Li = liver; Lu = lung; T = testes

(-C) = solvent control
(+C) = chemical control

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COMPOUND 9159

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	Mo	Li	+	+	+
A-PC	Mo	Lu	-	-	-
A-PC	Mo	T	-	-	-
A-H	Mo	Li	-	-	-
A-H	Mo	Lu	-	-	-
A-H	Mo	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 02498

000012

Interpretation of Test Results and Conclusions

Compound 9159 was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained.

A. *Salmonella typhimurium*

1. Plate tests

At a concentration of 0.63% (v/v), this chemical did not exhibit any mutagenic activity in non-activation or activation plate tests.

2. Non-activation suspension tests

These tests were all negative. Retest of a single high response with TA-1537 was negative.

3. Activation suspension tests

These tests were all negative. The original runs produced apparent positive responses at several dose levels with strains TA-1537 and TA-1538. Repeat tests indicated the original results were aberrant fluctuations in the spontaneous results and not true mutagenic responses.

B. *Saccharomyces cerevisiae*

1. Non-activation suspension tests

These tests were all negative.

2. Activation suspension tests

These tests were all negative.

C. Additional Comments

None

D. Conclusions

Compound 9159 was not genetically active, directly or in combination with metabolic enzyme preparations, in any of the *in vitro* assays used in this evaluation.

SUBMITTED BY:

David Brusick

David Brusick, Ph.D.
Director, Department of Genetics

SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND:

   9159

2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY OF THE TEST CHEMICAL UNDER TREATMENT CONDITIONS: This chemical produced on emulsion in the stock solution prepared in saline. The compound appeared to be soluble under test conditions except the D4 high dose.

3. OTHER COMMENTS: This chemical was stored at -80°C at all times and removed only when stock solutions were prepared for testing.

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TOXICITY AND DOSAGE DETERMINATIONS

COMPOUND 9159

		D4	TA-1537
	Dose No.	% Concentration	% Concentration
Range of concentrations of the test compound used to determine the 50% survival level	1		.0005
	2		0.005
	3		0.05
	4		0.5
	5		5.0
	Dose No.	% Survival	% Survival
Survival Results	Control	100	100
	1		62
Test Date: 10-11-74	2		100
	3		78
	4		93
	5		0
	Dose	% Concentration[a]	% Concentration
Concentrations of the test chemical required for mutagenicity tests	Plate Test	-	0.63
	¼ 50% Survival	0.63	0.30
	½ 50% Survival	1.26	0.63
	Other	-	-

[a] Yeast doses fixed at the bacterial 50% survival level and 1/2 that level.

Project No. 02493

000015

Litton BIONETICS

NON-ACTIVATION PLATE TESTS

DATE: 10-15-74

Test	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	EMS	0.05 ml undiluted chemical	+	+				
	QM	0.25 mg			+	+		
	NF	0.25 mg					+	+
SC	SALINE	..	-	-	-	-		
	DMSO	<10%					-	-

NOTE: PC = positive control
SC = solvent control
T-1 = trial 1
T-2 = trial 2
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimethyl sulfoxide
(c) = contamination present

Project No. 2493

000016

NON-ACTIVATION PLATE TESTS

DATE: 10-15-74

Test	Compound	Concentration	TA-1535		TA-1537		TA-1538	
			T-1	T-2	T-1	T-2	T-1	T-2
TC	9159	0.63%	-	-	-	-	-	-

NOTE: TC = test compound
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

Project No. 02498

000017

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: Mouse

DATE: 10-16-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 µmoles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

Project No. 2498

000018

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: MOUSE

DATE: 10-16-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2		TA-1537 T-1	TA-1537 T-2		TA-1538 T-1	TA-1538 T-2
TC	Li	9159	0.63%	-	-		-	-		-	-
	Lu	9159	0.63%	-	-		-	-		-	-
	T	9159	0.63%	-	-		-	-		-	-

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

Project No. 02408

000019

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: Rat

DATE: 10-16-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 µmoles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

Project No. 2498

000020

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: RAT

DATE: 10-15-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2		TA-1537 T-1	TA-1537 T-2		TA-1538 T-1	TA-1538 T-2
TC	Li	9159	0.63%	-	-		-	-		-	-
	Lu	9159	0.63%	-	-		-	-		-	-
	T	9159	0.63%	-	-		-	-		-	-

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

Project No. 02498

000021

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: Monkey

DATE: 10-16-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 µmoles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 µmoles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

Project No. 2498

000022

Litton BIONETICS

ACTIVATION PLATE TESTS

SPECIES: MONKEY

DATE: 10-16-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2		TA-1537 T-1	TA-1537 T-2		TA-1538 T-1	TA-1538 T-2
TC	Li	9159	0.63%	-	-		-	-		-	-
	Lu	9159	0.63%	-	-		-	-		-	-
	T	9159	0.63%	-	-		-	-		-	-

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

Project No. 02498

000023

NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 10-15-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	TA-1535	EMS	0.05 %	7.90	2,400	303.78
	TA-1537	QM	0.01 mg/ml	2.53	554	218.97
	TA-1538	NF	1.25 mg/ml	5.86	428	73.04
SC	TA-1535	SALINE	-	8.13	19	2.34
	TA-1537	SALINE	-	9.04	107	11.84
	TA-1538	DMSO	-	5.19	44	8.48

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimthyl sulfoxide
(c) = contamination present

Project No. 2498

NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

DATE: 10-15-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
TC	TA-1535	9159	H	6.67	21	3.15
TC	TA-1535	9159	L	7.02	18	2.56
TC	TA-1537	9159	H	0.23	41	178.26
TC	TA-1537	9159	L	5.82	82	14.09
TC	TA-1538	9159	H	4.65	59	12.69
TC	TA-1538	9159	L	7.40	41(c)	5.54

NOTE: TC = test compound
H = high dose
L = low dose
(c) = contamination present
( ) = percent survival

Project No. 02498

NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

Repeated Dose

DATE: 11-1-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	TA-1535	EMS	0.05 %			
	TA-1537	QM	0.01 mg/ml			
	TA-1538	NF	1.25 mg/ml			
SC	TA-1535	SALINE	-			
	TA-1537	SALINE	-	15.89	192	12.08
	TA-1538	DMSO	-			

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimthyl sulfoxide
(c) = contamination present

Project No. 02498

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NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

Repeated Dose — DATE: 11-1-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
TC	TA-1535		H			
TC	TA-1535		L			
TC	TA-1537	9159	H	15.49	134	8.65
TC	TA-1537		L			
TC	TA-1538		H			
TC	TA-1538		L			

NOTE: TC = test compound
H = high dose
L = low dose
(c) = contamination present
( ) = percent survival

Project No. 02498

Litton BIONETICS

ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Mouse

DATE: 10-15-74				Strain TA-1535		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/ 10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	8.43	1399	165.96
	Lu	DMNA	100 µmoles/ml	6.87	9	1.31
	T	DMNA	100 µmoles/ml	8.75	18	2.06
SC	-	DMNA	100 µmoles/ml	9.78	22	2.25
	-	SALINE	-	11.76	16	1.36

DATE: 10-16-74				Strain TA-1537		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/ 10^8 Survivors
PC	Li	AAF	1.25 mg/ml	4.01	90	22.44
	Lu	AAF	1.25 mg/ml	5.53	33	5.97
	T	AAF	1.25 mg/ml	5.48	55	10.04
SC	-	AAF	1.25 mg/ml	5.61	48	8.56
	-	DMSO	-	7.75	75	9.68

DATE: 10-21-74				Strain TA-1538		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/ 10^8 Survivors
PC	Li	AAF	1.25 mg/ml	3.69	175	47.43
	Lu	AAF	1.25 mg/ml	2.79	31	11.11
	T	AAF	1.25 mg/ml	3.46	28	8.09
SC	-	AAF	1.25 mg/ml	5.59	57	10.20
	-	DMSO	-	5.25	45	8.57

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 2498

ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

SPECIES: MOUSE

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 10-15-74				Strain TA-1535		
TC	Li	9159	H	2.16	4	1.85
		9159	L	7.53	58	7.70
	Lu	9159	H	0.51	2	3.92
		9159	L	8.12	18	2.22
	T	9159	H	2.10	4(c)	1.90
		9159	L	4.15	5	1.21
DATE: 10-16-74				Strain TA-1537		
TC	Li	9159	H	-	-	-
		9159	L	0.73	18	24.66
	Lu	9159	H	0.16	5(c)	31.25
		9159	L	0.90	11(c)	12.22
	T	9159	H	0.33	13	39.39
		9159	L	4.54	44	9.69
DATE: 10-21-74				Strain TA-1538		
TC	Li	9159	H	1.31	14	10.69
		9159	L	1.11	32	28.83
	Lu	9159	H	0.71	6	8.45
		9159	L	1.22	12	9.84
	T	9159	H	1.06	15	14.15
		9159	L	2.90	24	8.28

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

Project No. 02498

ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE Repeated Doses

DATE: Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml			
	Lu	DMNA	100 µmoles/ml			
	T	DMNA	100 µmoles/ml			
SC	-	DMNA	100 µmoles/ml			
	-	SALINE	-			

DATE: 11-6-74 Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml			
	Lu	AAF	1.25 mg/ml			
	T	AAF	1.25 mg/ml			
SC	-	AAF	1.25 mg/ml	7.96	39	4.90
	-	DMSO	-	10.11	26	2.57

DATE: 11-6-74 Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml			
	Lu	AAF	1.25 mg/ml			
	T	AAF	1.25 mg/ml			
SC	-	AAF	1.25 mg/ml	4.08	39	9.56
	-	DMSO	-	4.37	25	5.72

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 02498

ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

SPECIES: MOUSE Repeated Doses

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE:				Strain TA-1535		
TC	Li		H			
			L			
	Lu		H			
			L			
	T		H			
			L			
DATE:	11-6-74			Strain TA-1537		
TC	Li		H	7.32	28	3.83
			L	9.39	19	2.02
	Lu		H	8.12	17	2.09
			L	9.42	18	1.91
	T		H	16.09	74	4.60
			L	-	-	-
DATE:	11-6-74			Strain TA-1538		
TC	Li		H			
			L	2.28	20	8.77
	Lu		H			
			L			
	T		H			
			L			

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

Project No. 02498

NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 10-17-74

Strain D4

Test	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	EMS	1.0 %	6.11	775	916	126.84	149.92
SC	Saline	-	6.25	42	42	6.72	6.72

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2498

NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 10-17-74

			Strain D4			
Test	Compound	Concentration	Total Population Screened[a]	Number Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC 1	9159	H	12.54	69	72	5.50	5.74
	9159	L	8.14	65	43	8.00	5.28

NOTE: TC = test compound
H = high dose
L = low dose
a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present
( ) = percent survival

Project No. 02498

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Mouse DATE: 10-17-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	4.87	46	42	9.45	8.62
	Lu	DMNA	150 µmoles/ml	6.72	29(c)	28	4.32	4.17
	T	DMNA	150 µmoles/ml	5.67	19	26	3.35	4.59
SC	-	DMNA	150 µmoles/ml	4.66	10	14	2.15	3.00
	-	SALINE	-	7.67	45	20	5.87	2.61

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2489

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ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: MOUSE DATE: 10-17-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	9159	H	-	Toxic		-	-
		9159	L	6.49	45	31	6.93	4.78
	Lu	9159	H	4.98	10	6	2.01	1.21
		9159	L	6.62	39	39(c)	5.89	5.89
	T	9159	H	6.61(c)	43	29	6.51	4.39
		9159	L	6.83	40	36	5.86	5.27

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 02498

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Pub/Journal Name	—	9					
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Author(s)	—	10					
Organ. Name	E I DUPONT DENEMOURS & CO INC	11					
Dept/Div	—	12					
P.O. Box	—	13	Street No./Name	—	14		
City	WILMINGTON	15	State	DE	16	Zip	19898 17	Country	—	18
MID No. (7)	—	19	D & B NO. (11)	—	20		
Contractor	LITTON BIONETICS	21					
Doc Type	• RI • U.P • H.E.A.S.D 8D., S.U H.S F.N	22					
Doc Title	MUTAGENIC EVALUATION OF COMPOUND 9762	23					

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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3/8/85 BS

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(13)

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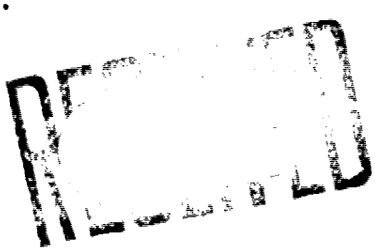

MUTAGENIC EVALUATION OF
COMPOUND

9762

1,3-Butadiene, 2-chloro-

SUBMITTED TO

HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE 19711

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

AUGUST 15, 1975

Litton BIONETICS

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DATE: August 15, 1975

SPONSOR: Haskell Laboratory for Toxicology, Newark, Delaware

SUBJECT: Plate Test Mutagenicity Evaluation of Compound 9762

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: July 30, 1975
2. Description: Clear Liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae* D4

Bacteria Strains: *Salmonella typhimurium* TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests: (components 1-4 make up the "Core Reaction Mixture"):

Component	Final Concentration/ml
1. TPN (sodium salt)	6 μM
2. Isocitric acid	49 μM
3. Tris buffer, pH 7.4	28 μM
4. $MgCl_2$	1.7 μM
5. Supernatant fraction equivalent to 70 mg of fresh tissue	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver tissues of the following mammalian species: Sprague Dawley adult male rats pretreated with Arochlor 1254 (500 mg/kg IP injection five days before sacrifice).

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Ethyl methanesulfonate (EMS)	Water or saline	BPS[b]
	2-Nitrofluorene (NF)	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard (QM)	Water or saline	FS[b]
Activation	Dimethylnitrosamine (DMNA)	Water or saline	BPS[b]
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide[c]	FS[b]
	7,12-Dimethylbenzanthracene (DMBA)	Dimethysulfoxide[c]	FS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor driven homogenizing unit at 4C. The whole organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C. Samples from these preparations were used for the activation studies.

B. Plate Tests (Overlay Method)

Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

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IV. SUMMARY OF TEST RESULTS

Plate Test:

A. Name or code designation of the test compound: 9762 (DuPont)

B. Test Date: August 4, 1975

C. Concentrations of the test compound: (1) .1 µl (2) 1 µl (3) 10 µl (4) 100 µl/Plate

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4
1. Nonactivation								
Solvent control	--	--	10	11	23	21	42	56
Positive control[a]	--	--	$>10^4$	>500	$>10^3$	$>10^3$	102	203
Test compound (1)	--	--	10	10	10	22	30	55
(2)	--	--	13	9	20	22	24	63
(3)	--	--	14	11	23	21	45	65
(4)	--	--	11	12	22	19	14	70
2. Activation								
Solvent control	--	Liver	34 18**	15	16	30	85	40
Positive control[b]	Rat	Liver	325 215	184	$>10^3$	$>10^3$	211	56
Test compound (1)	Rat	Liver	12 6	a7	17	20	103	43
(2)	Rat	Liver	23 23	12	19	18	102	33
(3)	Rat	Liver	53 21	15	25	24	105	42
(4)	Rat	Liver	115 175	12	23	44	102	44

a

TA-1535	EMS	10	µl/plate
TA-100	EMS	10	µl/plate
TA-1538	NF	100	µl/plate
TA-98	NF	100	µl/plate
TA-1537	QM	10	µl/plate
D4	EMS	10	µl/plate

b

TA-1535	DMNA	50	µM/plate
TA-100	DMNA	50	µM/plate
TA-1538	AAF	10	µg/plate
TA-98	AAF	10	µg/plate
TA-1537	DMBA	100	µg/plate

* The chemical was completely toxic at 500 µl/Plate.
** Repeat trial

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V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound 9762 was tested for genetic activity in a series of plate assays using five (5) Salmonella typhimurium and one (1) Saccharomyces cerevisiae strain. The assays were conducted with the chemical directly and in the presence of rat liver microsomal enzyme preparations from Arochlor 1254-induced rats. The following results were obtained:

A. Toxicity

The test chemical showed slight toxicity at 100 µl/plate and essentially complete killing at 500 µl/plate.

B. Nonactivation Assays

There was no indication of mutagenic activity for 9762 in these assays. The data from 500 µl/plate tests are not shown because the numbers of colonies per plate was always less than 10 and usually zero.

C. Activation Assays

Strain TA-1535 responded to the test chemical at 10 µl and 100 µl/plate. The response although not strong, was reproducible in a repeat trial. None of the other indicator strains responded to the test chemical. Strain TA-100 did not respond to the mutagenic activity of 9762 in these tests.

D. Conclusions

The test chemical 9762 was weakly mutagenic for TA-1535 in activation assays at concentrations of 10 µl and 100 µl per plate. These concentrations were slightly toxic and the next higher dose of 500 µl/plate was completely toxic. None of the other indicators exhibited a response to the test agent.

Submitted by:

David Brusick

David Brusick, Ph.D.
Director of Genetics

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Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
Ø8Ø877		Ø22285		N			

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Pub/Journal Name					9		
					9		
Author(s)					10		
Organ. Name	E I DUPONT DENEMOURS & CO INC				11		
Dept/Div					12		
P.O. Box	13	Street No./Name			14		
City	WILMINGTON	15	State	DE	16	Zip	19898	17	Country	18
MID No. (7)	19	D & B NO. (11)			20		
Contractor					21		
Doc Type	• R.I • U.P • HEA.S.D 8.D. , S.U H.S F.N				22		

Doc Title 23

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO IN THE SALMONELLA/MICROSOME ASSAY

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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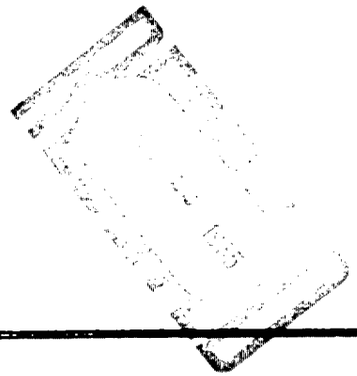

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

Material Tested: 1,3-Butadiene, 2-chloro- (Tested as a gas)*

Material Submitted By: R. S. Barrows, Elastomer Chemicals Department, Experimental Station

Sample Ready for Testing: 6-22-77

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN THE *SALMONELLA*/MICROSOME ASSAY

Materials and Methods: Four histidine-requiring strains of *Salmonella typhimurium* are used in the mutagen assays. Strains TA 1535 and TA 100 are used to detect base-pair substitution mutations, whereas TA 1537 and TA 98 are used to detect frame-shift mutations. (Ames, McCann and Yamasaki, Mutation Research 31 (1975) 347-364).

The assays are performed in the presence and absence of a rat-liver homogenate activation system. In the absence of metabolic activation, approximately 10^8 bacteria are added to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). The solution is mixed and poured on the surface of a Davis minimal agar plate (containing 20 ml of agar) prior to the exposure to the test gas. In the metabolically activated system 0.5 ml of S·9 mix is added to the bacteria-top agar mixture. The S·9 mix contains per ml: 0.3 ml of the 9000 X g supernatant of homogenized rat liver, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). This mixture is added to the top agar immediately before it is poured over the minimal agar plate.

The minimal plates with the bacteria overlay were exposed to the test gas in 9-liter glass chambers. The chambers, with Teflon® stopcocks and Viton® o-ring gaskets, were specially designed for this purpose. The petri plates, without lids, were held in the chamber by stainless steel racks, ten plates per chamber. Volumes of 0.01 ml, 0.05 ml, 0.1 ml and 0.2 ml of 1,3-butadiene, 2-chloro-, a liquid at room temperature, were placed in the exposure chambers in glass dishes. The chambers were then placed in a 37°C incubator for 48 hours. 1,3-Butadiene, 2-chloro- was completely volatilized at 37°C and the calculations of the concentration of vapor in the chamber was based on ideal gas laws. At the end of the exposure period the chambers were flushed with air for 5 volume changes, the plates removed and counted.

*See HL Report No. 571-77 for compound tested as a liquid.

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The concentration of the test gas in the exposure chamber was measured using a "Varian Aerograph" Model 600-D gas chromatograph. The concentrations of the test gas reported in the appended tables are the measured values.

Prior to testing for mutagenicity, the gas was tested for toxicity. TA 1535 is routinely used to determine the general toxicity range for all strains. Exposure in atmospheres that contained up to 1.12% of the compound did not inhibit bacterial growth.

The procedure used for Ethylene, chloro-, the positive control, is that for a standard gas using a flow-meter system.

Results: Tables I and II.

Summary: 1,3-Butadiene, 2-chloro- was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 in atmospheres that contained up to 1.12 per cent of the compound.

The compound was mutagenic in strains TA 1535 and TA 100 in the activated assays. TA 1535 and TA 100 also show slight activity in the nonactivated assays. The activated assay for TA 1535 in an atmosphere that contained 1.12% compound gave a 12-fold increase over the spontaneous mutation frequency.

Report by: Antje Koops

Antje Koops
Molecular Biologist

Approved by: David F. Krahn

David F. Krahn
Chief, Molecular Biology Section

AK:DFK:ms

Date: August 1, 1977

Revised: August 8, 1977

TABLE I

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 98 AND TA 100 WITH AND WITHOUT METABOLIC ACTIVATION

TRIAL I

	Histidine$^+$ Revertants Per Plate*			
1,3-Butadiene, 2-chloro-	TA 1535	TA 1537	TA 98	TA 100
WITH ACTIVATION				
0%	7	6	27	119
0.04%	24	4	25	180
0.5%	74	7	30	348
0.6%	96	4	27	491
Ethylene, chloro-				
10%	158			345
WITHOUT ACTIVATION				
1,3-Butadiene, 2-chloro-				
0%	13	8	21	122
0.04%	17	4	19	159
0.5%	35	8	17	154
0.6%	36	6	16	182
Ethylene, chloro-				
10%	164			295

*Activated Assay - Average number of revertants from 3 plates
Nonactivated Assay - Average number of revertants from 2 plates

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TABLE II

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN *SALMONELLA TYPHIMURIUM* STRAIN TA 1535 WITH AND WITHOUT METABOLIC ACTIVATION

TRIAL II

	Histidine+ Revertants Per Plate*
	TA 1535
WITH ACTIVATION	
1,3-Butadiene, 2-chloro-	
0%	9
0.035%	26
0.4%	57
0.66%	77
1.12%	109
Ethylene, chloro-	
10%	192
WITHOUT ACTIVATION	
1,3-Butadiene, 2-chloro-	
0%	13
0.035%	16
0.4%	34
0.66%	34
1.12%	40
Ethylene, chloro-	
10%	155

*Average number of revertants from 10 plates

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CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) •	OTS Ø2Ø6752	1	No. of Pages		2		
Doc I.D.	878215ØØ3	3	Old Doc I.D.	8DS	4		
Case No.(s)	OTS 84ØØ3A	5					
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
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Pub/Journal Name						9	
						9	
Author(s)						10	
Organ. Name	E I DUPONT DENEMOURS & CO INC					11	
Dept/Div						12	
F.O. Box	13	Street No./Name				14	
City	WILMINGTON	15	State	DE	16	Zip	19898 17
Country	18						
MID No. (7)	19	D & B NO. (11)	20				
Contractor	21						
Doc Type	• RI • U.P • H.E.A.S.D S.D. S.U H.S F.N	22					

Doc Title 23

MUTAGENIC ACTIVITY OF 1, 3-BUTADIENE, 2-CHLORO IN IN THE SALMONELLA/MICROSOME ASSAY

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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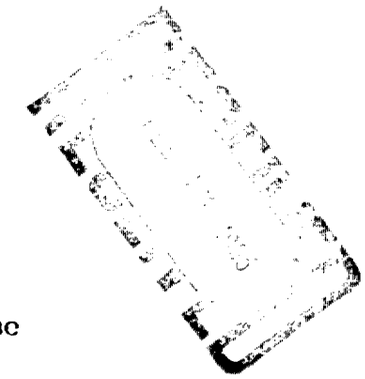

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

Material Tested: 1,3-Butadiene, 2-Chloro (Tested as a liquid)*

Sample Ready for Testing: 6-22-77

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN THE *SALMONELLA*/MICROSOME ASSAY

Materials and Methods: Five histidine-requiring strains of *Salmonella typhimurium* were used in the mutagen assays. Strains TA 1535 and TA 100 are used to detect base-pair substitution mutations, whereas strains TA 1537, TA 1538 and TA 98 are used to detect frame-shift mutations. (Ames, McCann and Yamasaki, Mutation Research 31 (1975) 347-364).

The tests were performed in the presence and absence of a rat-liver homogenate activation system (S-9). In the absence of metabolic activation, 0.1 ml of a solution of the test compound and approximately 10^8 bacteria were added to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). The solution was mixed and poured on the surface of a Davis minimal agar plate. The metabolic activation system involved the addition of 0.5 ml of S-9 mixture to the chemical-top agar solution. The S-9 mix contains per ml: 0.3 ml of the 9,000 X g supernatant of homogenized rat liver, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). This mixture was added directly to the top agar immediately before it was poured over the minimal agar plate.

Prior to testing for mutagenicity, the compound was tested for toxicity. TA 1535 is routinely used to determine the general toxicity range for all strains.

Appropriate controls were included for each strain. In the nonactivated system these controls consisted of a negative, or solvent control, and positive controls. A second negative control (-S-9 control) is included in the activated assay to measure any activity of the compound in the absence of the S-9 activator mixture.

All plates were incubated at 37°C for 48 hours.

*See HL Report 570-77 for compound tested as a gas.

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Results: Tables I and II.

Summary: 1,3-Butadiene, 2-chloro- was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 in concentrations up to 3000 µg per petri plate. The compound was classified as mutagenic in strains TA 1535 and TA 100 in the activated assay. Strains TA 1535 and TA 100 also show slight activity in the nonactivated assay. In TA 100 in the activated assay this activity represents a 15-fold increase over the spontaneous mutation frequency or 0.06 revertants per nmole of compound tested. All calculations are based on the amount of 1,3-Butadiene, 2-chloro- added to the plates at the beginning of the assays. Some of the compound may have volatilized during the 48 hour incubation period.

Report by: Antje Koops

Antje Koops
Molecular Biologist

Approved by: David F. Krahn

David F. Krahn
Chief, Molecular Biology Section

AK:DFK:ms

Date: August 1, 1977

TABLE I

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITH METABOLIC ACTIVATION

Compound Added			Histidine$^+$ Revertants Per Plate**				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO			15	14	19	28	105
11,321		µg/plate					
-S·9*	750	"	75	7	13	25	381
	600	"	46	7	16	21	256
	1200	"	167	9	25	26	605
	1800	"	220	12	20	60	1099
	2400	"	237	15	18	78	1527
	3000	"	172	10	27	49	881
2AA	5	"					2004
	10	"	273		2005	1549	
	100	"		141			

DMSO = Dimethylsulfoxide (solvent control)

11,321 = 1,3-Butadiene, 2-chloro-

2AA = 2-Aminoanthracene (positive control)

* = Control plate without S·9 activators

** = Average number of revertants from 2 plates.

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TABLE II

MUTAGENIC ACTIVITY OF 1,3-BUTADIENE, 2-CHLORO- IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 1538, TA 98 AND TA 100 WITHOUT METABOLIC ACTIVATION

Compound Added			Histidine$^+$ Revertants Per Plate*				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO			15	9	11	24	119
11,321		µg/plate					
	150	"	21	10	15	22	125
	300	"	33	8	9	22	162
	450	"	40	10	13	26	192
	600	"	40	13	16	21	195
	750	"	47	18	12	27	275
MNNG	2	"	3350				1342
2NF	25	"			1939	2029	
9AAc	50	"		2593			

DMSO = Dimethylsulfoxide (solvent control)

11,321 = 1,3-Butadiene, 2-chloro-

MNNG = N-Methyl-N'-Nitro-N-Nitrosoguanidine (positive control)

2NF = 2-Nitrofluorene (positive control)

9AAc = 9-Aminoacridine (positive control)

* = Average number of revertants from 2 plates

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CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) •	OTS 0206752	1	No. of Pages	2			
Doc I.D.	878215004	3	Old Doc I.D.	8DS	4		
Case No.(s)	OTS 84003A II				5		
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
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Pub/Journal Name					9		
					9		
Author(s)					10		
Organ. Name	E I DUPONT DENEMOURS & CO INC				11		
Dept/Div					12		
P.O. Box	13	Street No./Name			14		
City	WILMINGTON	15	State	DE	16	Zip	19898 17	Country	18
MID No. (7)	19	D & B NO. (11)			20		
Contractor					21		
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18-MONTH INHALATION CARCINOGENICITY STUDY OF BETA-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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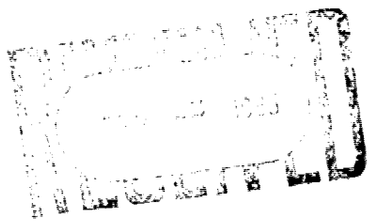

REPORT NO. R 6328

AN 18-MONTH INHALATION CARCINOGENICITY STUDY OF β-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

126-99-8

(Draft)

Authors : Drs. P.G.J. Reuzel
Drs. M.C. Bosland

At the request of : Joint Industry Committee on Chloroprene

Date : January 1980

Project number : B76/4975

Approved by : Dr. V.J. Feron

Start of exposures : September 30, 1976

Termination of exposures : March 29, 1978

Study director : Drs. P.G.J. Reuzel

All raw data and the final report are filed in the archives of the Department of Biological Toxicology under the reference "Joint Industry Committee on Chloroprene".

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Gehele of gedeeltelijke publikatie van dit rapport is zonder schriftelijke toestemming verboden.
Total or partial publication of this report is prohibited.

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SUMMARY

1. An 18-month inhalation carcinogenicity study with chloroprene was performed in hamsters. Three groups of 100 male and 100 female hamsters each were exposed to 0 (control group), 10 or 50 ppm chloroprene, 6 hours/day, 5 days/week, for a period of 18 months.

2. No abnormalities were observed in behaviour.

3. Mortality was lower in both test groups than in the controls.

4. Growth retardation was observed in males and females of the high-level group.

5. A slight reduction in amyloidosis was observed in hamsters of the high-level group.

6. At the 10 ppm level no adverse effect was found.

7. No indications were found for carcinogenic properties of chloroprene up to an exposure level of 50 ppm.

AN 18-MONTH INHALATION CARCINOGENICITY STUDY OF β-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

1. INTRODUCTION

At the request of the Joint Industry Committee on Chloroprene, a chronic (18-month) inhalation study with β-chloroprene was performed in hamsters. The exposure levels were selected on the basis of the results of a previous 4-week inhalation study with chloroprene in hamsters, in which compound-related effects were found at levels of 627, 162 and 39 (Reuzel et al. 1976). The effects were mortality, nose and eye irritation, incontinence of urine and faeces, microscopical changes in the respiratory tract and the liver and changes in relative weights of some organs. Since the effects observed at 39 ppm consisted of slightly abnormal behaviour and a minimal degree of nose and eye irritation, 50 ppm was selected as high-dose level for the present study, expecting that this dose would induce mild, but distinct toxicity. The low exposure level was fixed at 10 ppm.

2. PROCEDURES

2.1 Material and methods

Twelve bottles, each containing 200 ml freshly purified 1,3-butadiene, 2-chloro, synonym: β-chloroprene (further indicated as chloroprene), were received weekly from Bayer AG, Dormagen, W.-Germany, and stored at -20°C.

To generate the various test atmospheres, sufficient quantities of the test material were delivered to fritted glass vaporization vessels kept at 0°C. A measured, dried and filtered nitrogen flow was passed through the vessels. Each of the vapour/nitrogen mixtures thus produced was led into the inlet piece of an inhalation chamber, where it was mixed with the main air flow. Teflon and stainless steel transport tubes were used.

2.2 Analyses

Samples of the test atmospheres were taken from each inhalation chamber during the exposures (average three samples/hour) by means of a sample loop and then injected into an Intersmat IG 120 gas-chromatograph fitted with a flame ionisation detector. The following operating conditions were established:

Column:		300 x 4 mm parked with Carbowax 20M/chromosorb PAW
Temperature:	a. Injection port	95° C
	b. Oven	65° C
	c. Detector	150° C

Exposure chamber concentrations of chloroprene were established by comparing the peak areas produced by samples of the atmosphere with those produced by a standard chloroprene solution. The sample loop was calibrated by comparing peak surfaces of atmosphere samples taken with a gas-tight syringe with peak surfaces of simultaneously taken loop-samples.

2.3 Animals

Three hundred male and three hundred female Syrian golden hamsters were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, in September 1976. On arrival, they weighed 35-55 g and were four weeks old. After an acclimatization period of two weeks the animals were allocated equally, according to body weight and sex, to three groups of 100 males and 100 females each.

2.4 Exposure chambers

The chambers used had a capacity of 2.5 m^3 and were constructed of stainless steel with a glass door, which allows observation of the animals during the exposures. The chambers had a cylindrical form with a conical top and bottom. Inside they were fitted with a heptagonal frame, which was rotatable around the vertical axis of the chamber.

Forty two wire mesh cages, each with a floor surface of 930 cm^2, were suspended in the rotatable frame in seven vertical rows and six horizontal layers. The position of the rows was changed each day in a fixed order. The position of the animals in the layers was changed once every two months. The chambers were illuminated externally by normal laboratory TL-lighting, resulting in an intensity of light inside the chamber varying from 1 - 7 lumen, dependent on the position of the cage in the chamber. The light cycle was 12 hours light and 12 hours dark (light from 06.00 a.m. to 06.00 p.m.).

To prevent leakage of the test atmospheres into the room, the chambers were maintained at a negative pressure of 1-2 mm H_2O, relative to the room. The main airflow was drawn from the room, entered the chambers at the top, and had - when entering the inhalation chambers - a temperature of 21 $\pm$ 2° C and a relative humidity of 45 - 60 per cent. Before entering the inhalation chambers the main airflow was mixed with a nitrogen flow which was laden with the test material. In the chambers the airflow was equally spread by an air disseminator which was located in the top of the upper cone of the chambers.

The flow rate through the chambers was approximately 40 m^3/h during the exposures and 60 m^3/h thereafter.

2.5 Maintenance

All animals were housed in the inhalation chambers during 24 hours a day. For each group all conditions were the same, except for the concentration of the test material in the atmosphere during the exposures. Five males or five females were housed in each cage.

Neither food nor water was present in the inhalation chambers during the exposures. During the non-exposure periods the hamsters were provided with bottled unfluoridated tap water and a pelleted basal diet (Muracon I, Trouw & Co. N.V., Amsterdam - Putten, The Netherlands). The levels of contaminants in tap water were determined periodically (Appendix 1).

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2.6 Exposures

Groups of 100 hamsters of each sex were exposed to one of three different atmospheres, for 6 hours a day, 5 days a week during a period of 18 months. The nominal concentrations were 0, 10 and 50 ppm chloroprene. The exposures were generally started between 08.30 and 10.30 a.m. On public holidays the animals were not exposed.

2.7 Conduct of the study

2.7.1 Condition and behaviour

All animals were checked daily and all changes were recorded. Special attention was paid to the occurrence of tumours. During the last seven months of the test period all animals were palpated monthly for the presence of tumours. Time of appearance, size, location and progression were recorded. Moribund animals were killed to avoid spontaneous death and subsequent loss by autolysis or cannibalism.

2.7.2 Body weights

All animals were weighed immediately before the first exposure, weekly during the first 8 weeks and then at 4-week intervals. Animals loosing more than 10 per cent weight between two weighings were observed daily, until their weight gain was normal or they were sacrificed.

2.7.3 Pathology

After the 18-month exposure period the animals were sacrificed on 13 successive working days, in such a way that, on the average, time of sacrifice was approximately the same for each group. The hamsters were killed by exsanguination from the abdominal aorta under ether anaesthesia. A post-mortem examination was conducted during which the tissues were examined for gross abnormalities. The following organs were weighed *):

spleen	testes
adrenals	ovaries
kidneys	brain
liver	pituitary
heart	lungs with trachea and larynx

*) Organs of animals which died spontaneously or were killed in extremis were not weighed

Samples of the organs weighed and of those mentioned below were preserved in a 4 per cent aqueous neutral phosphate-buffered formaldehyde solution. The lungs were fixed (after weighing) by intratracheal infusion of the fixative under 10 cm water pressure

skin	thymus
external auditory canal with ceruminal (Zymbal's) glands	uterus
skeletal muscle	nose (four transverse sections)
sciatic nerve	eyes (with attached optic nerve)
mammary glands	sternum (bone marrow)
seminal vesicles	oesophagus
prostate	thyroid (with parathyroid)
epididymides	stomach
caecum	duodenum
colon	jejunum
pancreas	ileum
lymph nodes (axillary, cervical and mesenteric)	adipose tissue
urinary bladder	submaxillary salivary glands
spinal cord	exorbital lachrymal glands
femur (with knee joint)	parotid salivary glands
all gross lesions	sublingual salivary glands

The organs and tissues to be examined microscopically were processed to paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin. Nose, sternum and femur were decalcified prior to processing to paraffin wax. Transverse and longitudinal sections of the trachea including the bifurcation and longitudinal sections of the larynx and of each of the pulmonary lobes were prepared at three different levels.

Microscopic examination was carried out

a) of all organs and tissues preserved from all control and all high level hamsters and
b) of the liver, spleen, pituitary, thyroid, adrenals and of all grossly visible tumours or tumour-like lesions from hamsters of the low-level group.

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Attention was paid to detection of possible metastases from malignant tumours.

2.7.4 Statistical analyses

Statistical analyses of body weight changes and organ-to-body weight ratios were carried out using Student's t-test. The Chi-square test was used for analysing differences in the incidence of alopecia, of mortality and of pathological changes, including tumours.

3. RESULTS

3.1 Exposure period

The nominal exposure period extended for 18 months, from 30 September 1976 to 30 March 1978. The subsequent autopsies were carried out over a period of 17 days, during which the exposures were continued, so that the last exposure day was 17 April, 1978. The nominal exposure period comprised 385 planned exposure days. As a result of maintenance and repair work to the inhalation chambers on one of the planned exposure days exposure could not take place: therefore the actual number of exposure days was 384 for both test groups.

3.2 Chloroprene concentrations (table 1)

The actual mean concentrations of chloroprene in the test atmospheres during all the test period were 10.0 and 49.7 ppm for the low- and high-level group respectively.

3.3 Condition and behaviour (table 2)

No abnormalities were observed in behaviour in any of the groups. Alopecia was only occasionally seen in each of the groups during the first 64 weeks of the study. During the next weeks increasing numbers of hamsters were seen with more or less diffuse alopecia of back and sacral region. In week 68 and at the end of the experimental period all animals were examined for alopecia. Because of the considerable differences in mortality between the groups, not only the number of hamsters showing alopecia was given, but also the incidences of the lesions expressed as percentages of the number of animals examined (table 2).

From this table it appears that the percentages of animals affected varied considerably both at week 68 and at the end of the study. Moreover, the differences between the test groups and the control group could not be confirmed by the Chi-square test. Therefore, chloroprene exposure was not considered to play a role in the development of the alopecia observed.

3.4 Mortality (table 3)

The death rate was negatively correlated with the concentration of chloroprene. In the high level group mortality started at a considerably later point of time and was clearly lower than in the controls, the differences being more pronounced in females than in males. In the low level group the differences with the controls were less pronounced and only statistically significant in females towards the end of the study.

3.5 Body weights (table 4; figures 1 and 2)

Slight growth depression was observed in hamsters of the high level group throughout the study. Body weight of the low level hamsters were similar to or differed only very slightly from those of the controls. A wave-like course of the body weight curves (figures 1 and 2) occurred in all groups. This phenomenon is quite normal in hamsters, and is assumed to be associated with seasonal factors.

3.6 Organ weights (table 5)

Relative brain weight was statistically significantly higher in hamsters of the high level group than in the controls. Relative lung weight was slightly, but statistically significantly higher in males of the high level group than in the controls. A few isolated statistically significant changes of relative organ weights were: low relative weights of kidneys, liver and adrenals in males of the low-level group and a low relative spleen weight in males of the high-level group.

3.7 Pathology

Macroscopic examination (table 6)

Macroscopic examination at autopsy revealed a slight, dose-related decrease in incidence of pale adrenals in males. No other remarkable differences in gross changes were noticed between the various groups.

Microscopic examination (table 7 and 8)

a) Non-neoplastic lesions

The site, type and incidence of non-neoplastic changes observed in hamsters of the control and high-dose group are presented in table 7. The incidence of generalized amyloidosis was slightly lower in the high-dose group than in controls. Amyloid deposits in the liver, kidneys, spleen and adrenals were less frequently observed in high-dose animals than in controls, the differences often being statistically significant.

Several other differences between control and high-dose groups were statistically significant. These differences, however, were small and the alterations in question were of a minor degree, and are common in aged Syrian golden hamsters of the strain used. Therefore, the differences are considered not to be related to chloroprene exposure but rather to the large variability in incidence of this type of lesions.

b) Neoplastic lesions

The site, type and incidence of the neoplastic lesions found in the various groups are presented in table 8. The incidence of cystadenomatous polyps of the gall-bladder was slightly elevated in the males of the low-dose group. All other tumours observed were about equally distributed among test and control groups, or occurred only once or twice. Moreover, they are all common neoplastic lesions as is shown in recent papers on the background pathology in the strain of hamsters used (Pour et al. 1976, a, b, c and d).

4. DISCUSSION AND CONCLUSIONS

During the previous 4-week range-finding inhalation study with chloroprene in hamsters severe toxic effects were found at levels of 627 and 162 ppm in air (Reuzel et al. 1976). The changes observed at a level of 39 ppm were only very slight and consisted of minimal irritation of the mucous membranes of the nasal cavity, transient restlessness and some eye irritation. On the basis of these results it was expected that in a chronic inhalation study with hamsters 50 ppm chloroprene would be an effect level, but would not induce marked toxicity. This expectation was confirmed by the present chronic study from which it appeared that at the 50 ppm level a slight reduction in body weight gain occurred.

Though in the 4-week study with hamsters (Reuzel et al. 1976) one animal exposed to 162 ppm chloroprene showed severe alopecia and moreover, hair loss as a result of chloroprene exposure has been observed in rats (Reuzel et al. 1979) and also in men (Nyström, 1943; Ritter and Carter, 1948; Swartz,1945), the results of the present study do not provide evidence of chloroprene inducing or enhancing the formation of alopecia in hamsters.

The increase in relative brain weight in the high-level group was not accompanied by treatment-related histopathological changes in this organ. Since it is known that in rats an inverse relationship exists between body weight and relative brain weight (Feron et al. 1973),it is most likely that the increase in relative brain weight in high-level hamsters is the result of the relatively low body weight of these animals rather than the expression of an effect of chloroprene on this organ.

A few isolated statistically significant changes in relative organ weight in no case were accompanied by compound-related histopathological changes. They were, therefore, regarded as fortuitous findings unrelated to chloroprene exposure.

The pathological examinations revealed some indications that high-level hamsters were less affected by generalized amyloidosis than the controls. This finding may have some relation to the better survival of the high-dose animals as compared to the controls. In the 4-week study with chloroprene (Reuzel et al. 1979) some flattening of the lining epithelium of the nasal cavity was found in some hamsters exposed to 39 or 162 ppm of the test substance. In

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the present study there was no evidence of chloroprene exposure affecting the epithelium of the nasal mucosa.

In conclusion, the present study in hamsters provides evidence that exposure to 50 ppm chloroprene resulted in:

a. slight depression of body weight gain;
b. better survival;
c. slight reduction of amyloidosis

At the level of 10 ppm no effect of chloroprene was detected.

There was no evidence of chloroprene possessing carcinogenic activity in Syrian golden hamsters.

5. REFERENCES

1. Feron, V.J., A.P. de Groot, M.T. Spanjers and H.P. Til
   An evaluation of the criterion "organ weight" under conditions of growth retardation
   Food Cosmet. Toxicol. 11 (1973) 85-94

2. Nyström, A.E.
   Health hazards in the chloroprene rubber industry and their prevention
   Acta Med. Scand. 132 (1948) Suppl. 219, 1-125

3. Pour, P., N. Kmoch, E. Greiser, U. Mohr, J. Althoff and A. Cardesa
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 a.) 931-935
   I. Incidence and sites

4. Pour, P., U. Mohr, A. Cardesa, J. Althoff and N. Kmoch
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 b.) 937-948
   II. Respiratory tract and digestive system

5. Pour, P., U. Mohr, J. Althoff. A. Cardesa and N. Kmoch
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 c.) 949-961
   III. Urogenital system and endocrine glands

6. Pour, P., U. Mohr, J. Althoff, A. Cardesa and N. Kmoch
   J. Nat. Cancer Inst., 56 (1976 d.) 963-974
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   IV. Vascular and lymphatic systems and lesions of other sites

7. Reuzel, P.G.J., W. Tichelman, H.R. Immel and V.J. Feron
   Sub-acute (4-week) inhalation toxicity study with β-chloroprene in hamsters
   Central Institute for Nutrition and Food Research TNO 1976
   Report number R 5085

8. Reuzel, P.G.J., M.C. Bosland, H.C. Dreef van der Meulen and V.J. Feron
   A (2-year) inhalation carcinogenicity study of chloroprene in rats
   Central Institute for Nutrition and Food Research TNO
   1980 Report number R 6077

9. Ritter, W.L. and A.S. Carter
   Hair loss in neoprene manufacture
   J. Ind. Hyg. Toxicol. 30 (1948) 192-195

10. Schwartz, L.
    Skin hazards in the manufacture and processing of synthetic rubber
    J. Am. Med. Assoc. 127 (1945) 389-391

We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedure herein described, and that this report represents a true and accurate record of the results obtained.

p.o.

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Table 1. Actual mean chloroprene concentrations, their standard deviation and standard error of the mean during 18 months

Group	Mean concentration of chloroprene					
	in ppm			in mg/m^3 air		
	Mean	SD[1)]	SEM[2)]	Mean	SD	SEM
Low dose	10.0	0.3	0.0	36.7	1.2	0.1
High dose	49.3	1.3	0.1	182.4	4.9	0.2

[1)] SD = Standard deviation

[2)] SEM= Standard error of the mean

Table 2. Degree and incidence of alopecia

Degree of alopecia	Number of hamsters with alopecia					
	males			females		
	chloroprene concentration (in ppm)			chloroprene concentration (in ppm)		
	0	10	50	0	10	50
	week 68					
a. slight	23	16	26	32	45	33
b. moderate	5	2	4	7	3	18
c. severe	1	0	0	0	0	1
total incidence	29	18	30	39	48	52
	at terminal sacrifice (week 79-81)[1]					
total incidence	32	20	28	17	16	24
	Incidence of alopecia expressed as percentage of the number of animals examined					
	week 68					
a. slight	25	17	27	41	54	35
b. moderate	5	2	4	9	4	19
c. severe	1	0	0	0	0	1
total incidence	32	19	31	49	58	55
	at terminal sacrifice (week 79-81) [1]					
total incidence	37	22	31	27	21	41

[1] The degree of alopecia was not recorded at autopsy

Table 3. Cumulative mortality in groups initially consisting of 100 male and 100 female hamsters

Chloroprene concentration (in ppm)	Total number of deaths at the end of week																	
	4	8	12	16	20	28	36	40	44	48	52	56	60	64	68	72	76	78
Males																		
Control	0	1	5	6	6	6	6	7	7	7	7	7	8	8	9	10	11	12
10	1	2	3	3	3	3	3	3	3	4	4	4	4	4	5	5	8	8
50	0	0	0*	1	1	2	2	2	2	2	3	3	3	3	3	6	7	7
Females																		
Control	0	0	0	1	2	3	3	3	3	4	7	10	13	16	21	30	37	37
10	0	0	0	0	0	0	2	4	7	8	9	10	11	12	17	20	23*	25*
50	0	0	0	0	0	0	0	0	0	0*	1*	3*	4*	5*	6**	11***	17*	23*

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$

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Table 4. Mean body weights of groups initially consisting of 100 male and 100 female hamsters

Chloroprene concentration (in ppm)	Mean body weights (in g) at the end of week													
	0	1	4	8	12	17	24	32	40	48	56	64	72	78
Males														
0	83	91	103	109	111	112	107	105	104	108	108	90	94	101
10	83	92	104	109	111	109	107	102	101	105	107	98***	101**	101
50	83	89	101	107	106*	106**	99***	98**	97***	99***	99**	91	91	94***
Females														
0	84	97	112	120	124	130	127	117	115	117	117	102	95	99
10	85	94	110	120	127	131	128	115	110	111**	112*	102	95	98
50	85	91***	104**	111***	111***	116***	110***	99***	100***	106***	99***	90***	86***	90***

*P≤0.05; **P≤0.01; ***P<0.001

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Table 5. Mean body weights (g), mean relative organ weights (g/100 g body weight) and the standard error of the mean of groups initially consisting of 100 female hamsters

group no.	β-chloroprene ppm	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	pituitary	adrenals	lungs	number of survivors
						MALES						
8168	0	101.	.523	1.25	5.11	.197	1.10	2.30	.0068	.0311	.85	86
		(2.)	(.008)	(.02)	(.11)	(.005)	(.02)	(.05)	(.0003)	(.0012)	(.01)	
8169	10	101.	.507	1.17**	4.75*	.190	1.11	2.21	.0067	.0279*	.84	92
		(2.)	(.006)	(.02)	(.09)	(.007)	(.02)	(.05)	(.0003)	(.0003)	(.01)	
8170	50	93.***	.526	1.22	4.91	.174**	1.19***	2.36	.0078	.0294	.90**	92
		(1.)	(.006)	(.02)	(.11)	(.005)	(.02)	(.05)	(.0005)	(.0009)	(.01)	
						FEMALES						
8168	0	99	.628	1.48	6.73	.253	1.13	.036	.0094	.0340	1.01	60
		(2.)	(.013)	(.03)	(.19)	(.010)	(.02)	(.001)	(.0004)	(.0011)	(.02)	
8169	10	98	.595	1.50	6.54	.269	1.16	.036	.0084	.0356	.97	74
		(2.)	(.011)	(.03)	(.17)	(.011)	(.02)	(.001)	(.0004)	(.0016)	(.02)	
8170	50	90.***	.625	1.50	6.37	.286	1.24***	.042	.0100	.0383	1.01	72
		(2.)	(.011)	(.02)	(.18)	(.049)	(.02)	(.004)	(.0009)	(.0038)	(.02)	

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

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Table 6. Site, type and incidence of gross observations in survivors and non-survivors in the various groups

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
Initial number of animals	100	100	100	100	100	100
Effective number of animals	100	97	97	94	93	97
Mode of death:						
a) found dead	12	5	8	32	21	24
b) killed in extremis	2	2	1	5	5	6
c) scheduled sacrifice	86	93	91	63	74	73
Autolysis/Cannibalism:						
a) slightly autolytic	4	1	2	4	0	0
b) partially lost	8	3	3	27	14	17
c) completely lost	0	3	3	6	7	3
SKIN						
1. Scale	4	1	5	3	2	6
2. Alopecia	32	20	31	21	19	30
3. Abscess	1	0	0	0	0	0
4. Edema formation	0	0	0	0	0	1
AXILLARY LYMPH NODES						
1. Enlarged	2	0	2	0	0	1
PRAEPUTIAL / CLITORIAL GLANDS						
1. Enlarged	1	0	0	0	1	0
MAMMARY GLANDS						
1. Tumour or suspected of tumour	0	0	0	0	0	1
2. Evidence of secretory activity	0	0	0	2	3	1
ABDOMINAL CAVITY						
1. Tumour or suspected of tumour	0	0	0	0	0	1
2. Ascites	0	0	0	1	0	3
3. Haemorrhagic fluid	0	0	0	4	1	2
4. Peritonitis	2	0	1	0	0	0
SPLEEN						
1. Tumour or suspected of tumour	0	1	3	0	0	1
2. Splenomegaly	0	1	0	8	4	4
3. Atrophy	2	0	0	2	1	4
4. Granular surface	0	0	0	0	0	1
5. Pale.	1	0	1	0	0	0
6. Enlarged	1	0	1	0	0	0

Table 6. Continued 1

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
7. Cyst	0	0	1	0	0	0
ADRENALS						
1. Tumour or suspected of tumour	8	4	5	3	7	5
2. Small - unilateral	0	1	1	0	0	1
- bilateral	1	1*	1**	0	0	1
3. Discoloured - pale	11	3	0	4	8	3
- dark	3	3*	0	7	6	2
4. Spotted	9	2	7	2	5	1
KIDNEYS						
1. Tumour or suspected of tumour	2	1	0	0	0	3
2. Granular surface	6	2	10	27	33	27
3. Discoloured - pale	16	9	10	36	31	30
- dark	0	1	0	0	2	0
- greenish	3	4	2	18	17	12
4. Cyst(s)	1	0	0	1	0	1
5. Enlarged	2	0	0	0	0	0
STOMACH						
1. Tumour or suspected of tumour	1	1	4	1	1	0
2. Thickened cardia	2	0	0	0	0	0
3. Haemorrhage / erosion	1	1	0	2	2	0
4. Trichobezoar	3	2	2	1	0	0
5. Diverticulum	0	0	0	0	2	0
SMALL INTESTINES						
1. Prominent Peyer's patches	0	1	0	0	0	0
2. Enlarged/swollen	0	2	0	2	1	0
3. Haemorrhagic fluid	0	0	0	0	1	0
4. Dark mucosa	1	0	0	0	0	0
5. Evidence of inflammation	0	0	0	0	1	0
COECUM						
1. Enlarged/swollen	1	0	0	3	2	3
2. Thickened wall	1	0	0	0	0	0
COLON						
1. Tumour or suspected of tumour	0	0	0	1	0	0

Table 6. Continued 2

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
PANCREAS						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Dark	1	0	0	3	2	0
3. Hyaline aspect	0	0	0	1	0	0
4. Cyst	0	0	0	0	0	1
MESENTERIC LYMPH NODES						
1. Enlarged	1	1	2	3	1	1
2. Small	0	1	0	0	0	0
3. Greenish	0	0	0	0	1	1
URINARY BLADDER						
1. Proteïnaceous plug	1	0	0	0	0	0
SEMINAL VESICLES						
1. Evidence of inflammation	1	1	1			
2. Hyaline	59	57	61			
3. Cyst	0	1	0			
4. Enlarged	13	14	13			
5. Atrophy	8	4	7			
TESTES						
1. Atrophy - unilateral	9	9	10			
- bilateral	5	4	12			
2. Abscess	3	1	2			
3. Unilateral agenesis	5	3	4			
EPIDIDYMIDES						
1. Abscess	19	5**	11			
OVARIES						
1. Tumour or suspected of tumour				0	0	1
2. Cyst(s)				1	3	0
3. Enlarged				1	0	0
4. Haemorrhages				0	0	1

Table 6. Continued 3

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
LIVER						
1. Tumour or suspected of tumour	0	2	1	1	0	1
2. Prominent lobular pattern	11	5	6	5	6	11
3. Swollen	4	2	1	3	0	4
4. Discoloured - pale	2	1	6	4	0	5
- dark	2	2	1	0	4*	4*
- greenish	3	1	2	0	2	0
- yellow	0	0	0	0	0	1
5. Cysts	60	70	69	72	86	79
6. Small surface lesions (rosette, constriction)	0	0	2	2	1	0
7. White spots	1	0	0	1	0	0
8. Small right lateral lobe	0	1	0	0	0	0
GALL BLADDER						
1. Tumour or suspected of tumour	0	0	0	0	1	0
2. Enlarged	0	0	0	1	0	0
THORACIC CAVITY						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Hydrothorax	0	0	0	2	0	0
3. Haemothorax	0	1	0	4	0*	1
HEART						
1. Enlarged atrium (thrombus)	1	1	0	9	1**	1**
LUNGS						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Evidence of chronic respiratory disease	0	0	0	1	1	0
3. Spongy	1	1	0	9	6	7
4. Spotted	11	18	20	12	22	13
5. Atelectasis	0	0	0	1	2	0
6. Haemorrhage	1	0	0	3	0	1
7. Pleuritis	0	1	0	1	0	0
CERVICAL LYMPH NODES						
1. Enlarged	0	0	0	1	0	0

Table 6. Continued 4

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
SUBMAXILLARY SALIVARY GLANDS						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Edema	1	0	0	1	0	0
EXORBITAL LACHRYMAL GLANDS						
1. Discoloured - pale	1	0	0	1	0	0
- dark	1	0	0	0	0	0
THYROID						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Pale	0	0	0	0	0	1
EYES						
1. Pale	1	0	0	0	0	0
BRAIN						
1. Pale	0	0	0	0	0	1
2. Increase in cerebro-spinal fluid	1	1	0	0	0	0
PITUITARY						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Discoloured - pale	2	1	0	0	1	0
- dark	0	0	1	0	0	0
MISCELLANEOUS						
1. Small injuries in hind limb	0	3	4*	0	0	1
2. Cheek-pouch: evidence of inflammation	0	0	0	0	0	1

* $P < 0.05$, ** $P < 0.01$

Table 7. Site, type and incidence of non-neoplastic histopathological changes in animals of the control and the high-dose group[1)]

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
Initial number of animals	100	100	100	100
KIDNEYS	(92)	(95)	(84)	(88)
1. Some focal infiltrates of mononuclear inflammatory cells	9	4	30	12*
2. Tubular nephrosis: - slight	63	62	20	25
- moderate	18	7*	37	43
- marked	4	3	26	19
3. Degenerated glomeruli: - a few	34	37	19	19
- several	8	10	33	35
- many	2	2	29	32
4. Some calcareous deposits in the inter-cortico-medullary layer and/or papilla	22	22	59	50
5. Dilatation of tubules: - slight	17	6*	24	27
- moderate to marked	10	5	23	21
6. Casts within tubules: - a few	20	12	40	32**
- several to many	5	4	0	8
7. Slight urothelial hyperplasia in the renal pelvis	0	2**	2	3**
8. Slight to moderate amyloidosis	14	3*	44	27
9. Moderate urothelial degeneration	0	1	0	0
10. Moderate accumulation of proteinaceous droplets within tubular epithelium	0	0	2	0
11. Small cyst	0	0	1	0
12. Cortical infarct	0	0	0	1
13. Slight accumulation of yellow pigment in glomeruli	0	0	0	1
LIVER	(99)	(96)	(90)	(92)
1. A few periportal infiltrates of mono-nuclear inflammatory cells	13	9	18	7*
2. Slight periportal fibrosis	0	2	0	0
3. Slight to moderate vacuolation of liver cells: - centrilobular	2	0	1	0*
- focal	0	1	7	1
- diffuse	0	0	3	0
4. Slight focal necrosis	3	1	7	10
5. Proliferation of bile-ducts: - slight	39	31**	63	61
- moderate to marked	15	4*	20	24

Table 7. Continued 1

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
6. Foci of cellular alteration:				
- clear cell foci: - a few/small	2	3	22	20
- several/large	0	0	8	11
- one or a few basophilic foci	0	0	0	1
- one or a few eosinophilic foci	0	0	2	0
- one or a few mixed cell foci	0	0	0	2
7. Some RES-cell aggregates accompanied by necrotic hepatocytes	47	45	23	36
8. Moderate to marked centrilobular degeneration	0	0	2	2
9. Amyloidosis: - slight	9	6	41	29
- moderate to marked	7	1	15	16
10. Slight to moderate accumulation of yellow-brown pigment	32	27	83	81
11. Cyst(s) lined with flattened epithelium	47	53	73	71
12. Cyst(s) lined with cuboidal or cylindrical epithelium and showing papilliform outgrowths	0	0	1	1
13. Cholangiomatosis	3	8	5	2
14. A few-several infiltrates of polymorpho-nuclear inflammatory cells	1	3	1	0
15. A few giant cells	0	1	0	0
16. Abscess	0	1	0	0
17. Some extra-medullary haematopoiesis	1	1	1	0
18. Granuloma	2	0	0	0
19. Slight nuclear polymorphism	1	0	1	0
20. Moderate congestion	0	0	1	0
21. Slight focal calcification	0	0	1	2
GALLBLADDER	(84)	(80)	(67)	(72)
1. Cyst(s) - small	8	3	5	5
- medium to large	3	1	0	1
2. Cystic hyperplasia of epithelium:				
- slight	4	0*	0	1
- moderate to marked	2	2	2	5
SPLEEN	(94)	(94)	(84)	(85)
1. Slight fibrosis of red pulp	3	1	2	1
2. Slight/moderate increased amount of yellow-brown pigment in red pulp	17	20	8	5
3. Increased extramedullary haematopoiesis:				
- slight	12	3*	8	5
- moderate to marked	1	0	5	5
4. Depletion of the white pulp:				
- slight	9	9	21	12
- moderate to marked	13	9	16	14

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Table 7. Continued 2.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
5. Slight to moderate atrophy	2	3	2	1
6. Amyloidosis: - slight	2	4	14	9
- moderate	0	1	20	15
- marked	1	1	10	10
7. (Haemo) cyst(s)	0	4*	0	0
8. Abscess	0	1	0	0
PANCREAS	(89)	(92)	(84)	(78)
1. Some focal infiltrates of mononuclear inflammatory cells	3	4	2	1
2. Slight periarteritis	0	0	1	0
3. Slight focal vacuolation of exocrine cells	0	1	0	0
4. Slight focal loss of zymogen granules	0	2	1	1
5. Slight change of acini into (dilated) duct-like structures	3	11*	13	15
6. Degeneration of exocrine part: - focal, slight to moderate	4	3	9	4
- severe fatty atrophy	1	0	1	0
7. Cyst(s)	0	0	0	1
8. Granuloma	1	0	0	0
LUNGS	(85)	(96)	(89)	(93)
1. Slight focal infiltrates of mononuclear inflammatory cells	2	2	7	0**
2. Pneumonitis: - focal	0	2	4	5
- massive	1	0	0	0
3. Severe pleuritis	0	0	1	0
4. Thrombi in bloodvessels	0	1	0	0
5. Some foci of alveolar macrophages	3	1	1	3
6. Heart failure lung: - slight to moderate	8	5	5	7**
- marked	2	1	14	4
7. Slight focal epithelialization	0	0	1	2
8. A few small calcareous deposits in alveolar tissue	10	10	18	29
9. Granuloma	1	2	0	0
10. Slight focal oedema	0	0	1	0
11. Slight focal proliferation of mucoid cells in bronch(iol)i	1	0	0	0

Table 7. Continued 3.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
TRACHEA	(84)	(94)	(83)	(86)
LARYNX	(73)	(71)	(68)	(79)
1. Some subepithelial infiltrates of mononuclear inflammatory cells	2	0	0	2
2. Slight focal epithelial hyperplasia	1	1	1	0
3. Slight laryngitis	1	0	0	0
HEART	(95)	(96)	(87)	(93)
1. Some focal infiltrates of mononuclear inflammatory cells	9	6	2	6
2. Slight periarteritis	1	0	0	0
3. Slight myocardial fibrosis	0	0	3	3
4. Slight valvular oedema and fibrosis	0	0	1	1
5. Slight to moderate focal myocardial degeneration	5	4	11	7
6. Thrombus in atria and/or ventricles	4	3	29	22
7. Slight focal endocarditis	1	0	0	0
8. Slight focal epicarditis	0	0	1	0
9. Slight accumulation of yellow-brown pigment	1	0	2	0
10. Ectopic ossification	0	0	2	2
11. Slight focal necrosis	0	0	0	1
12. A few cholesterol clefts	0	0	0	1
STOMACH	(85)	(89)	(75)	(82)
1. Some focal infiltrates of mononuclear inflammatory cells	7	2	1	0
2. Large ulcer	1	0	0	0
3. Slight focal degeneration of fundic glands	2	1	0	0
4. A few cystic,dilated fundic glands	1	4	10	10
5. Slight hyperkeratosis of the forestomach	1	1	1	0
6. A few calcareous deposits in fundic glands	0	1	0	0
7. Slight focal necrosis	0	0	1	0
SKELETAL MUSCLE	(93)	(95)	(90)	(90)
1. Some focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Slight focal degeneration	1	0	0	0

Table 7. Continued 4.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
TESTES	(95)	(94)		
1. Abscess and/or granuloma	4	3		
2. Obstructive dilated seminiferous tubules	0	3		
3. Mineralization of seminiferous tubules	1	1		
4. Atrophy of seminiferous tubules: - a few	19	16		
- several	12	10		
- many	8	7		
5. Complete atrophy: - unilateral	3	3		
- bilateral	2	3		
6. A few multinucleated giant cells	1	4		
7. Slight to moderate proliferation of interstitial cells	0	2		
8. Unilateral agenesis	2	3		
9. Some focal infiltrates of mononuclear and/or polymorphonuclear inflammatory cells	0	3		
10. Slight accumulation of yellow-brown pigment	0	1		
11. Degeneration and desquamation of germinal epithelium	3	0		
EPIDIDYMIDES	(94)	(91)		
1. Ducts filled with cellular débris: - a few	2	6		
- several to many	7	11		
2. Epididymitis: - slight	6	2		
- moderate to marked	7	8		
3. Abscess(es)	12	7		
4. Granuloma(s)	7	2		
5. Large, obstructively dilated ducts	8	4		
6. Unilateral agenesis	2	3		
7. Mineralization within ducts	3	1		
COAGULATING GLANDS	(91)	(89)		
1. Moderate to marked adenitis	3	0		
2. Many glands containing cellular débris	1	0		
3. Slight mineralization of glands	2	2		

Table 7. Continued 5.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
SEMINAL VESICLES	(94)	(91)		
1. Moderate to marked vesiculitis	3	1		
2. Moderate, focal degeneration of glandular epithelium	1	0		
3. A few to several glands containing cellular débris	0	4*		
PROSTATE	(91)	(85)		
1. Slight to moderate prostatitis	4	2		
2. A few to several glands containing granular material and/or cellular débris	17	10		
3. Slight mineralization within glands	6	8		
4. A few cholesterol clefts	1	0		
UTERUS			(79)	(86)
1. A few cystic,dilated glands, often containing cellular débris			39	28*
2. Endometritis			1	0
3. Dilated uterine lumen			1	0
MAMMARY GLANDS	(0)	(0)	(3)	(1)
AORTA	(91)	(84)	(86)	(90)
1. Thrombus	0	0	1	0
OESOPHAGUS	(87)	(91)	(79)	(86)
PHARYNX[2)]	—	—	—	—
1. Subepithelial abscess	0	0	0	1
2. Focal pharyngitis	0	0	1	0
3. Focal degeneration of epithelium	0	0	1	0
CHEEK POUCH[2)]	—	—	—	—
1. Focal metaplasia of epithelium	0	0	0	1
SMALL INTESTINES	(89)	(95)	(73)	(81)
1. Slight hyperplasia of patches of Peyer	4	0*	0	0
2. A few focal infiltrates of mononuclear inflammatory cells	1	0	0	0
COECUM	(89)	(95)	(72)	(81)

Table 7. Continued 6.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
COLON	(89)	(95)	(72)	(81)
1. Several cystic,dilated crypts	0	0	0	1
URINARY BLADDER	(92)	(92)	(77)	(86)
1. Proteinaceous plug	8	3	0	0
SPINAL CORD	(81)	(79)	(67)	(77)
SCIATIC NERVE	(62)	(72)	(64)	(70)
BRAIN	(93)	(94)	(79)	(86)
1. Focal calcareous deposits	0	0	4	7
2. Slight focal degeneration	0	0	1	0
3. Slight focal meningitis	0	0	1	0
PITUITARY	(62)	(70)	(48)	(57)
1. Cyst in pars distalis	0	1	0	0
2. A few focal infiltrates of mononuclear inflammatory cells	0	0	1	0
3. Moderate focal calcification	0	0	0	1
THYROID	(76)	(75)	(75)	(80)
1. Some focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Activated appearance	2	0	0	0
3. Slight accumulation of yellow-brown pigment	0	0	0	1
4. Amyloidosis: - slight	0	0	18	14
- moderate to marked	0	1	14	9
5. Slight focal proliferation of parafollicular cells	2	2	0	1
6. A few cystic,dilated follicles, often containing cellular débris	14	12	22	35
7. Slight to moderate focal degeneration	2	8	9	10
PARATHYROID [2]	—	—	—	—
1. Slight hyperplasia	0	1	0	0
2. Cyst	0	0	0	1

Table 7. Continued 7.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
ADRENALS	(93)	(93)	(88)	(91)
1. Unilateral atrophy	1	0	0	0
2. (Haemo)cyst(s)	2	0	7	10
3. Slightly increased vacuolization of cortical cells	2	0	1	0
4. Slight extramedullary haematopoiesis	5	3	0	0
5. Slight to moderate focal degeneration of cortical cells	4	5	8	19*
6. Amyloidosis: - slight	9	9**	6	17
- moderate	16	3	34	37*
- marked	5	2	43	29
7. Focal hyperplasia of the zona glomerulosa: - slight	30	32	13	6
- moderate	6	1	1	0
8. Slight accumulation of yellow-brown pigment	11	4	1	1
OVARIES			(74)	(85)
1. Follicular cyst			1	0
2. Haemorrhages			1	2
3. Focal granulosa and theca cell hyperplasia			1	0
PAROTID SALIVARY GLANDS	(91)	(90)	(77)	(88)
1. A few focal infiltrates of mononuclear inflammatory cells	0	0	2	0
2. Slight focal degeneration of glandular epithelium	0	0	3	0
3. Slight focal proliferation of ductules	0	0	2	0
4. Slight mucoid metaplasia	4	6	1	0
SUBMAXILLARY SALIVARY GLANDS	(93)	(91)	(80)	(92)
1. A few focal infiltrates of inflammatory cells	3	2	3	4
2. Slight focal degeneration of glandular epithelium	1	0	3	0
3. Slight focal proliferation of ductules	0	0	1	0
4. Abscess	0	1	0	0
SUBLINGUAL SALIVARY GLANDS	(68)	(62)	(60)	(70)
1. A few focal infiltrates of mononuclear inflammatory cells	0	0	0	1

Table 7. Continued 8.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
EXTRA ORBITAL LACHRYMAL GLANDS	(96)	(89)	(77)	(85)
1. A few focal infiltrates of mononuclear inflammatory cells	0	2	5	0*
2. Slight dacryoadenitis	0	4*	5	4
3. Slight focal degeneration of glandular epithelium	2	2	0	0
EYES	(94)	(91)	(79)	(89)
1. Slight keratitis and/or conjunctivitis	3	6	2	2
THYMUS	(2)	(19)	(11)	(3)
1. Small colloid filled cysts	0	3	3	1
2. Slight proliferation of epitheloid cells	0	1	0	1
3. Involution: - slight to moderate	0	5	4	0
- marked	2	12	7	3
MESENTERIC LYMPH NODES	(74)	(71)	(68)	(66)
1. Slight accumulation of yellow-brown pigment	0	0	0	1
2. Dilated sinuses	0	0	1	0
3. Slight/moderate lymphoid hyperplasia	2	3	4	1
4. Slight/moderate sinushistiocytosis	22	17	22	19
5. A few amyloid deposits	1	0	0	0
6. Moderate atrophy	1	0	0	0
MEDIASTINAL LYMPH NODES	(24)	(16)	(17)	(25)
1. Slight accumulation of yellow-brown pigment	1	0	0	1
AXILLARY LYMPH NODES	(69)	(68)	(54)	(66)
1. Some focal fibrosis	0	2	0	0
2. Slight/moderate lymphoid hyperplasia	6	2	2	2
3. Slight accumulation of yellow-brown pigment	4	1	1	1

Table 7. Continued 9.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
CERVICAL LYMPH NODES	(70)	(75)	(67)	(73)
1. Slight-moderate accumulation of yellow-brown pigment	10	8	5	1
2. Lymphoid hyperplasia: - slight	11	21*	19	18
- moderate/marked	12	4	8	7
3. Slight erythrophagia	1	0	2	0
4. Cyst(s)	0	2	0	0
5. Dilated sinuses	0	0	0	1
6. Slight focal fibrosis	0	2	0	0
7. Marked lymphadenitis	0	0	0	1
SKIN/SUBCUTIS	(97)	(90)	(83)	(90)
1. Slight hyperkeratosis	0	0	5	0*
STERNUM	(78)	(91)	(85)	(85)
1. Increased ratio myeloid tissue/fatty tissue	0	1	0	0
ABDOMINAL WALL	(64)	(63)	(56)	(49)
1. Scar tissue	1	0	0	0
2. Peritonitis	0	0	0	1
ZYMBAL GLAND/EAR DUCTS	(77)	(85)	(65)	(75)
1. A few focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Slight adenitis	0	0	0	1
DEPOT FAT	(77)	(61)	(60)	(69)
1. Focal fat necrosis	0	0	4	3
NOSE	(93)	(95)	(77)	(89)
1. The presence within conchae of exudate containing cell débris and/or inflammatory cells	15	20	30	30
2. Focal rhinitis	6	1	1	0
3. Slight sinusitis	0	0	2	0
4. A few subepithelial infiltrates of mononuclear inflammatory cells	1	0	0	0
5. Slight focal hyperplasia of goblet cells	1	0	0	0
6. Slight/moderate periodontitis	3	2	1	3
7. Slight/moderate odontodystrophy	3	2	1	1
8. Haemorrhage	0	0	0	1

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Table 7. Continued 10.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
FEMUR/KNEE JOINT	(84)	(71)	(70)	(81)
1. Callus	0	0	0	0
2. Slight/moderate increase ratio myeloid tissue/fatty tissue	0	1	5	3
3. Focal osteodystrophy	0	0	0	1

1) The number of animals from which the organ(s) menioned was (were) examined is given in brackets. Some animals or organs were not examined because of too advanced autolysis or because of loss of autopsy material or cannibalism.

2) Occasional observations only are presented. Not all organs or sites were scored.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; X^2-test.

Table 8. INCIDENCE, SITE AND TYPE OF TUMOURS IN SURVIVORS AND NON-SURVIVORS AFTER AN EXPERIMENTAL PERIOD OF 18 MONTHS

Site and type of tumours	Incidence of tumours					
	MALES			FEMALES		
	chloroprene (in ppm)			chloroprene (in ppm)		
	0	10	50	0	10	50
Initial number of animals	100	100	100	100	100	100
Effective number of animals[1)]	100	97	97	94	93	97
Number of tumour-bearing animals[2)]	14	17	20	10	11	15
Total number of primary tumours[2)]	15	18	23	11	11	18
KIDNEYS						
1. Cortical adenocarcinoma	2	0	0	0	0	0
LIVER						
1. Neoplastic (hepatocellular) nodule	0	1	0	0	0	0
2. Unidentified tumour-like lesion[3)]	0	1	0	0	0	1
GALL BLADDER						
1. Cystadenomatous polyp.	1	6*	1	1	2	3
PANCREAS						
1. Islet cell adenoma	1	0	2	0	0	0
2. Islet cell adenocarcinoma	0	0	0	1	0	1
STOMACH						
1. Papilloma	0	0	2	0	0	0
2. Unidentified papilloma-like lesion[3)]	1	1	1	1	2	0
TESTES						
1. Leydig cell tumour	1	0	0			
COLON						
1. Adenomatous polyp	0	0	0	1	0	0
PITUITARY						
1. Adenoma	0	0	1	2	0	0
THYROID						
1. Parafollicular cell adenoma	2	0	0	0	2	1
2. Cystadenoma	1	0	0	0	0	0
3. Papillary adenoma	0	1	1	1	0	2
4. Follicular adenoma	2	1	0	1	2	1
PARATHYROID						
1. Adenoma	0	0	0	0	1	0

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Site and type of tumours	Incidence of tumours					
	MALES			FEMALES		
	chloroprene concentration (in ppm)			chloroprene concentration (in ppm)		
	0	10	50	0	10	50
ADRENALS						
1. Cortical adenoma	4	1	10	0	0	3
2. Cortical carcinoma	0	1	0	1	0	1
3. Phaeochromocytoma	0	4*	2	0	0	0
4. Malignant phaeochromocytoma	0	0	2	0	0	0
OVARIES						
1. Granulosa-theca cell tumour				0	2	1
PAROTID SALIVARY GLANDS						
1. Adenoma	0	0	0	0	0	1
SKIN						
1. Unidentified tumour-like lesion[3)]	0	1	0	0	0	0
ZYMBAL GLANDS						
1. Sebaceous adenoma	0	0	1	0	0	0
DEPOT FAT						
1. Lipoma	0	0	0	0	0	1
NOSE						
1. Adenoma of Bowman 's glands	0	0	0	1	0	0
2. Adenocarcinoma of Bowman 's glands	0	0	0	0	0	1
BONE (RIBS)						
1. Osteosarcoma	0	0	0	1	0	0
ABDOMINAL CAVITY						
1. Reticulum cell sarcoma	1	0	0	0	0	0

1) a few animals were not examined because of cannibalism or loss of autopsy material

2) some animals bore more than one tumour

3) macroscopically observed tumour-like lesion, but lost for microscopy

* $P<0.05$, χ^2 - test

Figure 1 Body weight curves of male hamsters

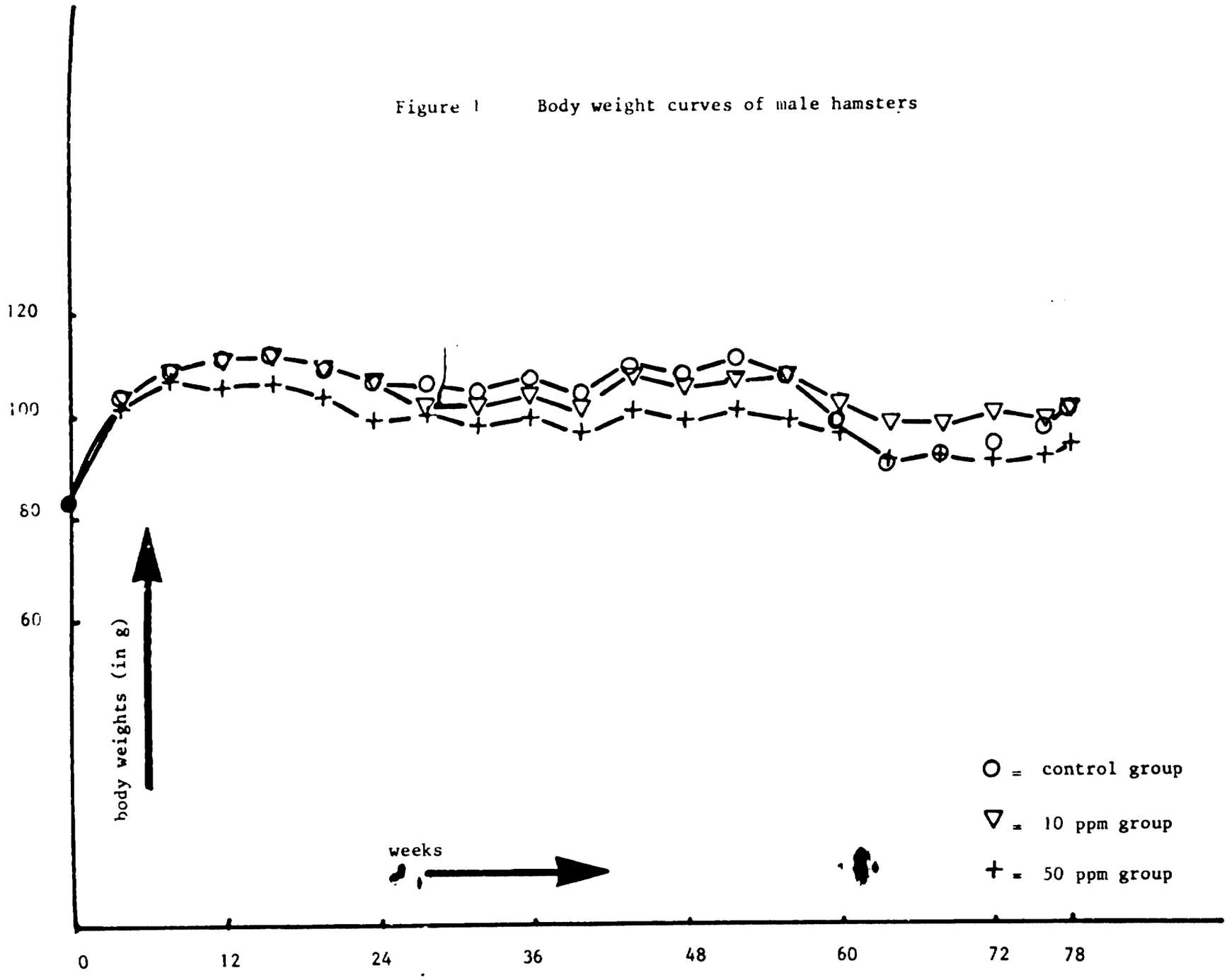

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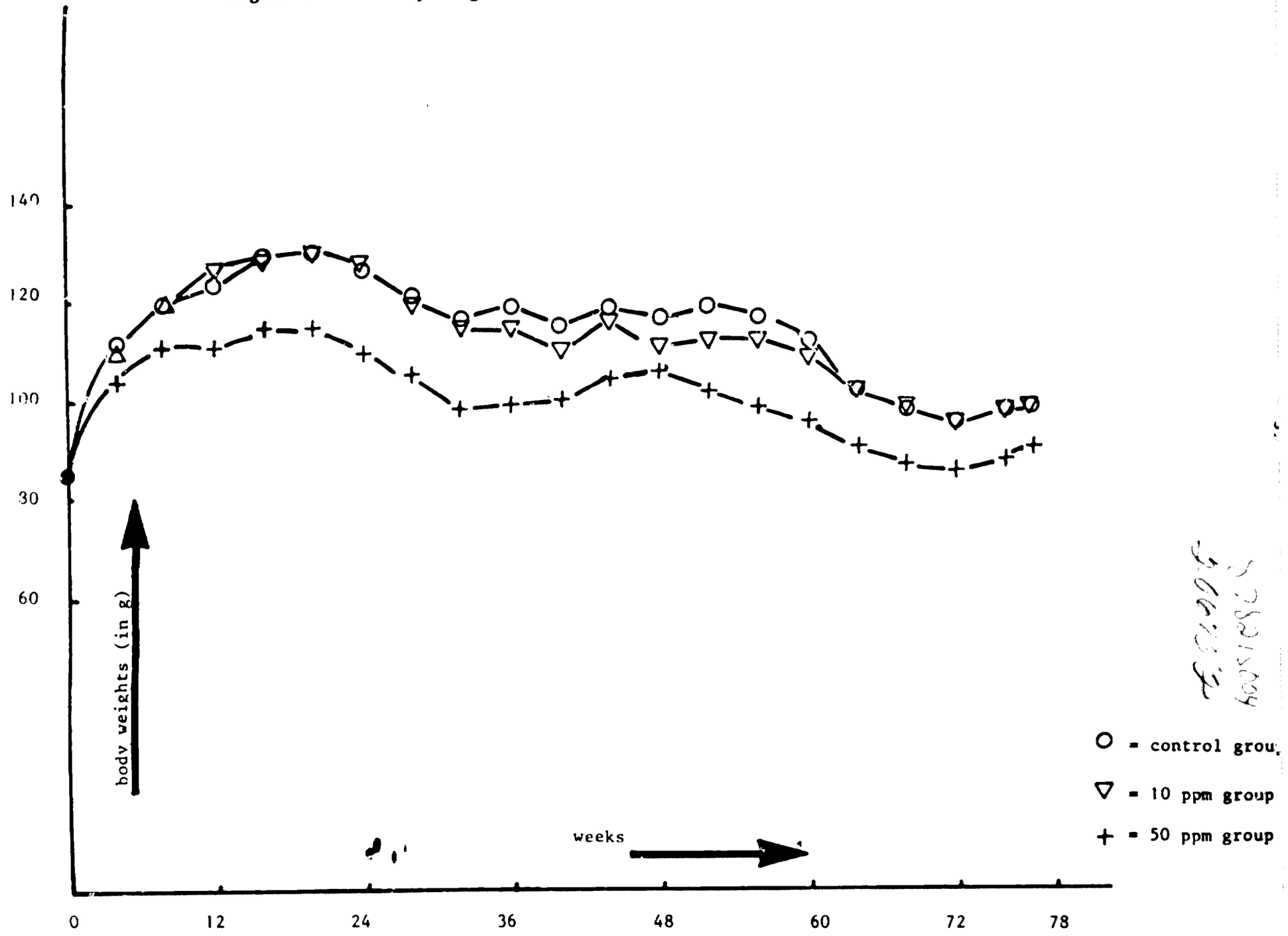
Figure 2 Body weight curves of female hamsters



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REPRODUCTION STUDY WITH BETA - CHLOROPRENE VAPOUR IN RATS	

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3- BUTADIENE		126-99-8	

1A

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REPORT NO. R 6225

REPRODUCTION STUDY WITH β-CHLOROPRENE VAPOUR IN RATS

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At the request of	: The Joint Industry Committee on Chloroprene
Project no.	: B77/2650
Date	: October 1979
Approved by	: Dr V.J. Feron
Start of study	: 23 December 1977
Termination of study	: 2 August 1978
Study director	: Drs P.G.J. Reuzel
Number of copies	: 15

All raw data and this report are stored in the archives of the department of Biological Toxicology under the reference "Joint Industry Committee on chloroprene".

000002

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Total or partial publication of this report without written assent is not allowed.

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SUMMARY

1. A reproduction study with β-chloroprene vapour was carried out, in which two successive generations of rats (F_0- and F_1-generation) were exposed to atmospheres containing 0, 10, 33 or 100 ppm β-chloroprene. The F_0-generation males and females were exposed 6 hours/day, 5 days/week for a period of 13 weeks, and thereafter, mated with untreated females and males respectively. F_1-generation rats were exposed 6 hours/day, 5 days/week for a 10-week period.

2. The actual mean concentrations of β-chloroprene in the test-atmospheres were 0, 9.80, 32.60 and 100.01 ppm for the F_0-generation and 0, 9.85, 32.99 and 100.58 ppm for the F_1-generation.

3. Fertility of males and females, number of young born per litter, general condition, appearance, male/female ratio, and mortality of the young were not adversely affected by the exposure to β-chloropren[redacted] There was no indication of increased intra-uterine mortality.

4. Growth retardation was observed in the F_0-generation at the high-dose level and in the F_1-generation at the mid- and high-dose levels.

5. The relative weights of the liver and the ovaries of the high-level female rats, descendants from untreated females and treated males, were significantly higher than those of the controls.

6. Gross and microscopic pathological examinations did not reveal any treatment-related abnormalities

7. It was concluded that under the conditions of the present two-generation experiment exposure to β-chloroprene at levels up to 100 ppm had no adverse effect on the reproductive performance of rats.

REPRODUCTION STUDY WITH β-CHLOROPRENE VAPOUR IN RATS

1. INTRODUCTION

At the request of "The Joint Industry Committee on Chloroprene" an inhalation toxicity study in rats over two successive generations was carried out with β-chloroprene.
The test animals were exposed to atmospheres containing 0, 10, 33 or 100 ppm β-chloroprene.

2. EXPERIMENTAL

2.1 Material

Twelve bottles, each containing 200 ml freshly purified β-chloroprene, were received weekly from Bayer AG, Dormagen, Western-Germany.

2.2 Animals

One hundred male and 100 female SPF reared rats (Cpb:Wu, Wistar random) were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands on December 13, 1977. They weighed about 50 g on arrival. The animals were allocated according to body weight and sex to four groups, 1 control group and 3 test groups, each consisting of 25 males and 25 females.

2.3 Diet

After the daily exposure to chloroprene the F_0-animals were provided ad libitum with a pelleted rat diet (Muracon I, Trouw & Co. N.V., Putten, The Netherlands) and with unfluoridated bottled water. The F_1-animals received the Institute's stock diet for rats and unfluoridated bottled tap water ad libitum. The nutrient composition and the levels of the various contaminants, as determined periodically in batches of the Institute's stock diet, are shown in appendices 1,2 and 3. During exposures neither food nor water was available to the animals.

2.4 Exposure chambers

The chambers used have a capacity of 2.5 m^3 and are constructed of stainless steel with a glass-door which allows observation of the animals during the exposures. The chambers have a cylindrical form with a conical top and bottom. Inside they are fitted with a heptagonal frame, which is rotatable around the vertical axis of the chamber.
Forty two tinned, wire mesh cages with a floor surface of 930 cm^2 each, were suspended over seven vertical rows with 6 layers each. The position of the rows within the chambers was changed daily in a fixed order.
The chambers are illuminated externally by normal laboratory lighting. The light cycle was 12 hours light and 12 hours darkness.

The chambers were maintained at a negative pressure of 1-2 mm H_2O relatively to the room, to prevent leakage of the test-atmospheres into the room.
The main airflow was drawn from the inhalation room and had, when it entered the chamber, a temperature of 22 ± 1 °C and a relative humidity of 45-60%. Before entering the inhalation chamber the main airflow was mixed with a nitrogen flow which was laden with the test material.
In the chamber the airflow is spred equally by an air disseminator which is located at the top. The flow rate through the chamber was approximately 40 m^3/h during exposures, and 60 m^3/h during the non-exposure periods.

2.5 Accommodation

The animals stayed in the exposure chambers, also during the non-exposure periods. During the mating period the animals were housed in stainless steel cages (one male and one female in each cage) which were suspended in an open rack in an animal room.
The temperature and the relative humidity were controlled at 22 ± 1 °C and 45-60% respectively.

2.6 Generation of the test atmosphere

β-chloroprene was evaporated by passing a small filtered and dried nitrogen flow through a glass evaporator, filled with an appropriate quantity of β-chloroprene and kept at 0 °C.
The β-chloroprene-saturated nitrogen flow was fed into the inlet system of the inhalation chamber and mixed with the air flow entering the inhalation chamber in such a way that the desired concentration was obtained.

2.7 Dose levels

Groups of rats were exposed, 6 hours/day, 5 days a week, to 0, 10, 33 or 100 ppm β-chloroprene. The exposure period was 13 weeks for the F_0-generation and about 10 weeks for the F_1-generation.

Group no.	number of animals				nominal concentration of β-chloroprene in ppm
	males		females		
	F_0	F_1	F_0	F_1	
9854	25	40	25	40	0
9855	25	40	25	40	10
9856	25	40	25	40	33
9857	25	40	25	40	100

2.8 Analysis

Analyses of the test atmospheres were performed by gaschromatography: the gaschromatograph used being an Intersmat GC 120, fitted with a flame ionization detector, operating at the following conditions:

Column : 300 cm x 3 mm internal diameter, packed with Carbowax 20M

Temperature : Injection port 105 °C
Detector 105 °C
Oven 65 °C

Carriergas : Nitrogen, with a flow rate of 65 ml/min

The gaschromatograph was calibrated with a standard solution of β-chloroprene in carbondisulfide, which was diluted to obtain the β-chloroprene concentration desired.
Peak surfaces, obtained by injecting samples of the dilutions of the β-chloroprene standard solution, were compared with those of samples of the test atmosphere.

To monitor the β-chloroprene concentration in each test atmosphere samples were taken automatically at regular intervals by means of a stainless steel sampling tube and sample loop using a membrane pump and a timer- controlled 7-port gas-sampling valve. The sample loop was calibrated by comparing peak surfaces of atmosphere samples taken with a gas-tight syringe with peak surfaces of simultaneously taken loop-samples.

2.9 Design and conduct of the study

2.9.1 The F_0-generation

Exposure period of the F_0-generation

The exposure of the F_0-generation was started on December 23, 1977. The last exposure day was March 17, 1978.

Condition and behaviour

The general condition and behaviour of all rats were checked regularly; all signs of ill health or reactions to treatment were recorded.

Body weights

Individual body weights were measured weekly.

Autopsy

After weaning their litters the females were killed and the uterus was treated in an ammonium sulfide solution for staining and counting the implantation sites. After the mating period (see 2.9.2) the males were killed and the testicles were collected and fixed in a 4% aqueous neutralized formaldehyde solution.

2.9.2 The mating period

After the exposure period of 13 weeks the rats were mated for 20 days: The males of the 3 exposure groups and of the control group were mated with females of the stock colony (1 male x 1 female). The females of the 3 exposure groups and of the control group were mated with males of the stock colony (1 female x 1 male) (see also figure 1).
After the mating period the females were caged individually until their litters had been weaned. Records were made of the number of pups in each litter at birth and the total number of survivors at day 1, 3, 14 and 28. The total weight of the litter was recorded at day 1, 3, 14 and 28. At the same time the male/female ratio was determined.
Litters containing more than 8 siblings were randomly culled to eight at day 4, in order to equalize the stress of lactations among the dams.

2.9.3 The F_1-generation

Exposure period of the F_1-generation

From the F_1-litters, 20 males and 20 females were selected randomly from each mating group one week after weaning, making a total of 160 males and 160 females. These animals were exposed for a period of 10 weeks. The exposure of the F_1-litters was started on May 22, 1978. The last exposure was on August 2, 1978.

Condition and behaviour

The general condition, behaviour and possible signs of intoxication were checked daily; all signs of ill health or reactions to treatment were recorded.

Body weights

Individual body weights were recorded weekly.

Haematology

Blood samples were collected from the tip of the tail of 15 rats/sex/group at an age of 4 weeks. All samples were examined for haemoglobin concentration (Hb) by the cyan-methaemoglobin method using Zap-Oglobin® of Coulter Electronics ltd.

Autopsy

Ten rats/sex/group were killed at the end of the exposure period and autopsied. The liver, lungs, ovaries and testes were weighed and fixed in a 4% aqueous neutralized formaldehyde solution.

2.10 Statistical analysis

Statistical analyses of body weights and organ-to-body weight ratios were carried out using the Student's t-test; the haematological data were evaluated statistically by means of the test of Wilcoxon.

3. RESULTS

3.1 Exposure levels

The mean actual concentrations of β-chloroprene, to which the rats in the different groups were exposed, are summarized in table 1.
From this table it can be seen that the actual levels were virtually identical to the design concentrations.

3.2 The F_0-generation

3.2.1 Condition and behaviour

The general condition and behaviour of the rats in the different groups did not show any abnormality, which could be ascribed to treatment with the test substance.

3.2.2 Body weights

The mean body weights are summarized in table 2. In the top dose group slight growth retardation was observed in males during the first 8 weeks and in females throughout the study. In the low and mid dose groups the mean body-weights sometimes differed statistically significantly from those of the controls, but there was no evidence of a constant pattern. Moreover, the mean body weights of the controls showed some irregularities, especially from week 9 till week 12. Inspection of the individual body weight figures learned that these irregularities were most propably due to occasional shortage of feed in several cages, which was almost surely caused by providing an insufficient daily portion of pellets.

3.2.3 Reproductive performance

The results of the breedings are summarized in the tables 3 and 4.
The percentage of females which casted a litter was high in all groups.
There were no consistent decreases in fertility observed in any of the groups.
The number of young born per litter did not show treatment related differences between the various test groups and controls.
The ratio of males/females showed some variation amongst groups, but there was no evidence of a dose-related response.
The mean body weights of the young descended from treated females and untreated males were significantly lower in the top-dose group than in the control group both at birth and during lactation.
The mortality figures varied somewhat among the groups, but there was no indication of a dose-related response.
The resorption quotients were similar in all groups and did not indicate any increase in foetal mortality as a result of exposure to β-chloroprene vapour.

3.3 The F_1-generation

3.3.1 Condition and behaviour

The general condition and behaviour of the young did not show any abnormality, which could be ascribed to the treatment.

3.3.2 Body weights

Growth retardation occurred at the 33 and 100 ppm levels in both sexes, the differences with the controls being statistically insignificant at 33 ppm in male descendants from treated females and untreated males (table 5).

3.3.3 Haematology

Haemoglobin levels were similar in all groups (table 6).

3.4 Alopecia

The incidence of focal alopecia is presented in table 7.
This table shows that alopecia was seen in a limited number of rats of each of the groups, the control group included. There was no evidence of chloroprene exposure inducing this skin lesion or enhancing its degree or incidence.

3.5 Organ weights

The relative weight of the liver of the high-level females, descendants from untreated females and treated males, was significantly higher than that of the controls (table 8); the relative lung weight of these females was significantly lower than that of the controls.

The relative weight of the testicles was in nearly all of the test groups higher than in the controls; there was no dose-effect relationship. These differences were considered a reflection of the lower body weights in these test groups, rather than an indication of an effect of β-chloroprene on this organ.

The relative ovary weight of the females of the highest dose level, descendants from untreated females and treated males, was clearly higher than that of the controls.

3.6 Pathology

3.6.1 Gross examination at autopsy

Gross examination at autopsy of the F_0- and F_1-generation rats did not reveal any abnormality that could be attributed to the test material.

3.6.2 Microscopic examination

Microscopic examination of the testicles of the F_0-generation rats (table 9) did not show any abnormality which could be ascribed to the treatment. The incidences of histopathological changes observed in F_1-generation animals (table 10) were about equal in the control groups and the test groups. None of these abnormalities, which are quite common findings in the strain of rats used, could, therefore, be ascribed to the test material.

The increase in relative liver and ovary weight of females of the highest dose level was not accompanied by treatment-related pathological changes in these organs.

4. DISCUSSION

It is evident from the results of the present two-generation study that β-chloroprene had no significant effect on :

a) the fertility of males and females
b) number of young born per litter
c) general condition, appearance, male/female ratio and mortality of the youngs.

Growth retardation occurred in males and females of both generations. The growth retardation of the F_1-generation animals was more marked than that of the F_0-generation animals, and occurred at the two highest dose levels, whereas in the F_0-generation only high-dose rats showed lower body weights. This difference between F_0- and F_1-generation rats is most probably not an indication of a greater sensitivity of the F_1-generation animals to β-chloroprene, but rather caused by the fact that the F_0-generation rats were fed another type of diet (viz. the Muracon I diet) than the F_1-generation animals (viz. the Institute's stock diet). This view is strongly supported by the results of a separate inhalation study with the same strain of rats, which demonstrated that animals exposed to 200 ppm β-chloroprene and fed the Institute's stock diet exhibited more severe growth retardation than similarly exposed animals fed the Muracon I type of diet (Appelman and Reuzel, to be reported). Although both types of diets are adequate rat diets which have been used for many years, it is obvious that growth depression due to β-chloroprene exposure is more likely to occur in rats fed the Institute's diet than in rats fed on the Muracon I diet.

The slight decrease of the relative lung weight, which was observed only in the high-level group of females, descendants from untreated females and treated males, is not regarded as the expression of an effect of β-chloroprene but rather as a fortuitous finding without any toxicological significance. This is supported by the observation that the histopathology of the lungs of these females was unremarkable.

Although special attention was paid to the histological appearance of the liver and the ovaries of the high-level females, descendants from untreated females and treated males, no changes were observed in these organs, which could be related to the exposure of β-chloroprene. It is, therefore, doubtful whether any toxicological significance can be attached to the slightly elevated relative liver weight in these females.

As to the relative ovary weight (0.041 g/100 g body weight) of these animals, it may be mentioned that this value indeed is fairly high as compared to those of all other groups, but is not beyond the range of values (0.028 - 0.042) found in controls used in subchronic toxicity studies with the same strain of rats. The relatively high ovary weight is, therefore, considered a fortuitous finding unrelated to β-chloroprene exposure.

In conclusion, the present two-generation study did not provide any evidence of β-chloroprene exerting any adverse effect on the reproductive performance of male and female rats exposed to the test compound at levels up to 100 ppm.

We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedure herein described, and that this report represents a true and accurate record of the results obtained.

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Drs P.G.J. Reuzel
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Pathologist

Dr V.J. Feron
deputy head of the dept.of Biological Toxicology

Table 1. Mean concentrations of β-chloroprene, the standard deviations and the standard errors of the mean.

nominal concentration of β-chloroprene in ppm		actual concentration of β-chloroprene in ppm		standard deviation		standard error of mean	
F_0	F_1	F_0	F_1	F_0	F_1	F_0	F_1
0	0	0	0	0	0	0	0
10	10	9.80	9.85	0.68	0.56	0.08	0.08
33	33	32.60	32.99	1.50	2.16	0.19	0.30
100	100	100.01	100.58	5.48	4.82	0.69	0.67

Table 2. Mean body weights of F_0-generation rats

β-chloroprene concentration in ppm	mean body weights (in g) at the end of week:													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
							males							
0	92	110	146	183	198	223	251	277	295	280	304	300	311	332
10	94	108	144	182	208	238	249	276	292	292	298	298	300	324
33	92	107	142	178	204	222	245	264	275*	282	293	285	292	313
100	93	112	141	173*	188	219	237*	258*	269**	275	287*	295	301	317
							females							
0	85	92	112	129	139	151	161	172	178	178	186	189	194	200
10	85	93	111	129	139	150	155*	166*	172	175	179*	178**	186*	193*
33	84	93	111	128	139	148	158	166*	171*	173	180	176***	185*	193*
100	82	92	106**	119***	131***	143*	150***	158***	165***	169*	177**	177**	182**	187***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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Table 3. Reproductive performance

Litters of females, which were exposed to β-chloroprene

β-chloroprene concentration in ppm	females		mean litter size at birth		male/female ratio
	no. mated	% with litters	dead	alive	
0	25	100	0.04	10.5	1.00
10	25	100	0.04	10.9	1.38
33	25	100	0.60[1)]	9.6	1.13
100	25	96	0.16	9.8	1.13

percentage males at birth	percentage females at birth	mean body weight of young at day			
		1	3	14	28
50	50	6.2	8.1	27.4	68.6
58	42	6.1	7.8	26.9	66.2
53	47	6.3	8.2	27.0	66.4
53	47	6.0*	7.9	25.7**	64.5**

% mortality of young during lactation at day				resorption[2)] quotient
1	3	4-14	4-28	
0.8	1.1	0	0.5	1.06
0.7	0.7	0	0.5	1.06
7.4	8.3	0	0	1.08
2.1	2.1	0	0	1.08

1) From one litter 9 of 10 young were dead at birth

2) Number of implantation sites/number of young born

*P < 0.05; **P < 0.01;*** P < 0.001

Table 4. Reproductive performance

Litters of females, which were not exposed to β-chloroprene

β-chloroprene concentration in ppm	females		mean litter size at birth		male/female ratio
	no. mated	% with litters	dead	alive	
0	25	100	0	11.0	0.85
10	25	92	0	10.2	1.17
33	25	96	0.25	11.0	0.85
100	25	100	0.08	11.2	1.13

percentage males at birth	percentage females at birth	mean body weight of young at day			
		1	3	14	28
46	54	6.0	7.9	28.2	68.6
54	46	6.2	8.0	28.2	69.3
46	54	6.0	7.8	27.8	67.3
53	47	6.0	7.8	28.2	67.4

% mortality of young during lactation at day				resorption[1] quotient
1	3	4-14	4-28	
1.8	3.3	0	0	1.07
0.4	0.4	0.6	0.6	1.08
3.0	3.0	0	0	1.10
1.8	1.8	0	0	1.06

1) Number of implantation sites/number of young born

Table 5. Mean body weights of F_1-generation rats

β-chloroprene concentration in ppm	mean body weights (in g) at the end of week:										
	0	1	2	3	4	5	6	7	8	9	10
descendants from treated females and untreated males											
males											
0	123	153	194	224	243	266	283	291	300	312	328
10	125	159	202	235	261*	280	296	309	313	324	336
33	121	148	186	217	237	257	271	277	291	302	314
100	119	142**	178***	206**	227*	248*	263*	278	288	298	305*
females											
0	104	123	139	152	164	173	179	182	186	190	196
10	104	122	139	153	164	173	180	185	187	191	191
33	104	117*	133*	146*	155**	166*	172*	178	181	186	191
100	99	110***	127***	141***	151***	161***	167**	175*	178**	182*	186**
descendants from untreated females and treated males											
males											
0	119	161	200	232	259	280	296	307	320	330	342
10	127	165	204	235	263	288	302	314	320	333	346
33	122	147*	183**	211***	233***	255***	267***	274***	286***	299***	310***
100	129*	151*	187*	211***	229***	252***	264***	274***	290***	300***	306***
females											
0	106	126	142	157	169	179	187	192	195	199	204
10	108	125	142	155	167	182	183	188	189	194	199
33	104	118**	135*	149*	158***	167**	173***	178**	182**	187**	194*
100	105	118**	133***	146***	154***	161***	167***	178***	182***	186***	190***

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8100

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 6. Mean haemoglobin values of F_1-generation rats

β-chloroprene concentration in ppm	Descendants from treated females and untreated males		Descendants from untreated females and treated males	
	haemoglobin (mmol/l)		haemoglobin (mmol/l)	
	males	females	males	females
0	7.2	7.4	7.3	7.5
10	7.0	7.2	7.4	7.5
33	7.0	7.6	7.3	7.3
100	7.3	7.2	7.3	7.5

Table 7. Incidence of focal alopecia.

β-chloroprene concentration in ppm	F_0-generation		Descendants from females, exposed to β-chloroprene		Descendants from females, not exposed to β-chloroprene	
	♂♂ males	♀♀ females	♂♂ males	♀♀ females	♂♂ males	♀♀ females
0	2	2	0	0	0	0
10	0	3[1]	0	0	0	8[4]
33	0	2[1]	0	0	0	0
100	2[2]	2	0	9[3]	2	0

1) Two in one cage of four.

2) Two males in one cage of three

3) Four of the nine were housed in one cage of 5 and three of the nine in one cage of four.

4) Four animals were housed in one cage of 5, and the other four were also housed in one cage of four.

Table 8 : MEAN BODY WEIGHTS(g), MEAN RELATIVE ORGAN WEIGHTS (g/100 g body weight) AND THE STANDARD ERROR OF THE MEAN OF F_1-GENERATION RATS, WHICH WERE EXPOSED TO β-CHLOROPRENE.

β-chloroprene concentration in ppm	body weight	liver	gonads	lungs	β-chloroprene concentration in ppm	body weight	liver	gonads	lungs
	descendants from treated females and untreated males					descendants from untreated females and treated males			
		males					males		
0	342 (6)	3.62 (0.07)	0.92 (0.02)	0.39 (0.01)	0	338 (6)	3.58 (0.09)	0.90 (0.05)	0.42 (0.01)
10	316 (14)	3.57 (0.19)	1.05* (0.05)	0.54 (0.07)	10	344 (8)	3.38 (0.05)	0.95 (0.02)	0.42 (0.01)
33	314 (9)	3.62 (0.07)	1.01* (0.02)	0.43 (0.01)	33	315 (16)	3.50 (0.23)	1.08* (0.05)	0.42 (0.02)
100	311 (13)	3.40 (0.10)	1.07** (0.04)	0.44 (0.02)	100	305** (8)	3.39 (0.10)	1.08* (0.03)	0.43 (0.01)
		females					females		
0	198 (3)	3.12 (0.08)	0.029 (0.003)	0.53 (0.01)	0	212 (4)	3.05 (0.05)	0.028 (0.002)	0.57 (0.02)
10	196 (4)	2.98 (0.04)	0.029 (0.001)	0.54 (0.02)	10	200* (4)	2.96 (0.06)	0.026 (0.003)	0.55 (0.02)
33	196 (5)	3.31 (0.08)	0.035 (0.002)	0.53 (0.01)	33	195* (5)	3.12 (0.05)	0.031 (0.002)	0.57 (0.02)
100	188 (4)	3.17 (0.06)	0.030 (0.002)	0.53 (0.01)	100	189*** (4)	3.33** (0.08)	0.041*** (0.002)	0.52* (0.01)

* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

Table 9

HISTOPATHOLOGY OF THE TESTICLES OF F_0-GENERATION MALE RATS

Site and type of lesions	Incidence of lesions			
	β-chloroprene concentration in ppm			
	0	10	33	100
Testes	(25)*	(25)	(25)	(25)
1. Interstitial edema	0	1	1	0
2. A few atrophic tubules	0	1	0	0

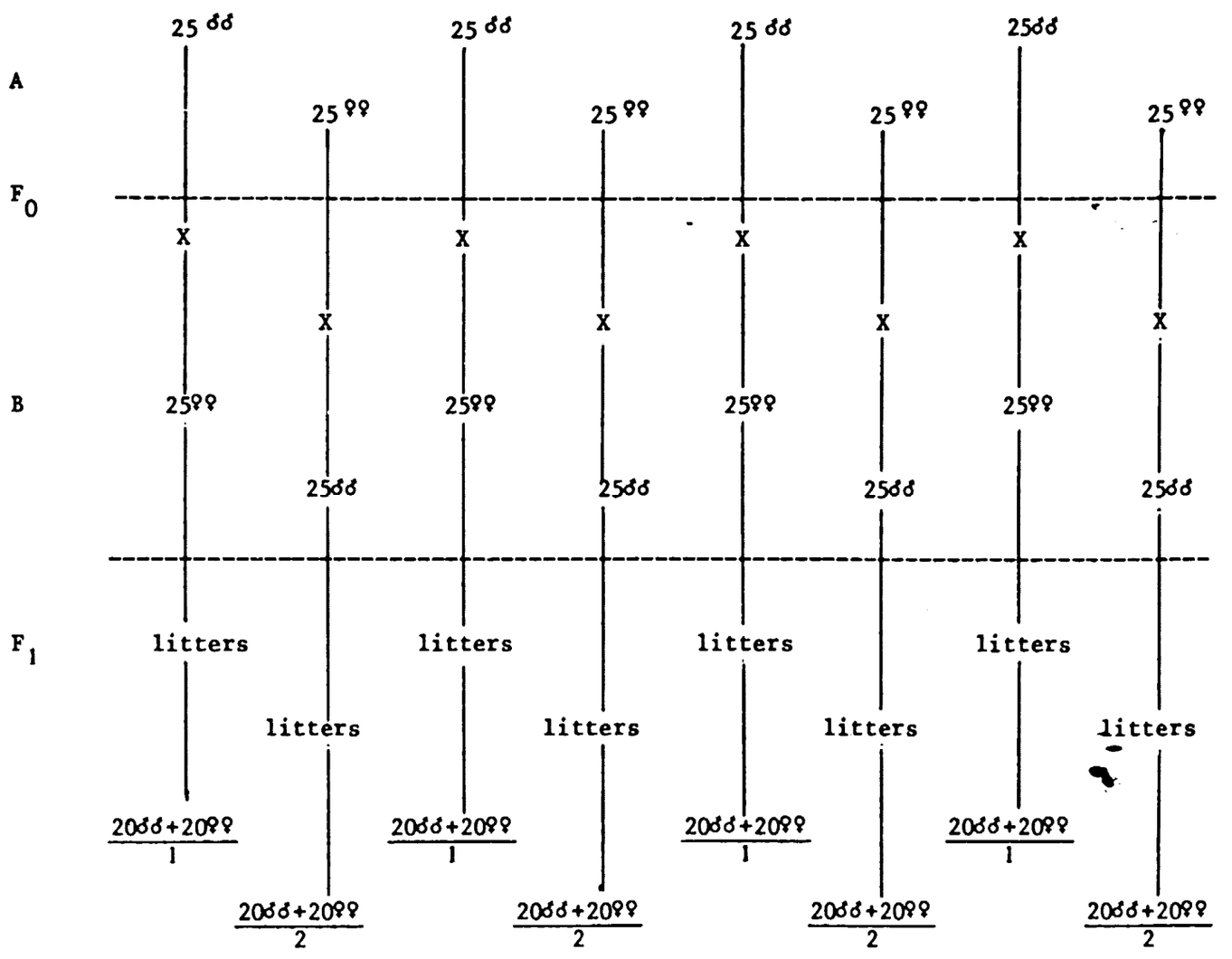
* The number of testes examined is given in brackets

Table 10 HISTOPATHOLOGY OF F_1-GENERATION ANIMALS, AND THE NUMBERS OF ANIMALS SHOWING THE OBSERVED LESIONS IN THE DIFFERENT GROUPS*)

Site and type of lesions	Incidence of lesions							
	Descendants from treated females and untreated males				Descendants from untreated females and treated males			
	β-chloroprene concentration in ppm				β-chloroprene concentration in ppm			
	Males		females		males		females	
	0	100	0	100	0	100	0	100
Liver	(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10
1. Slightly increased number of RES-cell aggregates accompanied by a few necrotic hepatocytes	2	3	8	6	4	2	7	4
2. Slight periportal aggregates of mononuclear inflammatory cells	1	1	0	2	1	0	0	5
3. Slightly enlarged hepatocytes	0	0	0	0	0	1	0	0
Lungs	(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10
1. Slight peribronchial, peribronchiolar and/or perivascular infiltrates of lymphocytes	9	10	8	9	10	7	6	7
2. Slight focal pneumonites	0	2	2	0	1	0	0	0
3. Calcareous deposits in the arterial wall	1	0	0	0	0	0	0	0
Trachea	(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10
1. Subepithelial aggregates of mononuclear inflammatory cells	1	0	1	0	0	0	0	0
2. Hyperplasia of the lining epithelium accompanied by large infiltrates of inflammatory cells	0	0	0	0	1	0	0	0
Larynx	(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10
Testes	(10)	(10)			(10)	(9)		
1. Slight interstitial edema	2	3			2	3		
Ovaries			(9)	(10)			(10)	(9

*) The number of organs examined is given in brackets

fig. 1: Mating diagram



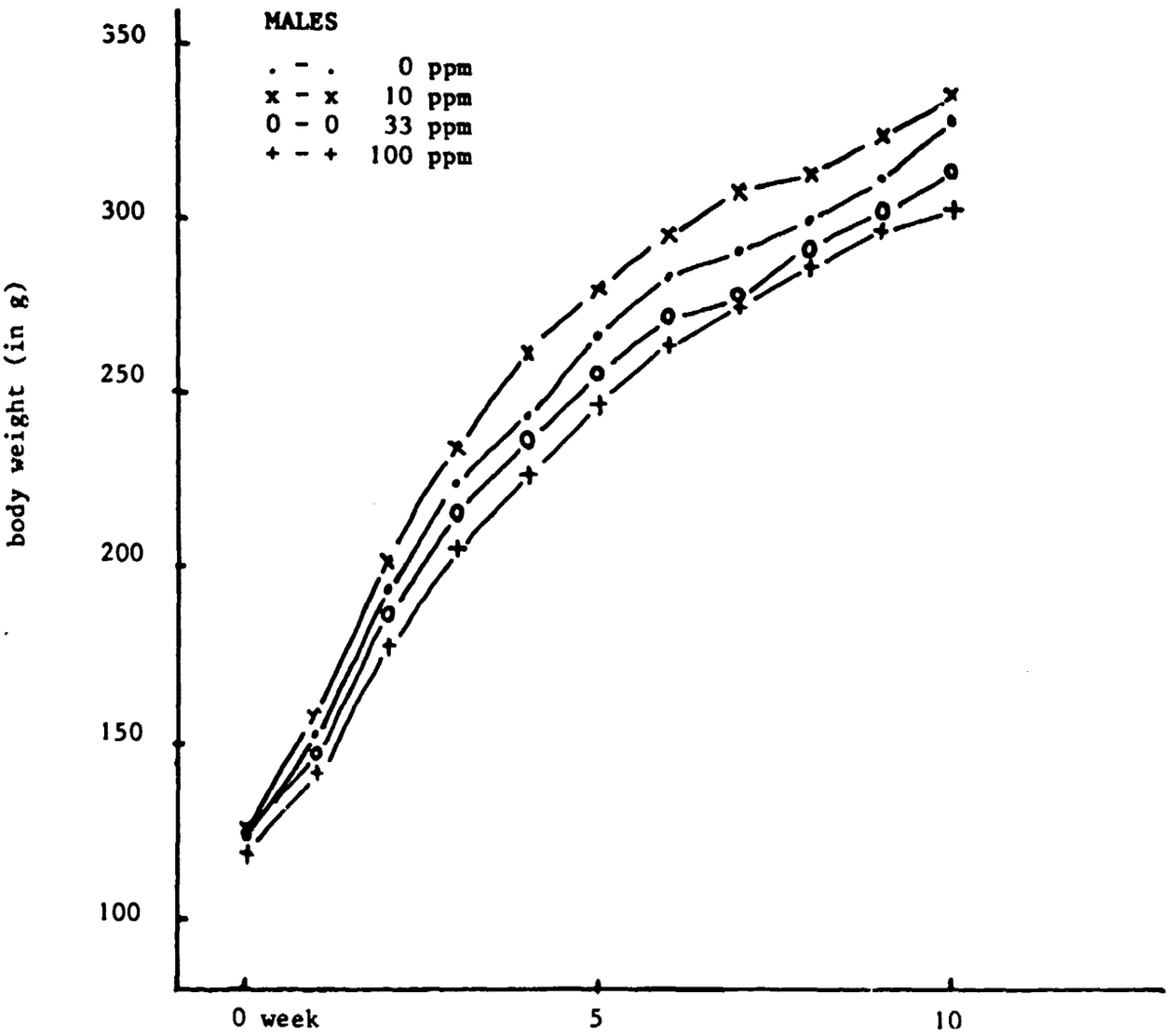
1 F_1-generation of treated males and untreated females

2 F_1-generation of untreated males and treated females

A Animals exposed to different concentrations of β-chloroprene

B Animals of the stock-colony

Figure 2. Mean body weights of the F_1-generation rats descended from females which were exposed to β-chloroprene



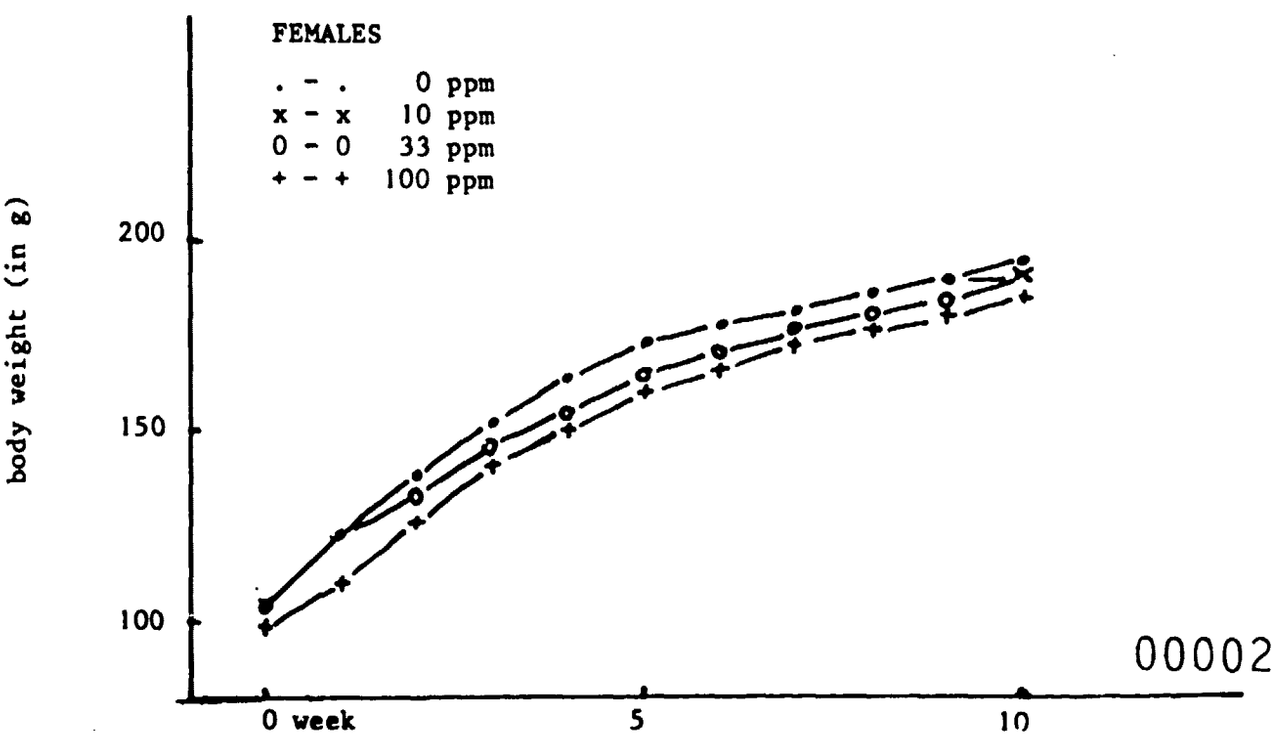
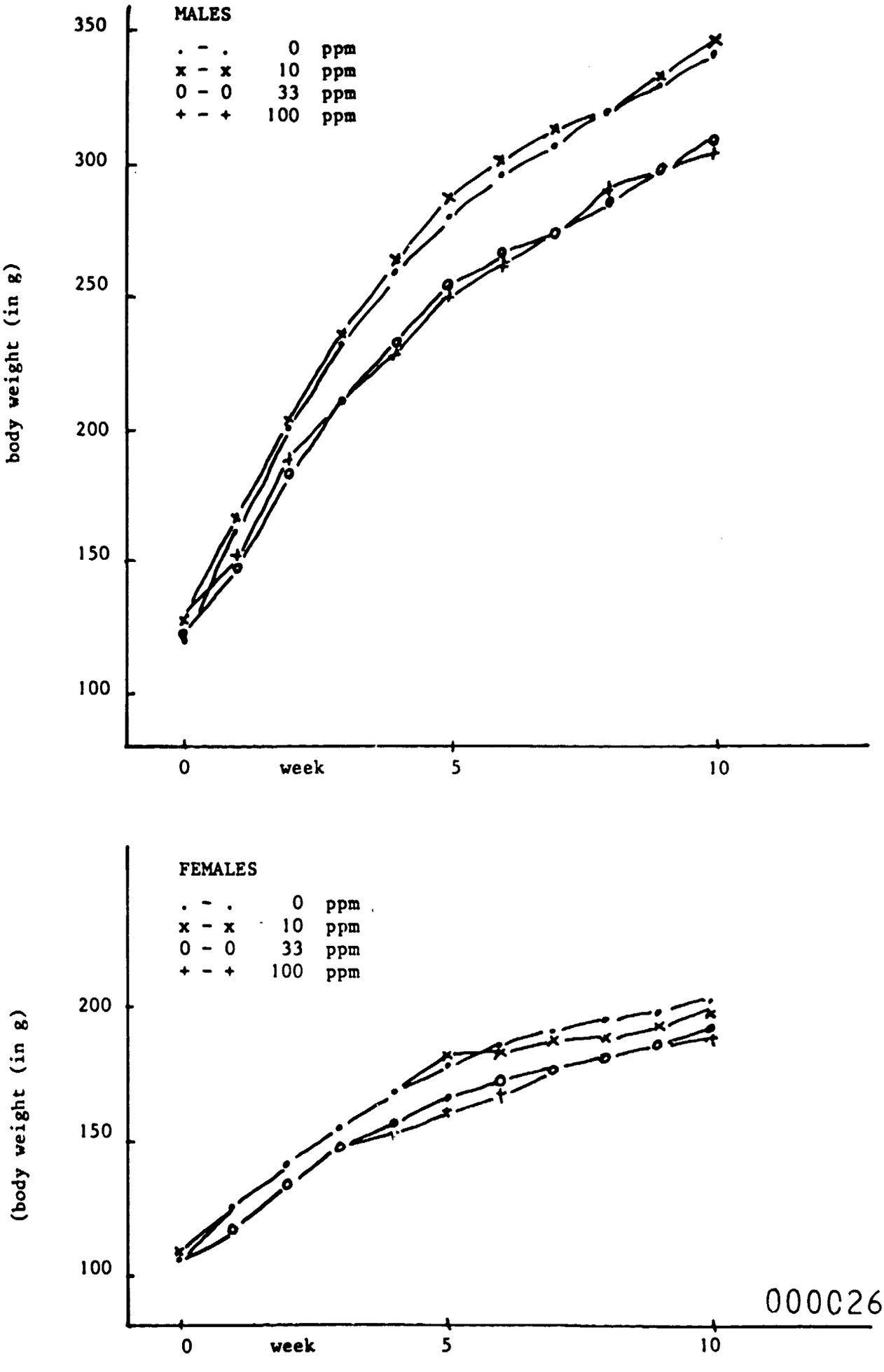


Figure 3 Mean body weights of the F_1 - generation rats descended from females which were not exposed to β-chloroprene



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Appendix 1

NUTRIENT COMPOSITION OF THE INSTITUTE'S STOCK DIET FOR RATS

Analyses of a 1000 kg batch produced in March 1978

moisture	12.0 %	iron	235 ppm
crude protein (N x 6.25)	20.1 %	manganese	84 ppm
crude fat	7.0 %	copper	17 ppm
calcium	0.86 %	zinc	49 ppm
phosphorus	0.71 %	cobalt	0.8 ppm
magnesium	0.14 %	chromium	1.5 ppm
		selenium	0.14 ppm

vitamin A	13.000	I.U./kg	vitamin B_1	8.5	mg/kg
vitamin D	3.800	I.U./kg	vitamin B_2	7.2	mg/kg
vitamin E	77.6	mg/kg	vitamin B_6	5.1	mg/kg
carotene	0.2	mg/kg	niacin	63	mg/kg
vitamin K_3	< 1	mg/kg	pantothenic acid	13	mg/kg
			folic acid	1.0	mg/kg
			choline	1500	mg/kg
			biotin	0.3	mg/kg
			vitamin B_{12}	18	µg/kg

	g/100 g	g/16 g N		g/100 g	g/16 g N
isoleucine	0.89	4.4	valine	1.10	5.5
leucine	1.64	8.2	arginine	1.18	5.9
lysine	1.06	5.3	histidine	0.45	2.2
methionine	0.40	2.0	alanine	1.12	5.6
cystine	0.35	1.7	aspartic acid	1.85	9.2
phenylalanine	0.90	4.5	glutamic acid	3.68	18.3
tyrosine	0.68	3.4	glycine	1.03	5.1
threonine	0.80	4.0	proline	1.32	6.6
tryptophan	0.21	1.0	serine	1.06	5.3

Appendix 2

CONTAMINANT ANALYSES

Compounds detected and lower detection limits

Organochlorine pesticides and PCB's (in mg/kg)

HCB	0.005	aldrin	0.01
α-HCH	0.01	dieldrin	0.01
β-HCH	0.02	endrin	0.02
γ-HCH, lindane	0.01	methoxychlor	0.05
heptachlor	0.01	p,p'-DDE	0.02
heptachlorepoxide	0.02	p,p'-TDE	0.0
α-chlordane	0.02	o,p'-DDT	0.03
γ-chlordane	0.02	p,p'-DDT	0.04
		PCB's	0.20

Organophosphorus pesticides (in mg/kg)

chlorpyrifos	0.01	malathion	0.01
diazinon	0.01	mevinfos	0.01
dichlorvos	0.01	parathion	0.01
fenithrothion	0.01	sulfotep	0.01

Dithiocarbamates (in mg carbondisulphide/kg)

maneb	0.5	ziram	0.5
zineb	0.5	mancozeb	0.5
nabam	0.5	thiram	0.5
ferbam	0.5		

Aflatoxin-B_1 0.01 mg/kg

Polycyclic aromatic hydrocarbons (in µg/kg)

3,4-benzpyrene	0.2
3,4-benzfluoranthene	0.2
indeno-1,2,3,c,d-pyrene	0.4
1,2-benzfluoranthene	0.4
fluoranthene	1.0

Nitroso compounds (in µg/kg)

nitrosodimethylamine	0.1
nitrosodiethylamine	0.2
nitrosomethylpropylamine	0.4
nitrosomethylbytylamine	0.2
nitrospyrrolidine	0.6
nitrosopiperidine	0.3
nitrosomorpholine	3

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Appendix 3

CONTAMINANTS IN THE INSTITUTE'S STOCK DIET FOR RATS

Sample of 1000 kg batch produced on March 10, 1978

lead		3.8 mg/kg
cadmium		0.07 mg/kg
mercury		0.02 mg/kg
tin		3 mg/kg
arsenic		0.1 mg/kg
selenium		0.14 mg/kg
organo-P-compounds		not detectable*)
malathion		0.02 mg/kg
organo-Cl-compounds		not detectable
dithiocarbamates		not detectable
aflatoxin B_1		not detectable
oestrogenic activity (Tiecco test)		negative
3,4-benzpyrene		2.8 µg/kg
3,4-benzfluoranthene		3.3 µg/kg
indeno-1,2,3,c,d-pyrene		2.8 µg/kg
1,2-benzfluoranthene		1.7 µg/kg
fluoranthene		25 µg/kg
K-nitrate		314 mg/kg
Na-nitrite		2 mg/kg
nitrosodimetylamine		3.2 µg/kg
nitrosopyrrolidine		1.2 µg/kg
other nitrosamines		not detectable
urease activity		0.05 Δ pH
trypsin inhibitor		0.3 % inhibition
haemagglutinins	positive dilution	1 : 20
	negative dilution	1 : 50

*) see list of compounds detected and lower detection limits

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CODING FORM FOR GLOBAL INDEXING

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Doc I.D.	878215ØØ6	3	Old Doc I.D.	8D S	4		
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Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
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Check One: ☐ Publication ☐ Internally Generated ☑ Externally Generated

Pub/Journal Name	—					9	
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Author(s)	—					10	
Organ. Name	E I DUPONT DENEMOURS & CO INC					11	
Dept/Div	—					12	
P.O. Box	—	13	Street No./Name	—		14	
City	WILMINGTON	15	State	DE	16	Zip	19898 17	Country	— 18
MID No. (7)	—	19	D & B NO. (11)	—		20	
Contractor	—					21	
Doc Type	• R.I • U.P • HEASD 8D. . S.U H.S F.N					22	

Doc Title 23

2-YEAR INHALATION CARCINOGENICITY STUDY OF CHLOROPRENE IN RATS

Chemical Name (300 per name)	25	C. S No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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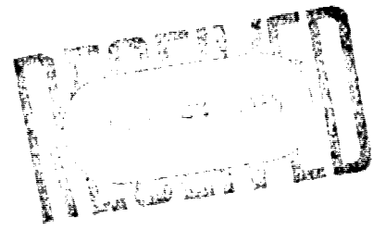

RAPPORT NR. R 6077

A 2-YEAR INHALATION CARCINOGENICITY STUDY OF CHLOROPRENE IN RATS

126-99-8

Authors	: Drs. P.G.J. Reuzel, Drs. M.C. Bosland, Drs. H.C. Dreef-van der Meulen and Dr. V.J. Feron
At the request of	: Joint Industry Committee on Chloroprene
Date	: January 1980
Project number	: B76/1171
Approved by	: Dr. A.P. de Groot
Start of exposures	: February 10, 1976
Termination of exposures	: February 10, 1978
Study director	: Drs. P.G.J. Reuzel

All raw data and the final report are filed in the archives of the Department of Biological Toxicology under the reference "Joint Industry Committee on Chloroprene".

000002

Gehele of gedeeltelijke publikatie van dit rapport is zonder schriftelijke toestemming verboden.
Total or partial publication of this report without written assent is not allowed.

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SUMMARY

1. A 2-year inhalation carcinogenicity study with chloroprene was performed in rats. Three groups of 100 male and 100 female rats each were exposed to 0 (control group), 10 or 50 ppm chloroprene respectively, 6 hours/day, 5 days/week, for a period of two years.

2. In week 72 an interruption of the ventilation in one of the inhalation chambers caused death by suffocation of 87 males and 73 females of the low-level group.

3. All treated animals showed slight restlessness during the exposure periods, but only during the first few weeks.

4. An increase in incidence and degree of alopecia was observed in rats of the high-level group. In the second year of the study both the incidence and degree of the alopecia decreased gradually. No microscopic skin lesions were observed.

5. Mortality was not influenced by exposure to chloroprene.

6. Growth retardation was observed in both males and females of the high-level group. The differences in body weight between control and high-dose rats diminished in the course of the second year, which resulted in similar body weights in all groups towards the end of the study.

7. Relative lung weights were significantly decreased at both exposure levels.

8. The animals in the high level group were less severely affected by chronic respiratory disease than the controls. The number of animals with small foci of cellular alteration in the liver, commonly seen in aged rats, was increased in the high level group.

9. No indications were found for carcinogenic properties of chloroprene up to an exposure level of 50 ppm.

10. Summarizing conclusions were:
    - exposure of rats to an atmosphere containing 50 ppm chloroprene caused alopecia and slight growth retardation;
    - chloroprene at the 50 ppm level may influence the incidence of foci of cellular alteration in the liver;
    - there was no evidence of carcinogenic activity of chloroprene in rats at the 50 ppm level.

A 2-YEAR INHALATION CARCINOGENICITY STUDY OF CHLOROPRENE IN RATS

1. INTRODUCTION

At the request of the Joint Industry Committee on Chloroprene, a chronic (2-year) inhalation study with chloroprene was performed in rats. The exposure levels were selected on the basis of the results of a previous 4-week inhalation study with chloroprene in rats, in which compound-related effects were found at levels of 625, 161 and 39 ppm (Reuzel et al. 1975). The effects were, mortality, alopecia, growth retardation, and microscopical changes in the respiratory tract and the liver. Since the effects observed at 39 ppm consisted of slightly abnormal behaviour and a moderate degree of growth depression, 50 ppm was selected as high-dose level for the present study, expecting that this dose would induce mild, but distinct toxicity. The low exposure level was fixed at 10 ppm.

2. PROCEDURES

2.1 Material and methods

Twelve bottles, each containing 200 ml freshly purified 1,3-butadiene, 2-chloro, synonym : β-chloroprene (further indicated as chloroprene), were received weekly from Bayer AG, Dormagen, W.-Germany, and stored at -20 °C.

To generate the various test atmospheres, sufficient quantities of the test material were delivered to fritted glass vaporization vessels kept at 0 °C. A measured, dried and filtered nitrogen flow was passed through the vessels. Each of the vapour/nitrogen mixtures thus produced was led into the inlet piece of an inhalation chamber, where it was mixed with the main air flow. Teflon and stainless steel transport tubes were used.

2.2 Analyses

Samples of the test atmospheres were taken from each inhalation chamber during the exposure (average three samples/hour) by means of a sample loop and then injected into an Intersmat IG 120 gas-chromatograph fitted with a flame ionisation detector.

The following operating conditions were established:

Column:		300 x 4 mm packed with Carbowax 20M/chromosorb
Temperature:	a. Injection port	95 °C
	b. Oven	65 °C
	c. Detector	150 °C

Exposure chamber concentrations of chloroprene were established by comparing the peak areas produced by samples of the atmosphere with those produced by a standard chloroprene solution. The sample loop was calibrated by comparing peak surfaces of atmosphere samples taken with a gas-tight syringe with peak surfaces of simultaneously taken loop-samples.

2.3 Animals

Three hundred male and 300 female SPF-reared rats (Cpb: WU; Wistar Random) were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, in January, 1976. On arrival, they weighed 35-50 g and were 3 weeks old. After an acclimatization period of two weeks the animals were allocated equally, according to body weight and sex, to three groups of 100 males and 100 females each.

2.4 Exposure chambers

The chambers used had a capacity of 2.5 m^3 and were constructed of stainless steel with a glass door, which allows observation of the animals during the exposures. The chambers had a cylindrical form with a conical top and bottom. Inside they were fitted with a heptagonal frame, which was rotatable around the vertical axis of the chamber.

Forty two wire mesh cages, each with a floor surface of 930 cm^2, were suspended in the rotatable frame in seven vertical rows and six horizontal layers. The position of the rows was changed each day in a fixed order. The position of the animals in the layers was changed once every two months

The chambers were illuminated externally by normal laboratory lighting. The light cycle was 12 hours light and 12 hours dark (light from 06.00 a.m. to 06.00 p.m.).

To prevent leakage of the test atmospheres into the room, the chambers were maintained at a negative pressure of 1-2 mm H_2O, relative to the room. The main airflow was drawn from the room, entered the chambers at the top, and had - when entering the inhalation chambers - a temperature of 21 $\pm$ 2 ^{o}C and a relative humidity of 45 - 60 %. Before entering the inhalation chambers the main airflow was mixed with a nitrogen flow which was laden with the test material. In the chambers the airflow was equally spread by an air disseminator which was located in the top of the upper cone of the chambers.

The flow rate through the chambers was approximately 40 m^3/h during the exposures and 60 m^3/h thereafter.

2.5 Maintenance

All animals were housed in the inhalation chambers during 24 hours a day. For each group all conditions were the same, except for the concentration of the test material in the atmosphere during the exposures.

During the first 16 weeks of the experimental period 5 males or 5 females were housed in each cage. Then the rats were re-distributed over the cages in such a way that each cage contained either 4 males or 6 females.

Neither food nor water was present in the inhalation chambers during the exposures. During the non-exposure periods the rats were provided with bottled unfluoridated tap water and the Institute's stock diet for rats ad libitum. The levels of nutrients and contaminants in stock diet, and contaminant levels in tap water, were determined periodically (Appendix 1).

2.6 Exposures

Groups of 100 rats of each sex were exposed to one of three different atmospheres, for 6 hours a day, 5 days a week during a period of 2 years. The nominal concentrations were 0, 10 and 50 ppm chloroprene. The exposures were generally started between 08.30 and 10.30 a.m. On public holidays the animals were not exposed.

2.7 Conduct of the study

2.7.1 Condition and behaviour

All animals were checked daily and all changes were recorded. Special attention was paid to the occurrence of tumours. During the last six months of the test period all animals were palpated monthly for the presence of tumours. Time of appearance, size, location and progression were recorded. Moribund animals were killed to avoid spontaneous death and subsequent loss by autolysis or cannibalism.

2.7.2 Body weights

All animals were weighed immediately before the first exposure, weekly during the first 8 weeks and then at 4-week intervals. Animals loosing more than 10 % weight between two weighings were observed daily, until their weight gain was normal or they were sacrificed.

2.7.3 Pathology

After the 2-year exposure period the animals were sacrificed on 11 successive working days, in such a way that, on the average, time of sacrifice was approximately the same for each group. The rats were killed by exsanguination from the abdominal aorta under ether anaesthesia. A post-mortem examination was conducted during which the tissues were examined for gross abnormalities. The following organs were weighed:*)

spleen	testes
adrenals	ovaries
kidneys	thyroid
liver	brain
heart	pituitary
lungs with trachea and larynx	

Samples of the organs weighed and of those mentioned below were preserved in a 4 % aqueous neutral phosphate-buffered formaldehyde solution. The lungs were fixed (after weighing) by intratracheal infusion of the fixative under 10 cm water pressure.

skin	thymus
external auditory canal with ceruminal (Zymbal's) glands	uterus
preputial glands	nasal cavity (four transverse sections)
skeletal muscle	eyes
sciatic nerve	sternum (bone marrow)
mammary glands	oesophagus
seminal vesicles	aorta
prostate	stomach
epididymides	duodenum
caecum	jejunum
colon	ileum
pancreas	adipose tissue
lymph nodes (axillary, cervical and mesenteric)	submaxillary salivary glands
urinary bladder	exorbital lachrymal glands
spinal cord	parotid salivary glands
femur (with knee joint)	sublingual salivary glands
all gross lesions	

*) Organs of animals which died spontaneously or were killed in extremis were not weighed.

The organs and tissues to be examined microscopically were processed to paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin. Nose, sternum and femur were decalcified prior to processing to paraffin wax.

Microscopic examination was carried out

a) of all organs and tissues preserved from all control and all high-level rats and

b)* of the liver, spleen, pituitary, thyroid, adrenals and all grossly visible tumours or tumour-like lesions from rats of the low-level group.

Attention was paid to detection of possible metastases from malignant tumours.

2.7.4 Statistical analyses

Statistical analyses of body weight changes and organ-to-body weight ratios were carried out using Student's t-test. The chi-square test was used for analysing differences in the incidence of histopathological changes and in tumour incidences.

3. RESULTS

3.1 Exposure period

The nominal exposure period extended for two years, from 10 February 1976 to 10 February 1978. The subsequent autopsies were carried out over a period of 17 days, during which the exposures were continued, so that the last exposure day was 24 February, 1978. The nominal exposure period comprised 512 planned exposure days. As a result of maintenance and repair work to the inhalation chambers and the analytical apparatus, the actual number of exposure days was 508 days for the low-level group and 509 days for the high-level group.

3.2 Chloroprene concentrations (table 1)

The actual mean concentrations of chloroprene in the test atmospheres during all the test period were 10.1 and 50.3 ppm for the low- and high level group respectively.

* Only applicable to animals which did not die by the ventilation accident (see § 3.7.1).

3.3 Condition and behaviour (table 2)

A slight restlessness was seen in rats of both treatment groups during the first few weeks of the exposure period only. Signs of slight eye irritation, such as closed or half-closed eyes and occasional lacrimation, were observed only in rats of the high-level group during the first two weeks. During the remainder of the study no differences in behaviour between test and control animals, or indications of eye irritation were observed.

The health condition of the rats was not affected by exposure to chloroprene, except that alopecia occurred more frequently in rats of the top-level group than in those of the low-level group or in the controls. The alopecia varied from small focal, mostly bilateral bald areas to severe, diffuse, generalized hairloss. Both the incidence and degree of alopecia were clearly more marked in females than in males. Alopecia, seen as small focal bilateral, bald areas in the lumbar or sacral region and in the cervical region in front of the scapulae, was seen for the first time in a few high-level rats after an exposure period of about 10 weeks. The degree and incidence then rapidly increased, but after a test period of 6 months the incidence and degree of the alopecia gradually decreased and in many animals complete regrowth of hair was observed. During the final 6 months of the study the number of rats showing alopecia again slightly increased.

3.4 Mortality (table 3; appendix 4)

The mortality rate was relatively low in all groups up to week 72; it ranged from 1 to 3 %. During the first night of week 72, however, 87 males and 73 females of the low-level group died from suffocation as a result of a failure of the ventilation system of the chamber. Though the mortality figures of this group are difficult to evaluate, it may be stressed that the mortality rate during the post-accidental period was proportionally similar to that of the controls.

Mortality rates of the high-level group was very similar to that of the controls throughout the study.

3.5 Body weights (figures 1 and 2; table 4 and appendix 3)

During the major part of the study the mean body weights of rats of the low-level group were slightly higher than those of the controls. As a result of the ventilation failure in week 72, the survivors of the low-level group showed a considerable loss of body weight; however, a partial recovery occurred during the next few months.

A slight but consistent growth retardation was found in males and females of the high-level group. The differences in weight, however, gradually became smaller, in females from week 56 and in males from week 80.

During the last 4 months of the study average body weights of controls and high-level groups gradually drew closer as a result of both a slight decrease in body weight in controls and a slight weight gain in the high-level rats.

3.6 Organ weights (table 5)

In both test groups mean lung weights expressed relative to body weight were statistically significantly lower than in the controls. The degree of weight depression was, however, no more marked in the high- than in the low-level animals.

Relative liver weights in the female test groups were slightly, though statistically significantly increased; again there was no dose-response relationship.

At the high-dose level, the mean relative spleen and thyroid weights were statistically significantly lower in females only. The kidney and pituitary weights in males of the low-dose group were significantly increased without any similar increase at the high-dosage level.

3.7 Pathology

3.7.1 Low level group

The animals of the low level group that died of suffocation in week 72 were subjected to gross autopsy which was restricted to the observation of subcutaneous, thoracic and abdominal tumours or tumour-like lesions. Two mammary gland tumours and one ovarian tumour were found in the females, and no tumours in the males. Subsequently, the animals were fixed in toto with opened thoracic and abdominal cavity. Only liver samples of all animals were processed for microscopical examination.

3.7.2 Macroscopic examination (table 6, appendix 4)

At autopsy the lungs in the test groups were observed to have a markedly lower incidence of signs of chronic respiratory diesease.

In females the incidence of tumours or tumour-like lesions of the mammary glands were slightly higher in the high-level group (viz. 34/100) than in the control group (viz. 23/99), the difference being statistically insignificant.

No other remarkable differences in gross changes were noticed between the groups.

3.7.3 Microscopic examination (tables 7-12, appendices 4 and 5)

a) Non-neoplastic lesions

The site, type and incidence of non-neoplastic histopathological changes observed in rats of the control and high-dose group are presented in table 7.

In addition, the incidence and type of foci of cellular alteration in the liver, which were classified according to Squire and Levitt (1975), are presented in more detail in table 8.

The number of rats with one or a few small foci of cellular alteration in the liver was higher in the 50 ppm group than in controls (tables 7 and 8). In males the higher incidence of these hepato-cellular lesions was due to a higher incidence of one or a few small clear cell foci ($P < 0.01$), and in females to a higher incidence of the combination of basophilic, clear cell and mixed cell type foci ($P < 0.01$).

The incidence of foci of hepato-cellular alteration in animals of the 10 ppm group which did not die from suffocation in week 72, was 1/13 and 6/24 for males and females respectively (table 8).

The livers of the rats of the 10 ppm group which died due to the accident in week 72 were all slightly to moderately autolytic, and therefore, foci of cellular alteration were difficult to assess, especially the clear cell foci. In the males 1/87 showed a small clear cell focus, and no foci of cellular alteration were observed in the females. Actually, the histological examination of these 10 ppm rats did not produce any evidence of a compound-related effect on the liver.

The degree of chronic respiratory disease, which is a common finding in aged rats of the strain used, was strikingly less severe in the high-level animals than in controls. Only mild changes such as lymphoid aggregates around bronchi, bronchioli and blood vessels were often observed in males and females of the 50 ppm group. The more severe lesions such as bronchiectasies, consolidation of alveolar tissue, accumulation of pigment-laden macrophages and medial hypertrophy of arterial walls were very seldom observed in test animals, but occurred rather frequently in control animals. Acute inflammatory processes, however, were found in high-level and control animals to a similar extent. The incidence of infiltrates of mononuclear cells, and hyper- and metaplasia of the epithelium of the trachea and larynx was distincly less in this test group than in the controls.

Most of the other lesions listed in table 7 were about equally distributed between the test and the control group or only occurred in one or a few animals. These lesions are considered to be related to normal ageing and not to chloroprene exposure.

Several statistically significant differences in the incidence of lesions between the control and test group (table 7) are considered not to be related to treatment. The differences were small and the alterations in question are quite common findings in aged rats of the strain used. The incidences in the test and the control group always fitted background data of recent 2-year studies carried out with the same strain of rats. Therefore, these differences are regarded as unrelated to treatment but demonstrate the large variability in incidence of these lesions that may occur.

b) Neoplastic lesions

Type, site and incidence of neoplastic lesions in the various groups are presented in table 9. Incidence and multiplicity of mammary gland tumours in females are presented in table 10.

The number of females bearing mammary tumours in the 50 ppm group was statistically significantly increased ($P < 0.05$). This increase was mainly due to animals that were killed or that died before the terminal sacrifice ($P < 0.01$). No difference was found between control and test group rats killed at the end of the study. Multiplicity of mammary tumours was not different between the top-dose group and the control group. The number of high-level rats bearing two or more mammary tumours was slightly and not statistically significantly higher than the number of controls with two or more of this type of tumours. The increased incidence of mammary tumours was almost entirely due to the relatively high number of animals of the test group bearing (benign) fibroadenomas. The number of animals with two or more fibroadenomas was also increased ($P < 0.05$). Multiplicity of fibroadenomas was not increased.

A typical localization of squamous cell carcinomas was found in 3/100 males of the 50 ppm group and in 1/99 females of the control group. These tumours were observed in the nasal cavity, the sinus maxillaris and subcutis, and, in three of the four cases, the skin was involved too. Neither macroscopic, nor microscopic examination could clarify the exact origin of these tumours. In case they are skin tumours, the total number of squamous cell carcinomas of the skin would amount to 5/100 males of the 50 ppm group, which is significantly different from the incidence in the control group, viz. 0/97 ($P < 0.05$).

All other tumours were about equally distributed between test and control groups or occurred in just one or two animals. The tumours observed represented the usual variety of neoplastic lesions found in this strain of rats at an age of approximately two years.

4. DISCUSSION

The results of the present study indicate the occurrence of minor adverse effects in rats exposed to an atmosphere containing 50 ppm chloroprene.

A slight transient restlessness occurred at the 50 ppm level during the first few weeks only. Such a pattern of behaviour is frequently encountered in inhalation studies during the exposures. It is probably due to an unpleasant smell or to slightly irritating properties of the compound in question.

From a previous 4-week inhalation study with chloroprene in rats (Reuzel et al., 1975) it appeared that the fur of the animals was affected by the test compound. This was confirmed in the present study in that an increased incidence of shedding of hair and of bald areas was found at the 50 ppm level.

It should be mentioned that two types of alopecia may occur in the strain of rats used:

- transient, focal, most often bilateral alopecia of unknown etiology. In our Institute its frequency ranges from 0 to 30 % in females and in males from 0 to 5 %;
- alopecia caused by plucking of hair by other rats in the same cage. This type is infrequently observed.

Since plucking of hair was not observed during the present study, and, moreover, the characteristic gatherings of hair were never seen on the pans under the cages it is very unlikely that this type of alopecia played an important role in the loss of hair seen after chloroprene exposure. At least part of the alopecia in the top-level group may be ascribed to the occurrence of spontaneous alopecia. However, the difference in degree and incidence of alopecia between the control and top-dose group in the present study was considerable. Therefore, it is justified to conclude that the increased alopecia was an effect of the treatment. This finding is in agreement with observations of hair loss in men exposed to chloroprene (Nyström, 1948; Ritter and Carter, 1948; Schwartz, 1945).

As could be expected from the results of the previous range finding study (Reuzel et al., 1975) growth retardation occurred in the present study. It was, however, minimal and restricted to rats of the top-level group.

The lungs of the high-level rats were remarkably less severely affected by chronic respiratory disease (CRD) than those of the controls. This might be the explanation for the decrease in relative lung weight found in top-dose animals. These observations are suggestive of chloroprene exposure reducing, in one way or another, the development of CRD in rats.

Another possibility is that the controls in this study were affected by CRD to an unusually large extent. This suggestion, however, must be rejected, since the incidence of CRD in the control group, viz. 35 % in males and 25 % in females, corresponds with the background incidence in rats of the same strain used as controls in 4 recently completed two-year oral toxicity studies, viz. 32 % (range 15 - 55 %) in males and 17 % (range 0 - 25 %) in females.

A minimal, though statistically significant increase in relative liver weight was found in females of both the low- and high-level group. Since such an increase was not seen in males, and, moreover, in females no dose-response relationship occurred, these relatively high liver weights in females of the test groups were not regarded as the expression of an effect of chloroprene on the liver but rather as fortuitous findings without any toxicological significance.

In comparison with the controls the incidence, but not the size, of foci of cellular alteration in the liver was slightly, though statistically significantly increased in rats exposed to 50 ppm chloroprene, viz. from 14 to 30 % in males and from 13 to 27 % in females[1]. However, the increase in incidence was not impressive and this type of liver alterations is common in aged rats (Burek, 1978), and may vary widely within one rat strain (Fears et al., 1977; Goodman et al., 1979). On the basis of these findings the possibility can not be ruled out that the increase observed is a chance effect.

Relative weights of spleen and thyroid were slightly lower in females of the 50 ppm group than in controls. Since no treatment-related histopathological changes were observed in these organs, no toxicological significance was attached to this finding.

---

1) The low level group was not taken into consideration because of the small effective number of animals in this group.

The incidence of fibro-adenomas of the mammary glands, but not that of malignant mammary tumours, was statistically significantly higher in females of the high dose group than in the controls.[1] The incidence of these benign lesions in the controls, viz. 30 %, was above the background incidence of 12 % (range 6 - 21 %), based on data from controls used in 5 recently completed two-year oral toxicity studies with the same strain of rats.

However, in 4 concurrent life-span studies with this strain of rats the mean incidence of palpable subcutaneous tissue masses in control groups after an experimental period of 104 weeks appeared to be 38 % (range 27-46 %) (table 11). The incidence of macroscopically observed subcutaneous tissue masses in the control- and high-dose group, viz. 23 and 34 % respectively, and that of microscopically confirmed mammary gland tumours in the same groups, viz. 30 and 45 % respectively, together with other subcutaneous tumours in these groups, viz. 2 and 3 % respectively, fall within the aforementioned range of incidences of gross subcutaneous tissue masses for the control groups in these 4 recent studies. On the basis of these data, chloroprene exposure is not considered to enhance the development of benign mammary gland tumours in rats.

The origin of the squamous cell carcinomas found in the nasal area of 3/100 males of the high dose group could not be established and may have been the nasal cavity, maxillary sinus or skin. If all three tumours originated in the skin the total incidence of skin squamous cell carcinomas in males of the high-dose group would have been 5/100, while none were found in male controls. However, this incidence is close to the background incidence in this strain of rats (this varies from 0 to 3,4 %, table 12) and there was no increase in the incidence of these tumours in female animals, only one occurring in the control group. Moreover, chloroprene was found to have no initiating nor promoting properties in skin painting experiments in mice (Zil'fyan et al., 1977).

In conclusion, the present study provides evidence that exposure of rats to a test atmosphere containing 50 ppm chloroprene resulted in alopecia and slight growth retardation; chloroprene may also has influenced the incidence of foci of cellular alteration in the liver, commonly observed in aged rats. No evidence was obtained of chloroprene possessing carcinogenic properties. This is in agreement with the rather incompletely reported studies on chloroprene of Zil'fyan et al. (1977) in rats and mice using oral and intratracheal administration as well as skin painting

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1) The low level group was not taken into consideration because of the small effective number of animals in this group.

and subcutaneous injection, and also with the results of a recent life-span study in rats with chloroprene administered by stomach tube (Ponomarkov and Tomatis, 1979).

In none of these experiments any indication was found of carcinogenic activity of chloroprene.

5. REFERENCES

1. Burek, J. D., Pathology of Aging Rats, CRC Press, West Palm Beach (1978)

2. Fears, R. T., Tarone, R. E. and Chu, K. C.,
False-positive and false-negative rates for carcinogenicity screens,
Cancer Res., 37, (1977) 1941 - 1945.

3. Goodman, D. G., Ward, J. M., Squire, R. A., Chu, K. C. and Linhart, M. S.,
Neoplastic and non-neoplastic Lesions in aging F334 rats, Toxicol. Appl.
Pharmacol., 48, (1979) 237 - 248

4. IARC Monographs on the evaluation of carcinogenic risk of chemicals to man
Vol. 19, Some monomers, plastics and synthetic elastomers, and acrolein,
International Agency on Cancer Research, Lyon (1979) pp. 131 - 156.
1979, pp. 131-156

5. Nyström, A. E.
Health hazards in the chloroprene rubber industry and their prevention
Acta Med. Scand. 132 (1948) Suppl. 219, 1 - 125

6. Ponomarkov, V. and L. Tomatis
Long-term testing of vinylidene chloride and chloroprene for carcinogenicity in mice and rats
Int. J. Cancer (1979) in press

7. Reuzel, P. G. J., A. Kruysse, V. J. Feron and H. R. Immel
Subacute (four week) inhalation study with β-chloroprene in rats
CIVO report R-4860 (1975)

8. Ritter, W. L. and A. S. Carter
Hairloss in neoprene manufacture
J. Ind. Hyg. Toxicol. 30 (1948) 192-195

9. Schwartz, L.
Skin hazards in the manufacture and processing of synthetic rubber
J. Am. Med. Assoc. 127 (1945) 389-391

10. Squire, R. A. and M. H. Levitt
Classification of specific hepatocellular lesions in the rat: Report of a workshop
Cancer Res. 35 (1975) 3214-3223

11. Zil'fyan, V.N., B. S. Fichidzhyan, D. K. Garibyan and A. M. Pogosova
Experimental study of chloroprene for carcinogenicity (Russ.)
Vopr. Onkol. 23 (1977) 61-65

We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedure herein described, and that this report represents a true and accurate record of the results obtained.

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Table 1. Actual mean chloroprene concentrations, their standard deviation and standard error of the mean during 24 months

Group	Mean concentration of chloroprene					
	in ppm			in mg/m^3 air		
	Mean	SD[1)]	SEM[2)]	Mean	SD	SEM
Low dose	10.1	0.8	0.0	37.1	2.9	0.1
High dose	50.3	3.8	0.2	184.6	13.9	0.7

[1)] SD = Standard deviation.

[2)] SEM = Standard error of the mean.

Table 2. Degree and incidence of alopecia[1)]

Degree of alopecia	Number of animals with alopecia					
	Males			Females		
	Chloroprene concentration (in ppm)			Chloroprene concentration (in ppm)		
	0	10	50	0	10	50
			Week 12			
a. Slight	-[2)]	-	-	-	-	-
b. Moderate	-	-	-	-	-	-
c. Severe	-	-	-	-	-	-
Total incidence	0	0	13	13	6	59
			Week 18			
a. Slight	-	-	-	-	-	-
b. Moderate	-	-	-	-	-	-
c. Severe	-	-	-	-	-	-
Total incidence	6	1	33	27	19	86
			Week 25			
a. Slight	2	5	9	20	14	16
b. Moderate	0	0	5	5	4	19
c. Severe	0	0	0	1	1	26
Total incidence	2	5	14	26	19	61
			Week 37			
a. Slight	1	0	3	21	12	28
b. Moderate	0	0	3	2	7	18
c. Severe	0	0	0	0	0	6
Total incidence	1	0	6	23	19	52
			Week 48			
a. Slight	0	0	3	15	10	17
b. Moderate	0	0	2	0	5	19
c. Severe	0	0	0	0	1	5
Total incidence	0	0	5	15	16	41
			Week 70			
a. Slight	0	0	0	3	2	22
b. Moderate	0	0	0	0	0	0
c. Severe	0	0	0	0	0	0
Total incidence	0	0	0	3	2	22
			Week 88			
a. Slight	3	2	23	10	1	26
b. Moderate	9	0	0	3	0	16
c. Severe	0	0	0	0	0	0
Total incidence	12	2	23	13	1	42

1) By mistake the degree and incidence of alopecia was not recorded at autopsy.

2) - = not recorded.

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Table 3. Cumulative mortality in groups initially consisting of 100 male and 100 female rats

Chloroprene concentration (in ppm)	Total number of deaths at the end of week												
	40	44	64	68	72	76	80	84	88	92	96	100	104
Males													
Control 0	0	0	0	2	3	3	3	4	6	11	15	17	23
10	0	1	1	2	89[1]	89	89	89	89	89	90	90	91
50	0	0	0	0	2	2	3	6	6	8	10	15	23
Females													
Control 0	0	0	0	0	1	2	3	3	4	7	12	14	19
10	1	1	2	3	77[1]	77	78	79	79	79	79	79	81
50	0	0	0	0	2	3	4	5	8	12	14	17	25

[1] 87 males and 73 females died in week 72 because of a failure of the ventilation system of the chamber.

Table 4. Mean body weights of groups initially consisting of 100 male and 100 female rats

Chloroprene concentration (in ppm)	Mean body weights (in g) at the end of week														
	0	1	4	8	12	17	28	40	52	64	76	84	92	100	104
Males															
0	103	134	231	298	343	366	404	435	460	487	505	499	487	495	487
10	98**	141***	237	310**	349	379*	413	449	461	492	490	502	501	500	501
50	102	123	208***	264***	305***	338***	371***	394***	419***	455***	474***	464***	480	494	491
Females															
0	88	111	154	184	201	212	226	243	260	281	298	291	299	311	306
10	89	115***	162***	189*	202	217	231	252*	266	287	286	298	293	306	309
50	89	102***	147***	171***	186***	200***	215***	226***	239***	267**	285*	276**	297	308	307

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$

000023

Table 5 .Mean body weights (g), mean relative organ weights (g/100 g body weight) and the standard error of the mean of groups initially consisting of 100 male and 100 female rats, after an experimental period of two years

Group no.	Chloroprene concentration (in ppm)	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	pituitary	thyroid	adrenals	lungs	effective number of animals
						MALES							
8465	0	494.	.307	.61	3.09	.154	.43	.68	.0034	.0056	.0099	.45	77
		(6.)	(.004)	(.01)	(.04)	(.004)	(.01)	(.01)	(.0001)	(.0001)	(.0002)	(.01)	
8466	10	500.	.319	.68*	3.31	.172	.43	.64	.0042**	.0056	.0096	.37**	9
		(14.)	(.011)	(.04)	(.09)	(.014)	(.02)	(.04)	(.0003)	(.0007)	(.0003)	(.01)	
8467	50	496.	.303	.64	3.15	.146	.42	.70	.0036	.0056	.0094	.38***	76
		(7.)	(.005)	(.01)	(.05)	(.003)	(.01)	(.01)	(.0001)	(.0001)	(.0003)	(.01)	
						FEMALES							
8465	0	308.	.370	.64	3.00	.180	.63	.030	.0062	.0080	.0180	.53	81
		(5.)	(.008)	(.01)	(.04)	(.005)	(.01)	(.001)	(.0002)	(.0002)	(.0005)	(.02)	
8466	10	309.	.396	.65	3.23*	.176	.63	.029	.0062	.0073	.0183	.45*	19
		(7.)	(.016)	(.01)	(.08)	(.006)	(.01)	(.002)	(.0005)	(.0002)	(.0007)	(.01)	
8467	50	307.	.368	.65	3.13*	.164**	.63	.028	.0057	.0070**	.0172	.45***	75
		(5.)	(.007)	(.01)	(.04)	(.003)	(.01)	(.001)	(.0002)	(.0002)	(.0004)	(.01)	

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

THE STANDARD ERROR OF THE MEAN APPEARS IN BRACKETS

Table 6. Incidence, type and site of gross observations at autopsy in the various groups (survivors as well as non-survivors)

Site and type of observation	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
Initial number of animals	100	100	100	100	100	100
Effective number of animals	97	13	100	99	24	100
Mode of death:						
a. Found death	9	91	9	5	83	1
b. Killed in extremis	14	1	22	13	3	22
c. Scheduled sacrifice	77	8	69	82	14	77
Autolysis/cannibalism:						
a. Slightly autolytic	2	0	0	0	0	0
b. Partially lost	2	0	4	1	2	0
c. Completely lost	1	0	0	0	0	0
Site and type of observation						
LIVER						
1. Tumour or suspected of tumour	0	0	0	2	0	0
2. Prominent lobular pattern	54	7	63	32	10	42
3. Hepatomegaly	7	0	12	4	1	11
4. Discoloured:						
a. Pale	11	3	38	20	7	28
b. Dark	4	0	4	4	1	4
c. Yellow	3	0	3	0	1	0
5. Cyst	6	0	8	12	1	12
6. Parasite	5	0	1	0	0	4
7. Small surface lesions (rosette, constrictions, fibrosis)	6	0	7	11	5	18
8. Evidence of inflammation	0	0	1	3	1	2
LUNGS						
1. Tumour or suspected of tumour	1	0	0	2	0	1
2. Evidence of CRD or inflammation	18	0	3	10	0	1
3. Spongy	3	1	2	0	1	0
4. Spotted	26	2	15	16	3	4
5. Atelectasis	45	0	1	37	2	2
6. Haemorrhage	1	0	3	0	0	1
KIDNEYS						
1. Tumour or suspected of tumour	2	0	2	3	0	1
2. Granular appearance	31	6	34	3	2	6
3. Unilateral hydronephrosis	0	0	2	1	0	0
4. Discoloured:						
a. Pale	2	0	5	0	0	0
b. Dark	3	0	1	1	1	0
c. Greenish	21	8	29	6	1	12
5. Enlarged	4	3	9	0	0	0
6. Cyst	4	1	3	1	0	1
7. Spotted	0	0	5	1	1	1
8. Haemorrhage	0	0	1	1	0	0

Table 6. Continued 1

Site and type of observation	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
SPLEEN						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Splenomegalia	3	0	5	6	0	2
3. Small spleen	0	0	0	1	0	0
4. Granular appearance						
THYMUS						
1. Tumour or suspected of tumour	0	0	0	1	[illegible]	1
PANCREAS						
1. Tumour or suspected of tumour	3	0	2	3	0	0
2. Discoloured:						
a. Pale	2	0	3	2	1	2
b. Dark	0	0	0	0	0	2
3. Granular appearance	1	1	5	2	0	0
4. Haemorrhage	1	0	0	2	0	0
HEART						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Enlarged	0	0	4	1	0	1
STOMACH						
1. Tumour or suspected of tumour	0	0	1	1	0	1
2. Haemorrhage/erosions	2	1	5	1	1	1
SMALL INTESTINES						
1. Tumour or suspected of tumour	0	0	1	0	[illegible]	0
2. Prominent Peyer's patches	1	0	2	0	[illegible]	0
3. Haemorrhage	3	0	0	2	0	0
CAECUM						
1. Tumour or suspected of tumour	0	0	2	1	0	0
2. Enlarged/swollen	2	0	0	3	0	0
COLON						
1. Tumour or suspected of tumour	0	0	1	0	0	0
URINARY BLADDER						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Proteinaceous plug	1	0	0	0	0	0
3. Haemorrhage	0	0	0	1	0	0

Table 6. Continued 2

Site and type of observation	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
THYROID						
1. Tumour or suspected of tumour	3	1	3	0	0	2
2. Small thyroid	0	0	0	6	3	5
3. Discoloured:						
a. Pale	0	0	0	3	0	0
b. Dark	14	4	17	6	2	4
4. Enlarged	4	0	2	0	0	0
5. Haemorrhage	0	0	0	1	0	0
PITUITARY						
1. Tumour or suspected of tumour	3	0	3	6	4	7
2. Haemorrhagic	6	1	1	4	1	7
3. Discoloured:						
a. Pale	0	0	2	3	0	1
b. Dark	0	0	0	1	0	0
ADRENALS						
1. Tumour or suspected of tumour	3	1	2	3	0	1
2. Small adrenal:						
a. Unilateral	0	0	2	0	0	0
b. Bilateral	17	5	23	0	0	0
3. Haemorrhagic	0	0	0	1	0	2
4. Discoloured:						
a. Pale	9	0	11	25	6	30
b. Dark	1	0	1	9	0	5
5. Spotted	10	2	12	30	8	38
6. Enlarged	3	0	5	9	2	9
SUBMANDIBULAR SALIVARY GLANDS						
1. Tumour or suspected of tumour	2	0	0	2	0	0
2. Edema formation	12	1	11	1	0	2
PAROTID SALIVARY GLANDS						
1. Spotted	1	1	0	0	0	0
EXORBITAL LACHRYMAL GLANDS						
1. Granular appearance	17	3	20	0	0	0
2. Discoloured:						
a. Pale	17	5	22	0	0	0
b. Dark	2	0	0	0	0	0
3. Enlarged	1	0	0	0	0	0

Table 6. Continued 3

Site and type of observation	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
MAMMARY GLANDS						
1. Tumour or suspected of tumour	3	0	0	23	10	34
2. Evidence of secretory activity	0	0	0	5	1	8
3. Evidence of inflammation	0	0	0	42	13	48
UTERUS						
1. Tumour or suspected of tumour				6	1	4
2. Hydrometria				5	2	4
3. Evidence of inflammation				1	0	1
4. Proliferation of endometrium				11	3	19
5. Polyp				7	2	5
OVARIES						
1. Tumour or suspected of tumour				1	1	0
2. Cyst(s)				23	3	21
3. Enlarged				2	0	0
TESTIS						
1. Tumour of suspected of tumour	1	0	0			
2. Atrophy: a. Unilateral	7	2	8			
b. Bilateral	3	2	3			
3. Enlarged	2	1	1			
4. Hydrocoel	1	1	4			
PROSTATE						
1. Tumour or suspected of tumour	0	0	1			
2. Evidence of inflammation	0	0	6			
SEMINAL VESICLES						
1. Evidence of inflammation	1	0	0			
2. Atrophy	1	0	0			
EPIDIDYMIDES						
1. Atrophy	0	0	1			
PRAEPUTIAL GLANDS						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Evidence of inflammation	6	1	4	5	1	8

Table 6. Continued 4

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
BRAIN						
1. Tumour or suspected of tumour	0	0	0	0	0	1
MESENTERIC LYMPH NODES						
1. Tumour or suspected of tumour	0	0	2	2	1	1
2. Enlarged	2	0	4	2	1	2
3. Small	0	0	0	1	0	1
4. Greenish discoloured	1	0	1	1	1	0
5. Haemorrhagic	1	0	0	0	0	1
AXILLARY LYMPH NODES						
1. Tumour or suspected of tumour	0	0	0	1	0	1
2. Enlarged	0	0	2	4	2	1
EYES						
1. Tumour or suspected of tumour	0	0	1	1	0	0
2. Enlarged	0	0	0	0	1	1
3. Blind	0	0	0	1	0	0
ABDOMINAL CAVITY						
1. Tumour or suspected of tumour	0	0	2	1	0	0
2. Hydrops ascites	1	0	1	0	0	0
THORACIC CAVITY						
1. Hydrothorax	2	0	5	2	0	2
2. Haemorrhage	2	1	2	1	0	0
SKIN						
1. Tumour or suspected of tumour	0	0	1	0	0	0
ORAL CAVITY						
1. Tumour or suspected of tumour	0	0	0	0	0	1

Table 6. Continued 5

Site and type of observation	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
MENINGES						
1. Haemorrhage	1	0	0	0	0	0
CERVICAL LYMPH NODES						
1. Tumour or suspected of tumour	1	0	2	0	0	0
2. Discoloured: Dark	1	0	0	6	2	0
3. Evidence of inflammation	0	0	0	[illegible]	1	0
4. Cyst	0	0	0	[illegible]	0	0
5. Enlarged	0	0	0	3	4	0
MISCELLANEOUS						
1. Subcutaneous tumour	2	0	4	7	0	0
RECTUM						
1. Tumour or suspected of tumour	2	0	0	0	0	0

Table 7. Site, type and incidence of non-neoplastic histopathological changes observed in animals of the control and the highest dose group[1)]

Site and type of changes[2)]	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
Initial number of animals	100	100	100	100
LIVER	(97)	(96)	(98)	(99)
1. FOCI OF CELLULAR ALTERATION (TOTAL)	14	30**	13	27*
2. Foci of RES-cells, occasionally accompanied by a few necrotic hepatocytes	10	17	20	12
3. Periportal infiltrates of mononuclear cells	24	26	13	9
4. Slight degree of bile-duct proliferation:				
a. Tubular type	40	40	24	13*
b. Oval cell type	3	6	13	9
5. Periportal fibrosis	4	0*	0	0
6. Vacuolation of liver cells:				
- Periportal	1	0	1	0
- Centrilobular	0	1	1	0
- Focal	0	4*	7	10
- Diffuse	3	5	2	1
7. Single cell necrosis	1	3	3	1
8. Increased number of Kupffer cells	0	0	3	5
9. Slight extra-medullary haematopoiesis	0	1	3	0
10. A few small foci of necrotic hepatocytes	0	4*	0	0
11. Large areas of necrosis	0	1	0	1
12. Granuloma	2	3	1	3
13. Focal hyperaemia, accompanied by liver cell atrophy	4	0*	2	0
14. Parasite	1	0	0	1
15. Cyst	2	0	0	4*
16. Centrilobular liver cell degeneration	1	0	0	0
17. Enlarged centrilobular liver cells	1	0	0	0
LUNGS	(97)	(97)	(99)	(99)
1. CHRONIC RESPIRATORY DISEASE:				
a. PERIBRONCHIAL, PERIBRONCHIOLAR AND/OR PERIVASCULAR LYMPHOID AGGREGATES:				
- Slight	23	58***	34	81***
- Moderate	18	30*	27	7***
- Marked	4	3	2	0
b. BRONCHIECTASIES WITH EPITHELIAL HYPER-/METAPLASIA AND/OR LYMPHOID AGGREGATES:				
- Slight	21	0***	13	0***
- Moderate	10	0**	12	0***
- Marked	4	0	0	0

Table 7. Continued 1

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
c. CONSOLIDATION OF ALVEOLAR TISSUE:				
- Slight	21	1***	18	1***
- Moderate	10	1**	6	0*
- Marked	4	0*	0	0
2. SLIGHT TO MODERATE MEDIAL HYPERTRCPHY OF ARTERIAL WALLS	25	4***	7	1*
3. PIGMENT LADEN INTERSTITIAL MACROPHAGES	12	0***	0	0
4. a. Focal acute pneumonia:				
- Slight	0	5*	1	1
- Moderate	0	0	2	2
- Marked	0	3	1	0
b. Massive acute pneumonia	2	0	3	0
5. Focal accumulations of alveolar macrophages	5	6	3	2
6. Foci of thickened, cell-rich alveolar septa (lymphoid infiltrations, epithelialization) accompanied by an increased number of alveolar macrophages:				
- A few foci	24	20	18	7*
- A moderate number of foci	2	3	0	1
7. Calcareous deposits in arterial wall	32	35	31	38
8. Adenomatosis	1	1	1	2
9. Scar tissue	3	0	0	0
10. Haemorrhages	1	0	0	0
11. Cholesterol clefts	1	0	0	0
12. Bone spicule	0	0	2	0
13. Focal pleuritis	0	2	1	0
14. Granuloma	0	0	1	0
15. Parasite	0	1	0	0
16. Foreign body	0	2	0	0
17. Slight degeneration of bronchiolar epithelium	0	0	0	1
TRACHEA	(98)	(95)	(100)	(100)
1. HYPER- AND/OR METAPLASIA OF EPITHELIUM:				
- Slight	4	4	11	0***
- Moderate	3	0	4	0*
- Marked	1	0	0	0
2. SUBEPITHELIAL AGGREGATES OF MCNONUCLEAR CELLS:				
- Slight	59	23***	53	6***
- Moderate	15	0***	12	0***
- Marked	3	0	1	0
3. Slight to moderate acute tracheitis	3	1	0	0
4. Slight degeneration of bronchiolar epithelium	0	0	0	1

Table 7. Continued 2

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
LARYNX	(93)	(87)	(88)	(83)
1. HYPER- AND/OR METAPLASIA OF EPITHELIUM:				
- Slight	3	3	9	1*
- Moderate	4	0*	1	0
2. SUBEPITHELIAL INFILTRATES OF MONONUCLEAR CELLS:				
- Slight	48	5***	42	1***
- Moderate	13	0***	11	0***
KIDNEYS	(95)	(94)	(94)	(98)
1. Tubular nephrosis:				
- Slight	36	32	42	50
- Moderate	36	34	14	19
- Marked	15	19	2	1
2. Degenerated glomeruli	37	44	10	10
3. Focal infiltrates of mononuclear inflammatory cells	70	72	33	37
4. Periglomerular sclerosis	2	1	0	0
5. Increased amount of brown pigment in cortical tubular epithelial cells:				
- Slight	26	20	11	28**
- Moderate	5	6	1	1
6. Calcareous deposits in the cortico--medullary layer and/or papilla	0	0	19	18
7. Dilatation of tubules	71	66	14	21
8. Casts within tubules	67	45**	15	15
9. Urothelial hyperplasia in the pelvis	2	3	10	0***
10. Microabscess	2	0	0	0
11. Acute nephritis	1	1	0	0
12. Hydronephrosis	0	1	1	0
13. Fibrosis	0	0	1	1
14. Periarteritis	0	0	2	2
15. Cyst	2	0	1	0
NASAL CAVITY	(98)	(97)	(97)	(99)
1. Rhinitis:				
- Slight	6	6	7	2
- Moderate	1	1	1	2
2. Sinusitis:				
- Slight	4	1	5	3
- Moderate	0	0	1	0
3. Periodontitis and odontodystrophy:				
- Slight	4	4	6	7
- Moderate	1	0	3	1

Table 7. Continued 3

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
SPLEEN	(96)	(95)	(99)	(100)
1. Increased haematopoietic activity:				
- Slight	4	16**	31	27
- Moderate/marked	6	4	3	4
2. Increased amount of brown pigment in the red pulp:				
- Slight	16	11	36	24
- Moderate/marked	1	8*	17	9
3. Marked fibrosis of the red pulp	0	2	0	0
4. Reticulum cell hyperplasia	1	0	0	0
5. Hyperplasia of the white pulp	2	1	1	1
6. Depletion of the white pulp	1	0	1	2
7. Atrophy	0	2	2	0
8. Medial hypertrophy of arteries	0	0	2	0
PANCREAS	(94)	(96)	(97)	(99)
1. Focal infiltrates of mononuclear inflammatory cells	2	6	7	2
2. Periarteritis	2	5	1	2
3. Focal vacuolation of exocrine cells	0	1	0	0
4. Loss of zymogen granules	3	5	8	8
5. Change of acini into duct-like structures	1	2	1	3
6. Focal atrophy	2	0	1	0
7. Hyperplastic islets	4	1	1	0
8. Atrophy of islets	2	0	0	0
9. Accumulation of brown pigment	1	3	0	0
10. Medial hypertrophy of arteries	0	0	2	0
HEART	(96)	(99)	(100)	(99)
1. Focal infiltrates of mononuclear inflammatory cells	13	17	2	4
2. Endocardial disease	1	0	0	1
3. Myocardial fibrosis	6	1*	0	2
4. Myocardial degeneration:				
- Slight	35	27	18	12
- Moderate	4	1	0	1
5. Ectopic cartilage/bone formation at apex	2	3	1	1
6. Thrombus	1	0	0	0
7. Focal endocarditis	0	0	1	0

Table 7. Continued 4

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
STOMACH	(97)	(97)	(96)	(97)
1. Focal infiltrates of mononuclear inflammatory cells	4	4	2	3
2. Small mucosal haemorrhages	1	0	0	0
3. Increased number of granulocytes in the submucosa	0	1	0	0
4. Cystic, dilated fundic glands:				
- Slight	47	39	51	48
- Moderate	13	13	3	0
5. Focal degeneration of fundic glands	0	1	0	0
6. Hyperkeratosis of the fore stomach	2	2	0	2
7. Periarteritis	0	1	0	1
SKELETAL MUSCLE	(97)	(97)	(99)	(98)
1. Focal infiltrates of mononuclear inflammatory cells	2	1	0	0
2. Focal degeneration	4	1	0	0
3. Fatty atrophy	2	0	0	0
TESTES	(97)	(97)		
1. Partial atrophy:				
- A few tubules	12	14		
- Several tubules	4	3		
- Many tubules	1	2		
2. Complete atrophy:				
- Unilateral	7	6		
- Bilateral	3	0		
3. Focal infiltrates of mononuclear inflammatory cells	1	0		
4. Interstitial oedema	9	7		
5. Periarteritis:				
- Slight	7	10		
- Moderate	3	5		
6. Mineralization of seminiferous tubules	4	2		
7. Multinucleated giant cells	1	1		
8. Proliferation of interstitial cells:				
- Focal	5	4		
- Diffuse	2	2		
9. Slight desquamation of epithelium	0	3		
10. Granuloma	0	1		
11. Medial hypertrophy of arteries	0	1		
EPIDIDYMIDES	(96)	(96)		
1. Tubules containing cell débris	0	3		

Table 7. Continued 5

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
COAGULATING GLANDS	(97)	(93)		
1. Focal infiltrates of mononuclear inflammatory cells	2	1		
2. Adenitis	0	1		
3. Degenerative changes	1	1		
SEMINAL VESICLES	(97)	(93)		
1. Focal infiltrates of mononuclear inflammatory cells	1	0		
2. Atrophy	1	0		
PROSTATE	(97)	(95)		
1. Prostatitis:				
- Slight	13	18		
- Moderate/marked	2	2		
2. Glands containing granular material:				
- Slight	34	42		
- Moderate/marked	16	6*		
3. Glands containing cellular débris:				
- Slight	25	19		
- Moderate/marked	9	2*		
4. Granuloma	1	0		
UTERUS/CERVIX			(100)	(98)
1. Endometritis/pyometra			12	3*
2. Cystic dilated glands, often containing cellular débris			10	7
3. Dilated uterine horn:				
- Slight			2	7
- Moderate/marked			5	9
4. Squamous metaplasia of endometrial epithelium			4	0*
5. Cystic endometrial hyperplasia			0	2
6. Uterine lumen containing exudate			1	0
7. Cervical leucoplakia			1	0
8. Endometrial hyperplasia			1	0
9. Periarteritis			0	1
MAMMARY GLANDS	(51)	(38)	(98)	(94)
1. Duct ectasia:				
- Slight			11	4
- Moderate			6	10
- Galactoceles			5	8
2. Lobular hyperplasia	0	0	12	6
3. Mastitis	0	0	0	1
4. Accumulation of brown pigment	1	0	0	0
5. Focal fibrosis	0	1	2	0

Table 7. Continued 6

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
OESOPHAGUS	(91)	(89)	(95)	(99)
1. Hyperkeratosis	0	0	0	1
SMALL INTESTINES	(96)	(94)	(96)	(99)
1. Subepithelial infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Hyperplasia of Peyer's patches	1	4	3	4
3. Parasites	0	1	0	0
CAECUM	(96)	(95)	(96)	(98)
1. Parasites	4	4	6	6
2. Hyperplasia of Peyer's patches	1	4	1	0
3. Periarteritis	0	0	0	1
4. Granuloma	0	1	0	0
COLON	(96)	(95)	(96)	(99)
1. Hyperplasia of Peyer's patches	2	3	1	0
2. Parasites	1	5	8	4
3. Granuloma	0	1	0	0
4. Periarteritis	0	0	0	1
URINARY BLADDER	(94)	(94)	(94)	(99)
1. Focal infiltrates of mononuclear inflammatory cells	2	5	2	5
2. Proteinaceous plug	22	17	0	0
3. Distended bladder filled with blood	0	1	0	0
4. Hyperplasia of epithelium	0	1	1	0
5. Acute cystitis	0	0	1	0
SPINAL CORD	(90)	(93)	(93)	(96)
1. Focal infiltrates of mononuclear inflammatory cells	1	0	0	0
BRAIN	(97)	(95)	(99)	(98)
1. Focal submeningial infiltrates of mononuclear inflammatory cells	1	0	0	0
PITUITARY	(91)	(88)	(87)	(93)
1. Cyst:				
a. In pars distalis	6	4	4	5
b. In pars intermedia	0	0	2	2
2. Vacuolation of cells in pars distalis	0	1	0	0
3. Accumulation of brown pigment	0	0	2	0
4. Haemorrhage	0	0	1	0
5. Focal degeneration	0	0	0	1

Table 7. Continued 7

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
THYROID	(93)	(95)	(93)	(96)
1. Infiltrates of mononuclear inflammatory cells	2	0	0	0
2. Activated appearance:				
- Slight	31	29	14	10
- Moderate	6	2	0	0
3. A few psammoma bodies	0	4*	1	4
4. Ultimobranchial remnant	1	0	0	[illegible]
5. Proliferation of parafollicular cells:				
- Diffuse	7	3	9	7
- Focal	13	18	17	18
6. Pigment in epithelial cells	2	5	0	0
7. Fibrosis of capsule	1	1	0	0
8. Cystic dilated follicles	0	0	0	1
ADRENALS	(94)	(97)	(99)	(99)
1. Degenerative cortical changes (haemorrhagic cysts and parenchymal necrosis)	14	9	76	68
2. Increased vacuolization of cortical cells	21	34	16	19
3. Infiltrates of mononuclear inflammatory cells	0	2	1	0
4. Accumulation of brown pigment	0	1	0	0
5. Extramedullary haematopoiesis	0	1	2	2
OVARIES			(99)	(98)
1. Diffuse fibrosis			1	[illegible]
2. Follicular cysts			10	[illegible]*
3. Atrophy			1	0
4. Luteinization of stroma			1	0
5. Focal haemorrhages			0	1
PAROTID SALIVARY GLANDS	(96)	(97)	(95)	(99)
1. Focal degeneration of glandular epithelium	0	0	0	1
2. Focal proliferation of ductules	0	2	1	3
3. Focal infiltrates of mononuclear inflammatory cells	0	2	0	0
4. Chronic adenitis	0	0	1	0
SUB-MAXILLARY SALIVARY GLANDS	(96)	(97)	(92)	(100)
1. Focal proliferation of ducts	2	2	1	0
2. Focal degeneration of glandular epithelium	0	2	0	2
3. Chronic adenitis	0	0	1	0

Table 7. Continued 8

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
SUBLINGUAL SALIVARY GLANDS	(93)	(95)	(85)	(95)
1. Focal infiltrates of mononuclear inflammatory cells	3	0	0	1
2. Focal proliferation of demi-lune cells	0	0	1	2
3. Slight hyper/metaplasia of ductular epithelium	1	9*	6	0*
EXTRA-ORBITAL LACHRYMAL GLANDS	(97)	(93)	(92)	(100)
1. Dacryoadenitis:				
- Slight	21	38**	8	10
- Moderate	12	4*	1	0
- Marked	5	0	0	0
2. Prosoplasia of glandular epithelium into Harderian type acini:				
- Slight	26	17	3	1
- Moderate	32	38	1	0
- Marked	33	27	0	0
3. Necrotic area	0	0	0	1
4. Granuloma	1	0	0	0
5. Accumulation of brown pigment	1	0	0	0
PREPUTIAL/CLITORAL GLANDS	(83)	(87)	(81)	(82)
1. A few focal infiltrates of mononuclear inflammatory cells	3	12*	3	2
2. Adenitis	5	8	0	1
3. Cystic, dilated duct	1	0	3	9
4. Granuloma	2	2	0	2
EYES	(97)	(94)	(95)	(99)
1. Slight degree of keratitis	0	4*	0	0
2. Marked conjunctivitis	0	1	0	1
3. Degeneration of retina	0	2	3	1
4. Focal degeneration of cornea	0	1	0	0
5. Degeneration of vitreous body	0	1	0	1
6. Ablation of retina	0	0	1	0
THYMUS	(43)	(63)	(61)	(59)
1. Colloid filled cysts	0	1	11	9
2. Proliferation of epitheloid cells	4	4	15	20
3. Involution	21	28	29	17*
4. Ratio cortex/medulla:				
- Increased	1	1	0	1
- Decreased	8	7	18	16
5. Accumulation of brown pigment	0	0	1	0
6. Hyperplasia, focal proliferation of foamy cells and focal fibrosis	0	0	0	1

Table 7. Continued 9

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
MESENTERIC LYMPH NODES	(94)	(90)	(95)	(94)
1. Increased accumulation of brown pigment	5	5	8	4
2. Erythrophagocytosis	2	4	6	2
3. Focal fibrosis	1	0	0	0
4. Lymphoid hyperplasia	1	1	4	1
5. Fatty atrophy	1	0	0	0
6. Sinus histiocytosis	0	0	1	[illegible]
7. Cystic dilatation	0	0	1	0
8. Haemorrhages	0	0	1	0
AXILLARY LYMPH NODES	(75)	(8[illegible])	(71)	(74)
1. Focal fibrosis	1	[illegible]	0	0
2. Lymphoid hyperplasia	1	0	2	1
3. Erythrophagia	3	2	0	3
4. Focal accumulation of brown pigment	4	4	0	5*
5. Proliferation of epitheloid cells	1	0	0	0
SKIN/SUBCUTIS	(92)	(93)	(96)	(93)
1. Acanthosis/hyperkeratosis	0	2	0	0
ABDOMINAL WALL	(96)	(96)	(97)	(94)
1. Chronic peritonitis	0	0	1	0
ZYMBAL GLANDS/EARS	(85)	(84)	(81)	(86)
1. Dilated duct	0	0	1	1
CERVICAL LYMPH NODES	(61)	(62)	(66)	(67)
1. Lymphoid hyperplasia	5	6	26	14*
2. Erythrophagia	0	1	1	0
3. Periarteritis	0	1	0	0
4. Accumulation of pigment	0	1	2	0
DEPOT FAT	(92)	(88)	(83)	(91)
1. Focal fat necrosis	0	1	1	0
2. Periarteritis	0	1	0	0
FEMUR/KNEE JOINT	(97)	(97)	(97)	(99)
STERNUM	(98)	(98)	(100)	(99)
AORTA	(93)	(94)	(97)	(100)

1) The number of animals from which the organ(s) mentioned was (were) examined is given in brackets.
2) Treatment-related changes are written in capitals.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Individual body weights 50 ppm males at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
D																
Z	96	118	172	195	212	224	238	249	262	278	285	292	300	303	306	318
R1	103	130	196	213	230	246	259	270	285	298	296	308	308	319	320	332
R2	112	134	192	213	220	236	250	265	276	296	295	308	310	320	328	342
L1	108	136	187	214	226	248	262	281	293	310	315	326	333	342	342	351
L2	116	136	195	217	225	240	260	271	289	313	314	328	338	352	358	379
R1L1	104	123	199	222	230	249	265	277	294	310	313	318	324	331	334	350
R1L2	86	101	152	176	186	207	220	233	241	262	266	278	287	289	293	311
R2L1	88	104	163	186	198	219	234	250	266	263	290	300	300	309	309	329
R2L2	106	136	214	238	246	271	285	300	314	335	335	348	351	359	365	379
R3	110	134	204	222	248	268	286	297	311	324	330	338	341	351	352	369
E																
Z	106	132	204	221	232	261	278	293	309	324	330	340	347	355	360	369
R1	114	134	202	218	224	237	246	251	255	266	265	268	272	278	276	285
R2	92	106	179	189	210	235	252	267	283	299	300	308	313	326	328	342
L1	97	118	171	192	202	219	238	243	257	272	274	280	286	292	296	309
L2	100	120	181	204	212	233	250	264	277	292	298	305	309	319	324	332
R1L1	91	103	164	184	196	218	235	250	259	280	272	294	304	314	315	332
R1L2	100	116	186	206	214	230	236	245	248	282	252	272	280	280	282	293
R2L1	104	109	176	195	204	223	237	248	264	270	266	292	306	316	315	330
R2L2	98	113	184	204	212	228	240	249	256	271	256	274	280	284	290	303
R3	102	109	172	194	205	225	235	251	263	280	267	290	294	305	306	319
F																
Z	93	115	178	197	206	228	238	251	263	285	286	296	290	314	316	341
R1	107	135	206	232	248	265	282	294	310	323	317	330	310	344	344	359
R2	96	123	190	214	226	245	263	278	289	304	302	312	306	330	338	353
L1	99	115	163	181	178	203	223	236	250	265	267	280	270	296	304	284
L2	98	123	198	222	238	257	267	280	293	305	307	318	304	329	334	348
R1L1	103	133	201	224	236	251	265	275	291	308	307	318	322	329	336	349
R1L2	100	126	196	223	232	256	264	273	283	298	262	220	254	286	298	317
R2L1	95	125	193	221	236	256	270	281	293	305	297	308	311	316	318	331
R2L2	100	120	173	189	192	203	213	223	236	258	253	266	270	277	282	298
R3	94	113	178	199	211	236	254	268	282	302	299	312	320	326	328	343

Individual body weights 50 ppm males at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D																
Z	328	336	347	355	362	377	381	392	402	410	422	442	455	467	470	480
R1	329	340	353	359	366	386	383	398	398	412	424	430	436	446	445	450
R2	344	354	372	377	380	407	413	427	430	438	460	480	485	495	506	514
L1	358	368	379	391	402	419	426	427	438	450	462	480	498	493	498	512
L2	389	405	421	437	430	446	451	459	472	482	498	515	523	535	538	552
R1L1	361	370	384	395	402	419	436	434	446	454	468	490	499	505	505	515
R1L2	318	324	331	332	334	348	349	357	376	380	398	410	417	428	435	440
R2L1	330	346	359	372	376	396	407	413	424	428	448	460	469	473	475	485
R2L2	387	396	407	424	433	456	452	474	492	499	516	535	542	553	559	572
R3	371	385	392	405	409	434	443	460	469	480	506	522	531	536	543	552
E																
Z	376	387	399	401	401	416	422	429	447	454	464	480	482	486	487	498
R1	287	298	305	311	320	337	338	349	356	358	374	390	388	388	401	402
R2	347	354	355	367	376	393	402	412	412	420	441	450	464	473	475	482
L1	311	318	330	340	339	356	364	355	372	380	400	420	430	443	443	450
L2	341	344	355	363	363	359	383	396	404	416	434	450	453	472	479	490
R1L1	324	336	350	357	362	368	374	391	398	396	410	432	434	444	446	441
R1L2	292	301	309	321	328	322	344	360	370	370	388	395	403	415	423	425
R2L1	322	337	353	360	366	369	382	398	411	424	435	445	462	468	467	473
R2L2	305	326	322	336	343	363	372	388	391	402	412	422	432	458	443	449
R3	319	330	340	351	350	368	375	388	396	408	422	432	435	443	451	460
F																
Z	342	355	361	371	372	395	394	398	407	420	436	448	458	474	483	490
R1	360	356	371	375	378	403	403	419	427	440	450	465	469	481	486	488
R2	356	369	381	397	388	418	424	424	432	446	456	475	483	485	488	500
L1	314	319	321	317	314	303	292	290	318	338	330	295	318	354	327	355
L2	352	361	371	380	381	397	403	412	414	422	432	445	451	458	461	466
R1L1	355	358	372	381	385	401	405	417	430	434	448	460	477	483	498	492
R1L2	296	327	333	348	346	371	367	384	379	389	394	402	411	423	428	430
R2L1	334	340	346	354	363	379	377	393	401	410	422	436	439	456	456	460
R2L2	304	311	322	327	332	341	341	349	355	364	374	390	398	416	419	427
R3	348	354	366	366	366	385	375	399	404	416	430	442	453	462	475	478

Individual body weights 50 ppm males at week:

	80	84	88	92	96	100	104
D							
Z	486	486	488	504	529	539	539
R1	450	450	456	467	476	481	480
R2	521	512	511	522	538	532	530
L1	512	509	506	517	525	520	506
L2	561	562	566	578	599	602	602
R1L1	517	514	513	524	540	536	534
R1L2	439	434	428	442	462	465	462
R2L1	484	479	483	490	492	486	467
R2L2	583	586	597	598	622	622	624
R3	559	558	568	583	592	604	614
E							
Z	494	484	481	496	514	516	510
R1	403	398	408	419	429	426	422
R2	492	492	484	490	510	510	513
L1	454	456	457	465	484	490	494
L2	490	489	486	493	510	506	496
R1L1	449	442	446	458	442	468	463
R1L2	428	424	421	432	442	444	451
R2L1	470	458	462	472	492	496	497
R2L2	443	446	454	460	474	470	476
R3	451	449	450	444	462	467	472
F							
Z	476	470	474	484	505	508	517
R1	452	23.8.77*					
R2	505	496	494	502	516	517	512
L1	340	355	356	355	385	389	400
L2	465	460	458	470	477	481	478
R1L1	498	498	490	3.11.77*			
R1L2	435	412	426	436	421	428	6.2.78*
R2L1	466	462	457	478	492	496	496
R2L2	429	430	432	436	445	449	452
R3	483	479	481	494	514	518	522

* = dead

0000043

Individual body weights 50 ppm males at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A I																
Z	107	135	210	237	250	268	280	293	303	323	321	326	337	344	352	359
R1	110	135	203	207	243	263	278	292	300	319	326	334	344	348	355	368
R2	95	120	182	230	220	247	265	280	298	314	315	326	339	349	356	362
L1	95	11[illegible]	177	196	208	223	234	245	250	265	267	274	280	283	288	291
L2	88	112	183	207	218	245	260	277	288	306	310	320	330	339	340	346
R1L1	99	119	178	200	214	230	244	256	261	274	279	284	290	298	304	319
R1L2	114	141	219	244	264	294	312	327	350	352	359	368	370	388	398	407
R2L1	108	129	192	213	224	243	256	269	272	289	290	298	306	314	318	327
R2L2	113	127	191	200	214	233	247	257	263	280	286	286	294	306	321	326
R3	105	125	184	202	209	220	234	247	257	270	272	287	284	294	294	309
B I																
Z	96	118	176	208	220	238	257	272	284	304	300	308	318	322	330	345
R1	114	136	168	197	204	225	242	253	266	284	280	290	303	311	317	330
R2	101	121	180	202	204	228	244	257	269	286	283	292	302	306	309	328
L1	104	122	168	204	210	231	240	252	262	284	284	288	294	299	306	314
L2	93	111	151	172	174	197	212	225	234	254	252	260	270	275	280	295
R1L1	99	127	206	238	252	279	292	306	322	322	335	342	359	365	372	383
R1L2	93	114	177	198	204	222	230	237	244	269	263	270	274	279	284	293
R2L1	94	118	192	221	232	256	270	289	303	338	323	330	342	355	360	375
R2L2	110	139	211	244	260	283	303	316	332	348	352	360	370	383	390	400
R3	104	131	201	225	240	259	277	295	308	333	331	334	344	353	356	370
C I																
Z	99	119	179	195	202	219	232	250	259	273	275	282	290	299	298	319
R1	99	126	196	224	236	254	271	284	298	314	314	320	326	337	330	350
R2	122	147	226	250	258	278	296	313	327	348	351	356	365	378	373	393
L1	111	142	210	236	246	265	281	294	309	332	332	336	350	358	345	371
L2	103	117	179	190	202	221	235	248	262	286	286	292	298	291	272	270
R1L1	115	130	202	224	224	243	256	274	285	308	305	310	327	333	342	361
R1L2	105	124	185	195	198	208	218	231	244	260	266	274	282	292	298	316
R2L1	97	119	166	179	182	199	210	223	237	256	261	268	280	294	296	323
R2L2	113	134	196	204	205	224	235	246	256	270	274	278	290	296	298	309
R3	107	124	214	246	252	275	288	303	317	340	336	346	352	360	368	383

000044

Individual body weights 50 ppm males at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A I																
Z	372	378	396	403	414	431	439	453	462	473	490	500	509	516	521	528
R1	375	378	392	395	384	412	406	411	424	432	444	460	475	475	475	478
R2	386	401	409	412	421	436	448	450	469	478	492	495	507	517	533	542
L1	301	316	320	323	326	343	345	355	353	366	374	388	394	401	402	410
L2	352	361	370	381	391	408	418	424	436	450	464	475	486	501	501	505
R1L1	319	333	340	347	352	364	366	373	380	390	400	418	425	430	435	442
R1L2	423	436	454	465	478	493	505	522	540	544	566	590	594	599	607	615
R2L1	334	340	355	361	362	384	384	393	350	314	394	388	357	378	381	340
R2L2	324	336	347	357	354	371	372	394	410	424	440	455	463	462	473	485
R3	304	327	335	332	338	349	354	359	362	368	376	392	400	414	416	424
B I																
Z	351	355	362	374	372	393	391	396	415	430	438	455	468	470	479	484
R1	342	351	362	368	367	381	383	386	399	406	422	435	446	458	463	478
R2	327	334	340	341	338	354	351	362	372	384	397	405	412	417	421	430
L1	323	329	339	350	346	358	363	378	381	386	398	404	413	418	426	430
L2	304	308	320	323	325	335	338	348	357	366	374	390	401	411	419	425
R1L1	393	400	415	429	439	462	468	474	490	502	525	538	555	568	574	588
R1L2	299	303	315	321	314	332	328	278	252	206	232	240	243	351	349	360
R2L1	397	411	414	429	420	423	424	422	428	438	446	465	468	484	484	492
R2L2	415	429	438	453	459	483	488	502	525	530	546	558	573	589	576	595
R3	377	387	397	403	392	411	400	414	421	428	440	454	464	473	471	480
C I																
Z	317	329	339	340	345	355	354	361	369	374	384	398	398	410	408	412
R1	353	359	371	376	374	395	395	410	417	424	436	448	451	460	462	464
R2	400	412	425	435	446	460	464	468	484	496	500	515	518	528	531	530
L1	385	395	401	419	423	457	466	488	486	496	518	528	543	563	565	565
L2	300	315	345	325	359	380	363	389	405	404	422	432	414	414	415	455
R1L1	372	387	405	409	412	416	426	434	443	458	464	480	490	498	507	512
R1L2	325	339	344	349	351	353	352	368	372	380	394	402	416	434	437	453
R2L1	332	348	360	375	372	394	396	406	417	424	440	460	468	480	488	501
R2L2	312	328	333	337	326	348	348	354	359	364	364	386	393	403	406	412
R3	390	398	408	417	430	450	439	464	474	486	500	508	518	529	526	530

0000045

Individual body weights 50 ppm males at week:

	80	84	88	92	96	100	104
A I							
Z	530	526	524	540	550	552	549
R1	480	477	457	474	502	508	518
R2	551	552	550	558	569	574	574
L1	412	402	403	407	420	386	12.1.78•
L2	512	506	502	516	530	532	530
R1L1	445	444	436	444	449	447	452
R1L2	615	623	615	626	633	620	616
R2L1	374	353	350	334	333	6.1.78•	
R2L2	494	499	496	499	532	534	540
R3	429	428	428	436	437	440	445
B I							
Z	490	479	484	497	505	498	485
R1	482	476	473	484	500	511	518
R2	425	410	408	422	432	440	437
L1	423	412	420	434	445	443	408
L2	428	422	430	433	442	448	454
R1L1	594	592	598	608	640	635	627
R1L2	358	348	348	349	365	4.1.78•	
R2L1	500	493	486	498	506	507	439
R2L2	610	610	590	593	510	416	11.1.78•
R3	482	464	458	457	469	477	475
C I							
Z	416	406	403	402	414	418	418
R1	472	464	463	480	494	499	507
R2	530	522	512	512	513	509	477
L1	570	561	566	558	518	29.12.77•	
L2	418	464	446	450	481	471	485
R1L1	510	494	512	508	517	516	522
R1L2	464	460	456	469	490	495	514
R2L1	503	507	514	526	530	540	536
R2L2	413	414	408	411	424	430	428
R3	529	522	504	520	536	530	504

• dead

000046

Individual body weights 50 ppm males at week:

D I	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
Z	91	110	169	187	198	215	228	239	249	264	265	270	274	276	276	293
R1	93	114	178	201	214	235	248	247	271	298	304	314	325	327	330	346
R2	13	134	196	226	238	255	272	272	288	310	314	324	330	336	334	357
L1	83	97	145	166	178	199	216	224	237	256	260	268	277	283	284	303
L2	94	115	156	173	181	195	211	208	225	240	243	250	258	240	256	279
R1L1	95	113	171	199	208	231	249	264	271	300	298	308	314	321	328	337
R1L2	95	119	166	183	191	209	220	229	238	256	267	282	290	302	310	329
R2L1	99	122	180	200	212	238	249	269	279	288	312	321	330	344	346	359
R2L2	104	126	187	203	210	231	241	248	256	270	277	282	292	302	304	319
R3	102	124	202	229	238	265	278	291	302	309	322	334	344	351	356	371
Total	10167	12321	18572	20813	21927	23759	25116	26357	27780	29447	29594	30511	31222	32081	32440	33771
Average	102	123	186	208	219	238	251	264	278	294	296	305	312	321	324	338
Number of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

0000047

Individual body weights 50 ppm males at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D I																
Z	294	304	310	321	320	334	336	349	358	364	364	384	388	394	395	400
R1	348	354	367	367	371	384	392	394	406	409	426	440	457	462	469	480
R2	352	363	371	388	390	408	405	409	420	428	440	454	464	477	485	500
L1	297	313	320	332	328	346	340	350	356	366	380	396	406	409	417	436
L2	276	285	289	300	304	326	306	337	340	351	358	370	374	389	394	400
R1L1	332	343	351	361	358	370	361	366	379	384	390	402	411	419	419	428
R1L2	331	343	345	352	350	359	366	369	378	384	398	407	409	423	428	430
R2L1	372	385	393	402	402	421	422	432	449	456	470	478	485	490	501	505
R2L2	327	336	340	342	350	356	360	364	371	374	388	395	401	420	431	431
R3	378	398	405	420	434	456	470	488	508	526	538	556	554	576	583	600
Total	34348	35369	36406	37182	37532	39091	39482	40443	41224	41930	43342	44738	45452	46423	45714	46452
Average	343	354	364	372	375	391	395	404	412	419	433	447	455	464	466	474
Number of animals	100	100	100	100	100	100	100	100	100	100	00	100	100	100	98	98

0000048

Individual body weights 50 ppm males at week:

	80	84	88	92	96	100	104
D I							
Z	393	398	396	414	422	422	427
R1	478	476	476	476	491	494	492
R2	500	496	492	503	514	516	514
L1	431	424	429	432	452	455	461
L2	401	392	398	405	421	424	419
R1L1	428	414	418	422	434	438	434
R1L2	428	415	418	419	434	442	438
R2L1	504	492	494	500	518	526	499
R2L2	430	422	431	435	445	452	459
R3	597	605	606	626	642	648	640
Total	46133	44516	44419	44164	44248	42057	39297
Average	476	474	473	480	492	495	491
Number of animals	97	94	94	92	90	85	80

000049

Individual body weights controlgroup females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A																
Z	88	117	141	147	153	166	169	176	182	187	190	189	194	196	195	202
R1	96	128	161	174	185	194	205	210	208	224	226	232	237	234	240	244
R2	89	109	144	148	159	171	176	183	188	188	190	193	194	198	201	180
L1	96	117	160	160	170	189	195	201	208	216	218	222	235	236	242	248
L2	86	102	127	134	139	152	154	160	164	169	167	174	177	176	177	203
R1L1	92	112	155	165	173	182	188	187	194	197	198	205	204	206	207	214
R1L2	104	130	174	187	199	207	219	218	226	231	230	242	239	244	246	254
R2L1	96	118	151	163	171	182	188	188	195	198	195	207	207	210	209	218
R2L2	98	108	142	143	153	169	173	174	180	188	182	191	189	192	193	197
R3	89	118	159	165	175	187	195	197	201	210	212	218	219	222	224	222
B																
Z	87	110	153	159	167	179	187	194	194	202	201	195	207	212	215	222
R1	104	123	158	159	166	179	190	188	195	203	203	211	207	215	216	224
R2	91	114	154	162	170	183	187	191	196	198	199	210	206	206	214	216
L1	89	115	155	168	175	187	194	196	202	207	207	216	212	212	217	223
L2	98	126	162	170	181	191	197	198	200	202	202	210	210	208	215	220
R1L1	88	117	154	159	165	177	184	184	188	195	203	206	203	210	210	208
R1L2	88	108	142	141	151	163	162	183	184	190	190	196	195	198	200	196
R2L1	90	113	154	160	166	174	186	185	190	193	200	201	198	204	205	203
R2L2	84	106	138	147	155	163	170	171	175	181	185	187	190	192	192	196
R3	84	112	150	159	167	180	187	191	198	202	208	212	214	216	215	222
C																
Z	102	128	160	163	163	192	192	202	204	211	214	223	220	224	228	230
R1	88	114	152	167	162	182	193	194	197	204	207	214	212	216	220	228
R2	84	105	148	150	148	174	188	196	194	195	200	208	207	213	218	224
L1	94	116	162	169	176	210	213	210	222	229	229	229	227	232	235	240
L2	107	128	168	171	166	191	204	205	210	219	223	225	225	230	229	234
R1L1	90	110	143	146	155	163	172	179	180	182	182	183	183	184	189	184
R1L2	92	115	146	154	166	172	182	185	189	193	196	205	205	208	210	212
R2L1	89	110	140	149	159	166	176	178	182	190	188	[illegible]	192	195	194	200
R2L2	82	109	141	151	[illegible]9	166	177	184	189	196	198	206	206	208	210	214
R3	86	112	141	143	152	158	168	175	179	182	186	193	193	192	194	196

000050

Individual body weights controlgroup females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A																
Z	205	211	213	220	222	232	238	251	253	264	284	288	302	311	314	318
R1	250	259	259	270	271	282	298	303	313	332	344	352	352	369	375	384
R2	205	210	211	210	224	223	234	242	240	242	269	274	276	301	314	310
L1	259	260	263	270	268	266	258	260	282	287	314	328	322	346	356	354
L2	186	191	195	195	200	200	212	228	217	214	223	224	224	234	248	235
R1L1	222	223	231	234	240	244	252	281	289	290	305	314	320	333	332	330
R1L2	259	262	277	286	307	333	332	338	356	366	384	389	394	416	426	425
R2L1	222	226	232	236	241	250	259	275	288	290	306	338	338	343	353	359
R2L2	202	206	213	212	218	228	228	231	242	252	264	268	262	271	280	282
R3	228	234	242	242	250	256	260	263	269	288	288	306	306	318	20.6.77◆	
B																
Z	227	230	230	228	232	220	212	225	245	252	250	238	220	239	244	249
R1	230	234	233	238	237	240	248	249	254	262	282	302	300	309	320	327
R2	229	226	232	234	242	251	276	274	281	304	312	325	326	343	349	344
L1	228	230	246	246	248	256	266	264	270	278	282	314	304	322	343	346
L2	228	221	218	218	227	236	246	247	259	276	277	290	294	288	310	304
R1L1	218	219	224	218	232	232	236	243	241	246	255	253	260	275	286	282
R1L2	208	210	216	196	195	220	220	221	222	223	230	228	234	244	256	265
R2L1	214	212	220	226	227	232	242	235	248	254	268	295	288	320	322	320
R2L2	200	208	208	209	219	222	232	236	233	236	247	251	250	265	280	278
R3	227	229	233	236	248	254	266	262	280	270	311	320	322	338	348	354
C																
Z	239	239	248	248	258	270	270	283	284	296	313	326	334	342	352	358
R1	224	233	237	240	252	261	264	268	272	272	275	298	310	315	319	318
R2	226	228	236	240	246	252	262	262	280	268	271	298	297	313	318	318
L1	244	252	254	262	283	279	290	291	298	310	294	314	317	326	328	330
L2	243	250	263	258	270	282	283	281	289	308	313	316	313	323	328	324
R1L1	196	196	205	190	211	210	210	215	238	236	239	244	255	248	266	262
R1L2	217	222	226	230	240	245	248	254	261	274	285	313	308	328	326	341
R2L1	202	208	216	212	220	226	230	235	257	258	268	276	284	297	300	300
R2L2	218	226	235	232	239	243	244	252	256	253	260	264	262	275	282	277
R3	202	209	214	214	223	223	226	225	228	234	239	244	239	254	258	272

◆ means dead

000051

Individual body weights controlgroup females at week:

	80	84	88	92	96	100	104
A							
Z	320	332	335	334	339	350	348
R1	385	386	386	367	387	400	392
R2	321	328	337	336	337	342	346
L1	360	360	343	328	344	350	338
L2	241	248	270	252	6.12.77		
R1L1	334	336	329	312	322	322	322
R1L2	451	463	466	474	452	476	450
R2L1	359	370	378	356	336	29.12.77	
R2L2	290	290	293	261	279	290	286
R3							
B							
Z	250	244	244	232	244	252	246
R1	332	333	336	339	342	346	344
R2	359	358	364	352	325	279	26.1.78
L1	356	354	344	311	349	360	369
L2	308	313	321	319	311	312	306
R1L1	286	290	295	248	280	292	292
R1L2	264	277	274	282	284	292	292
R2L1	334	340	351	360	359	370	376
R2L2	280	276	294	299	279	287	296
R3	361	362	369	364	369	378	382
C							
Z	363	358	358	339	355	356	352
R1	321	328	329	336	322	334	336
R2	320	328	325	290	299	315	316
L1	329	330	339	334	322	316	314
L2	339	342	337	334	338	344	334
R1L1	266	272	280	272	258	276	277
R1L2	349	344	348	359	352	365	368
R2L1	306	306	308	308	247	292	299
R2L2	284	292	292	305	284	294	300
R3	270	265	269	266	254	281	272

means dead

000052

Individual body weights controlgroup female at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
D																
Z	80	102	132	138	145	156	162	165	170	175	173	180	182	184	183	192
R1	91	108	143	152	158	174	179	182	184	188	189	195	193	193	196	201
R2	96	120	155	165	171	180	191	194	196	202	203	207	204	207	211	230
L1	81	104	150	160	167	178	187	189	192	196	198	204	206	210	212	218
L2	89	112	144	156	163	178	179	180	187	187	188	198	194	200	199	208
R1L1	91	122	156	163	170	179	190	188	196	198	200	205	204	209	207	212
R1L2	86	103	127	129	134	148	150	157	160	165	168	173	171	173	176	182
R2L1	98	126	166	176	185	200	204	206	212	215	214	220	203	204	209	221
R2L2	83	106	139	146	149	159	169	168	171	174	175	181	181	182	184	188
R3	84	103	134	139	147	159	160	164	169	173	174	182	180	184	186	193
E																
Z	72	98	124	125	128	137	149	144	152	148	151	153	157	158	154	162
R1	96	118	152	162	172	182	194	199	199	208	206	212	216	220	219	228
R2	94	120	160	167	174	192	193	202	207	214	208	214	214	221	224	228
L1	76	98	131	139	146	159	161	164	171	175	172	179	178	182	184	192
L2	85	108	144	145	152	166	169	176	179	183	185	193	194	196	199	204
R1L1	84	108	144	152	159	170	174	172	180	171	170	165	170	188	187	196
R1L2	86	108	142	147	156	165	178	180	183	190	193	198	202	201	203	211
R2L1	88	110	148	158	159	174	186	188	193	200	202	207	209	210	210	216
R2L2	84	106	138	139	145	163	164	166	172	171	180	182	180	181	183	197
R3	88	116	166	169	178	192	201	198	205	212	216	221	219	224	223	228
F																
Z	101	125	166	169	180	190	196	198	208	215	208	216	214	222	222	226
R1	74	96	125	150	159	161	170	165	178	181	187	190	184	204	228	200
R2	83	112	147	149	159	170	177	182	189	192	194	196	195	200	204	292
L1	89	110	149	145	161	175	182	189	196	205	208	212	204	218	234	219
L2	86	110	141	158	176	180	189	192	189	191	190	197	198	212	229	221
R1L1	94	120	159	168	179	196	201	193	206	203	208	217	210	214	211	221
R1L2	91	111	150	147	154	171	167	177	181	185	189	195	194	196	196	201
R2L1	96	118	152	155	170	186	184	195	200	217	212	220	219	222	218	234
R2L2	96	118	153	163	170	186	190	191	197	196	195	206	200	208	[illegible]	213
R3	85	104	135	139	146	155	159	156	166	167	168	175	170	174	174	180

000053

Individual body weights controlgroup females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D																
$\bar{Z}$	192	200	202	204	210	216	224	226	250	236	245	258	260	267	268	259
R1	204	209	212	209	218	220	216	220	222	224	234	238	236	243	244	246
R2	221	224	226	226	230	238	242	244	272	254	269	277	286	297	306	307
L1	217	223	228	226	238	252	260	263	282	288	293	302	304	319	320	321
L2	210	214	213	214	222	225	232	233	234	236	243	260	254	271	268	275
R1L1	219	219	218	222	234	244	248	267	267	272	287	294	295	302	312	311
R1L2	185	189	192	190	194	201	204	200	212	212	217	222	238	246	248	252
R2L1	226	222	222	226	230	244	250	250	255	254	267	296	278	291	288	288
R2L2	192	192	191	198	201	211	216	213	222	235	238	240	244	251	245	242
R3	200	205	203	210	211	228	242	242	266	278	289	296	298	307	307	313
E																
$\bar{Z}$	165	164	166	169	172	178	178	181	191	194	203	204	207	206	206	206
R1	236	225	250	242	244	256	250	261	275	263	269	276	295	304	306	316
R2	231	234	240	240	246	266	261	264	284	295	299	306	306	309	322	317
L1	195	200	299	201	204	202	208	212	219	224	241	239	255	268	276	280
L2	210	215	215	218	224	225	236	240	246	252	256	266	270	284	294	291
R1L1	198	181	212	208	211	217	214	218	220	226	231	230	222	249	248	235
R1L2	212	218	217	222	230	230	244	244	263	262	276	286	280	286	277	268
R2L1	220	225	226	228	234	234	240	245	247	253	274	288	278	289	298	300
R2L2	207	208	208	202	201	210	208	215	219	216	216	218	214	218	224	233
R3	233	237	240	242	244	247	250	253	264	287	268	296	295	314	318	317
F																
$\bar{Z}$	231	236	237	238	241	259	260	256	262	278	278	293	301	296	316	320
R1	209	212	214	214	220	222	224	213	227	226	233	236	247	246	246	259
R2	232	220	220	228	229	244	261	244	256	258	271	266	285	296	289	299
L1	233	236	237	236	243	265	257	276	299	292	303	312	321	335	326	335
L2	219	216	219	218	223	225	222	225	231	232	238	244	243	251	252	261
R1L1	227	231	237	240	244	254	256	270	275	280	294	314	308	323	326	327
R1L2	212	216	221	224	229	231	242	234	256	245	268	276	274	282	286	297
R2L1	242	239	242	252	260	273	276	290	311	308	323	340	343	353	362	362
R2L2	217	219	224	232	238	251	254	280	281	268	301	312	311	315	322	316
R3	185	189	191	190	202	203	203	212	209	216	219	242	238	260	266	264

0000054 /15

Individual body weights controlgroup females at week:

	80	84	88	92	96	100	104
D							
Z	268	268	265	270	256	265	260
R1	254	256	260	265	276	304	306
R2	310	318	323	300	20.11.77		
L1	322	314	304	290	293	283	260
L2	279	282	282	259	283	295	290
R1L1	302	314	312	306	314	319	303
R1L2	258	258	258	260	262	272	270
R2L1	293	300	302	268	301	306	300
R2L2	250	257	260	248	259	270	271
R3	315	322	326	316	5.12.77		
E							
Z	210	209	209	214	216	218	31.1.78
R1	316	318	315	320	259	292	6.2.78
R2	332	324	328	306	325	332	31.1.78
L1	280	284	284	280	282	294	284
L2	303	302	306	292	299	320	316
R1L1	247	240	238	254	251	259	252
R1L2	23.8.77						
R2L1	310	298	295	295	286	268	224
R2L2	232	226	224	223	217	238	230
R3	322	322	322	320	306	314	290
F							
Z	316	325	310	324	328	323	324
R1	265	264	258	266	272	272	266
R2	310	308	312	315	292	320	308
L1	341	340	340	340	326	342	334
L2	257	258	260	249	242	248	220
R1L1	332	334	326	325	334	340	327
R1L2	296	292	284	287	300	305	304
R2L1	362	363	359	357	362	360	350
R2L2	328	332	300	314	31[illegible]	318	299
R3	261	273	256	266	268	284	273

•) mean dead

000055

Individual body weights controlgroup females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A1																
Z	85	102	151	159	170	183	192	191	201	206	209	210	210	210	215	225
R1	80	104	144	153	164	180	183	187	192	201	201	210	208	210	213	216
R2	89	106	149	151	163	174	174	179	187	190	189	197	194	196	200	202
L1	92	112	153	160	165	177	188	189	193	198	202	206	206	212	214	216
L2	90	107	147	157	163	174	179	176	185	189	188	193	196	198	199	204
R1L1	92	110	144	152	162	177	181	181	190	176	187	201	205	209	206	215
R1L2	81	100	149	158	164	175	186	173	190	196	202	206	202	208	210	213
R2L1	82	110	143	156	163	183	183	182	190	195	194	186	181	154	173	195
R2L2	80	100	132	137	145	156	164	166	174	177	181	186	186	190	187	198
R3	86	112	149	157	163	176	183	184	191	194	199	203	200	206	205	212
B1																
Z	85	104	134	140	151	164	164	166	170	173	172	173	179	180	182	188
R1	77	96	128	135	141	153	156	162	165	170	172	180	177	180	185	186
R2	97	126	173	184	193	208	214	216	222	224	227	235	235	234	237	243
L1	72	90	130	137	140	154	157	156	160	161	162	168	167	172	172	174
L2	92	108	154	165	164	175	186	185	190	192	198	199	199	202	201	210
R1L1	97	127	169	175	181	194	196	192	204	207	215	217	216	222	220	228
R1L2	80	114	155	163	172	185	196	194	201	205	207	214	216	216	215	219
R2L1	82	110	152	166	174	185	188	187	193	204	202	208	210	200	215	214
R2L2	85	107	144	156	163	174	181	177	188	193	197	201	197	200	203	213
R3	76	100	142	145	152	161	170	173	181	185	188	197	194	200	201	204
C1																
Z	83	108	138	143	150	166	173	170	179	184	184	188	190	194	198	214
R1	90	108	146	131	139	149	142	158	160	166	170	172	167	176	175	186
R2	89	113	155	161	169	186	193	192	199	205	207	210	211	214	217	221
L1	84	110	145	153	165	179	187	192	196	201	204	205	207	210	212	215
L2	86	106	139	146	151	167	175	176	183	175	191	192	194	199	200	203
R1L1	88	113	150	162	171	188	194	196	201	205	207	212	212	212	214	219
R1L2	90	114	151	153	161	179	180	185	189	195	199	202	298	202	207	211
R2L1	78	101	136	145	154	167	168	171	177	181	181	186	185	186	187	191
R2L2	106	124	161	165	179	199	206	208	208	218	219	222	225	222	230	236
R3	102	124	164	168	173	190	196	195	202	203	204	208	202	188	200	205

000056

Individual body weights controlgroup females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	60	68	72	76
A1																
$\bar{Z}$	224	224	229	238	238	248	258	250	270	275	286	292	291	308	308	312
R1	220	227	231	232	242	247	251	257	272	278	294	288	289	297	296	295
R2	213	216	226	226	235	238	244	246	250	256	264	270	271	294	300	301
L1	220	227	225	232	240	235	248	246	252	254	265	282	276	298	300	304
L2	208	216	215	216	225	225	236	244	261	262	278	286	289	294	292	297
R1L1	221	229	234	236	248	266	256	267	280	286	295	310	303	316	320	317
R1L2	218	226	225	226	230	237	238	237	249	246	207	220	239	234	256	249
R2L1	214	222	220	224	239	236	236	244	245	240	248	266	256	248	264	271
R2L2	207	218	211	204	208	225	215	223	221	234	230	218	228	219	230	239
R3	216	220	222	226	224	230	232	240	237	243	252	262	262	276	275	278
B1																
$\bar{Z}$	191	198	207	210	219	222	226	223	231	232	254	264	274	288	292	290
R1	195	200	199	200	191	208	216	217	241	233	241	236	241	242	238	241
R2	249	251	256	274	271	292	292	305	319	318	327	334	331	342	350	345
L1	179	181	184	188	190	197	196	205	209	223	227	232	234	232	238	235
L2	216	215	215	220	227	256	242	259	260	276	277	280	289	305	301	305
R1L1	236	230	244	252	258	262	274	280	291	296	329	340	347	349	362	367
R1L2	225	236	240	248	252	262	260	269	287	276	296	304	309	320	320	318
R2L1	219	221	221	220	223	227	232	232	250	238	252	248	256	257	262	260
R2L2	215	220	224	224	233	236	248	246	251	256	259	280	285	312	314	321
R3	209	211	215	220	229	233	236	234	242	248	258	266	267	275	274	280
C1																
$\bar{Z}$	204	209	216	218	233	230	236	232	238	252	253		271	269	272	284
R1	185	182	179	182	188	190	197	196	198	196	209		210	213	228	226
R2	224	228	227	234	237	242	248	267	265	261	277	298	289	303	307	314
L1	221	226	226	228	235	232	238	240	249	252	268	273	272	282	286	285
L2	205	215	215	220	226	254	257	252	263	270	279	292	292	299	302	305
R1L1	222	228	237	239	250	249	266	256	270	276	294	293	299	320	326	328
R1L2	213	214	222	220	229	218	232	241	245	244	255	254	277	292	296	296
R2L1	190	193	200	201	208	207	216	215	216	218	229	228	245	247	254	258
R2L2	236	234	241	249	262	260	266	273	280	276	301	305	316	325	324	328
R3	222	222	220	224	234	234	237	238	244	247	262	260	260	272	266	262

0000057

Individual body weights controlgroup females at week:

	80	84	88	92	96	100	104	
A1								
$\bar{Z}$	316	316	315	319	328	334	330	
R1	308	304	309	306	311	312	313	
R2	311	311	310	281	258	294	285	
L1	305	314	313	302	320	320	317	
L2	304	306	313	269	295	303	282	
R1L1	324	326	330	325	330	338	336	
R1L2	259	260	261	231	253	19.12.77♠		
R2L1	285	272	273	277	276	284	241	
R2L2	235	234	244	252	260	277	266	
R3	282	270	274	260	290	15.12.77♠		
B1								
$\bar{Z}$	297	301	293	278	292	296	290	
R1	243	242	264	260	269	272	270	
R2	357	351	347	352	338	344	342	
L1	239	240	233	230	242	246	242	
L2	305	312	313	318	307	322	314	
R1L1	371	378	376	385	376	396	406	8.2.78♠
R1L2	325	334	338	336	9.12.77♠			
R2L1	259	262	264	244	263	271	266	
R2L2	329	324	325	328	332	352	348	
R3	280	290	284	15.11.77♠				
C1								
$\bar{Z}$	292	286	262	252	253	262	270	
R1	230	237	233	234	230	238	224	
R2	320	322	318	323	334	334	330	
L1	293	292	290	270	292	296	289	
L2	305	304	306	4.11.77♠				
R1L1	333	355	337	326	348	352	349	
R1L2	300	298	304	285	298	312	302	
R2L1	261	262	262	259	264	272	263	
R2L2	334	348	350	350	362	376	366	
R3	265	278	14.10.77♠					

♠) mean dead

850058

Individual body weights controlgroup females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
D1Z	84	114	157	170	178	197	203	203	211	213	218	226	224	228	226	245
R1	89	110	144	149	154	165	168	168	180	187	188	193	192	198	197	206
R2	71	99	142	145	153	169	180	186	188	195	199	204	206	210	213	215
L1	84	103	134	137	144	159	167	169	173	181	183	192	191	202	202	200
L2	81	102	137	142	149	163	165	166	172	176	174	181	180	182	183	193
R1L1	86	108	144	152	158	179	187	190	199	205	211	219	220	218	222	236
R1L2	96	116	149	159	165	183	188	187	195	199	201	208	204	214	216	220
R2L1	84	108	151	159	166	179	182	188	194	196	204	205	208	206	211	215
R2L2	88	112	154	165	170	185	199	198	202	210	208	219	214	216	219	223
R3	79	98	139	149	159	175	171	171	179	194	193	190	191	198	196	197
Total	8816	11106	14785	15463	16224	17562	18171	18388	18956	19404	19590	20119	20049	20355	20612	21215
average	88	111	148	155	162	176	182	184	190	194	196	201	200	204	206	212
nr. of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

650059

Individual body weights controlgroup females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
p1																
$\bar{z}$	241	246	245	250	252	254	264	281	276	292	300	316	311	319	329	327
R1	192	221	236	236	234	245	268	253	274	266	291	296	301	303	306	14.7.77*
R2	222	222	227	228	233	245	246	245	250	264	264	268	281	286	292	293
L1	206	212	216	216	218	223	226	228	248	254	263	230	277	286	294	300
L2	195	199	202	202	210	212	216	217	218	222	234	232	230	232	240	238
R1L1	241	247	250	250	250	256	258	264	261	266	275	285	288	299	306	308
R1L2	232	238	246	248	253	258	260	259	266	272	288	292	295	302	308	318
R2L1	218	226	227	238	242	250	254	260	271	284	292	302	309	309	310	308
R2L2	224	231	235	236	240	248	260	256	260	267	265	290	283	290	300	302
R3	200	206	216	216	218	226	240	232	238	246	259	264	271	279	284	284
Total	21626	21969	22378	22570	23191	23854	24341	24714	25615	25999	26997	27847	28113	29116	29323	29172
average	216	220	224	226	232	239	243	247	256	260	270	278	281	291	296	298
nr. of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	98

000060 /59

Individual body weights controlgroup females at week:

	80	84	88	92	96	100	104
D1							
Z	332	326	326	313	328	339	336
R1							
R2	289	300	316	316	327	344	324
L1	306	268	266	280	274	298	290
L2	252	248	246	244	250	254	246
R1L1	311	322	330	328	332	330	322
R1L2	333	330	329	332	336	340	342
R2L1	318	314	320	7.11.77✦			
R2L2	310	304	300	296	283	286	278
R3	292	296	298	292	294	302	292
Total	29419	29543	29304	27851	26838	26722	25090
average	303	304	305	299	302	311	306
nr. of animals	97	97	96	93	89	86	82

✦) means dead

000061 .1/E

Individual body weights 10 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A																
Z	95	128	175	179	197	199	211	208	224	228	224	231	240	229	240	259
R1	86	120	154	168	176	186	195	199	204	208	211	216	223	219	224	230
R2	90	124	137	142	158	165	165	173	179	180	185	190	192	184	192	192
L1	92	112	143	162	160	171	167	184	180	196	196	199	200	191	205	205
L2	86	110	138	153	165	169	176	176	183	192	186	194	206	188	200	208
R1L1	88	116	145	160	167	168	177	184	183	196	196	199	203	201	206	208
R1L2	92	126	160	170	180	188	192	198	199	210	212	214	218	215	220	226
R2L1	92	119	151	161	172	176	183	186	193	198	215	208	210	208	206	213
R2L2	88	120	150	168	176	184	187	201	204	206	212	216	224	222	222	230
R3	96	126	164	179	182	189	197	200	204	208	213	214	218	217	217	223
B																
Z	90	122	150	165	169	178	186	192	201	206	207	209	210	214	212	218
R1	104	140	168	182	191	194	200	204	211	216	218	206	228	225	228	231
R2	98	132	168	186	200	193	209	209	213	218	220	219	224	223	220	232
L1	80	120	152	164	177	186	199	191	199	204	203	226	210	211	216	221
L2	89	122	163	173	180	192	203	207	214	220	221	224	230	230	230	236
R1L1	88	116	149	163	169	179	183	185	193	200	199	204	207	207	204	215
R1L2	84	110	138	155	161	165	176	176	186	190	190	197	200	197	200	206
R2L1	90	126	153	165	169	171	187	193	191	202	205	206	209	215	212	219
R2L2	92	118	150	158	171	174	180	191	188	202	201	208	213	202	210	214
R3	98	128	158	167	180	180	190	190	200	204	206	206	210	208	208	219
C																
Z	94	122	160	175	182	190	197	200	203	212	215	208	218	222	220	228
R1	98	114	150	159	166	173	179	182	191	190	196	191	201	204	206	214
R2	86	108	148	170	182	182	184	193	191	194	201	190	206	205	214	267
L1	100	130	160	173	179	185	180	195	198	202	211	194	213	219	210	230
L2	86	122	156	160	168	178	179	187	199	200	195	199	204	209	206	265
R1L1	95	112	162	180	189	198	207	213	214	224	226	227	232	232	228	234
R1L2	90	98	139	150	161	167	173	181	181	190	194	196	199	213	203	205
R2L1	82	98	143	155	162	171	177	181	189	194	192	194	199	202	200	206
R2L2	97	116	155	172	178	191	194	197	203	204	205	206	211	209	210	205
R3	82	98	145	168	169	179	183	190	195	200	202	200	208	208	208	211

000062 .16

Individual body weights 10 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A																
Z	253	258	248	260	272	267	298	302	308	314	336	338	333	349		
R1	249	241	240	244	252	254	275	280	272	258	284	294	282	296	242	248
R2	202	206	204	209	210	217	226	230	217	222	228	226	243	243		
L1	216	218	244	232	234	244	248	251	257	266	288	294	294	301		
L2	206	219	223	226	240	247	249	250	248	280	287	292	292	293	287	288
R1L1	215	218	220	224	236	241	251	255	272	273	292	312	316	334		
R1L2	229	238	240	248	247	255	264	271	278	293	310	314	310	323		
R2L1	213	223	227	232	238	249	259	275	286	294	294	310	316	323		
R2L2	233	236	239	242	246	254	15.4.76*									
R3	224	230	231	240	241	249	251	260	278	260	290	282	294	307		
B																
Z	222	225	225	170	229	234	238	248	259	249	261	248	266	278		
R1	243	243	253	256	264	286	283	285	289	316	324	332	332	343		
R2	235	238	243	244	239	257	258	267	268	282	293	298	294	311		
L1	224	228	233	234	221	244	256	270	279	284	305	320	318	325		
L2	240	241	247	248	239	256	260	270	283	278	298	306	304	314		
R1L1	217	220	220	226	217	255	254	264	263	270	278	288	294	301		
R1L2	208	209	217	222	212	226	245	242	251	262	266	286	285	301		
R2L1	225	229	231	234	229	249	253	258	283	290	302	316	320	333		
R2L2	219	231	231	234	260	269	267	290	294	302	310	384	336	346		
R3	222	228	225	234	236	241	244	242	253	273	283	290	294	304		
C																
Z	230	238	243	246	251	258	261	257	260	262	294	276	278	303		
R1	211	219	223	230	234	239	251	249	274	258	270	282	300	307		
R2	234	228	219	216	236	240	236	255	246	256	260	268	268	271		
L1	231	241	245	247	256	259	268	274	280	290	300	320	316	334		
L2	230	218	222	230	228	229	232	235	234	238	243	262	268	281	264	284
R1L1	240	243	250	250	260	279	291	289	298	314	330	332	330	348	336	335
R1L2	212	213	219	224	226	232	245	236	247	252	270	272	284	288	268	285
R2L1	208	214	220	220	224	228	237	239	240	242	248	256	260	267	270	267
R2L2	212	216	204	216	224	224	232	230	195	192	205	242	248	241	242	247
R3	218	222	223	226	224	231	240	245	246	262	280	288	297	303	302	329

* dead

000063

Individual body weights 10 ppm females at week:

	80	84	88	92	96	100	104
A≡Z							
R1	258	262	260	260	275	268	260
R2							
L1							
L2	302	326	320	334	342	342	344
R1L1							
R1L2							
R2L1							
R2L2							
R3							
B≡Z							
R1							
R2							
L1							
L2							
R1L1							
R1L2							
R2L1							
R2L2							
R3							
C≡Z							
R1							
R2							
L1							
L2	291	284	296	298	311	311	309
R1L1	347	334	330	318	333	331	326
R1L2	290	296	294	284	296	292	296
R2L1	14.8.77*						
R2L2	252	252	260	252	267	282*	286
R3	323	5.9.77*					

* dead

0000064

Individual body weights 10 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
D																
Z	79	100	119	130	138	144	150	154	160	168	169	171	176	178	170	181
R1	88	112	150	164	175	179	186	191	200	204	211	214	217	218	218	224
R2	86	110	129	156	169	187	185	194	195	200	208	210	210	214	210	233
L1	84	106	139	149	157	159	168	170	174	180	182	184	186	186	190	190
L2	86	110	142	152	155	166	171	169	176	180	182	186	187	183	190	192
R1L1	82	102	121	134	138	143	150	155	156	156	162	164	164	166	170	167
R1L2	101	134	166	179	187	191	205	205	210	216	213	222	228	225	230	231
R2L1	90	118	149	166	176	177	191	197	204	204	209	212	215	215	214	220
R2L2	82	112	143	153	158	173	180	181	187	194	202	200	203	205	206	210
R3	93	122	152	164	176	182	191	180	173	190	169	171	200	201	214	218
E																
Z	82	110	138	155	159	170	175	177	185	188	192	194	198	200	200	211
R1	88	118	154	171	177	187	189	197	202	206	212	214	214	224	218	229
R2	82	104	134	148	152	163	168	168	179	184	187	186	192	196	192	196
L1	86	114	150	162	168	180	184	188	197	194	202	203	208	215	210	218
L2	82	110	145	155	161	169	171	178	186	190	191	190	197	200	196	208
R1L1	98	128	168	185	195	197	208	218	220	224	232	234	233	233	238	243
R1L2	89	114	144	161	159	174	182	190	190	192	200	204	205	209	210	217
R2L1	85	120	166	182	195	199	207	216	219	220	223	230	231	227	234	241
R2L2	82	100	125	138	140	148	149	162	161	168	173	176	176	183	184	194
R3	95	122	148	159	163	172	180	182	182	190	195	197	200	204	205	208
F																
Z	83	122	165	180	191	200	202	212	218	218	226	233	238	236	234	248
R1	92	121	160	184	186	196	204	211	218	216	227	224	234	229	234	240
R2	96	128	175	186	202	215	216	231	236	246	249	259	266	264	260	274
L1	90	122	160	172	188	198	206	215	220	220	223	231	238	234	230	237
L2	86	110	141	156	162	167	174	179	187	192	193	197	202	200	198	205
R1L1	86	112	136	149	156	164	172	174	176	184	190	190	190	196	196	199
R1L2	90	120	154	171	178	192	198	204	206	210	214	214	220	221	224	231
R2L1	92	116	143	154	163	174	175	182	186	190	193	199	203	206	206	212
R2L2	86	104	136	146	153	158	168	172	175	180	184	182	190	192	196	197
R3	84	116	150	166	176	185	191	200	201	204	212	216	220	222	220	231

0000065 /6

Individual body weights 10 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D																
Z	182	176	191	192	190	195	197	188	188	192	201	196	206	216		
R1	228	236	237	236	240	255	258	250	260	277	287	286	290	302		
R2	226	237	236	244	252	275	284	290	294	308	322	339	348	361		
L1	191	196	197	200	206	213	214	217	220	222	228	244	240	255		
L2	196	200	206	214	215	228	226	233	253	264	282	314	25.4.77*			
R1L1	176	181	181	180	183	189	195	193	194	198	194	200	202	209		
R1L2	233	234	237	244	244	260	273	267	258	266	290	302	304	316		
R2L1	222	228	224	228	232	233	208	228	235	242	245	268	268	264		
R2L2	218	223	225	237	227	235	253	244	257	266	272	294	292	299		
R3	221	211	227	228	234	242	247	244	241	248	256	246	260	265		
E																
Z	212	216	225	228	224	231	233	233	236	240	248	252	253	258		
R1	241	242	241	239	236	267	270	256	254	258	268	278	276	281		
R2	202	204	207	210	213	224	244	236	245	248	258	272	270	278		
L1	221	235	240	260	245	265	291	280	277	306	338	347	358	376		
L2	209	210	215	222	226	261	249	251	245	274	286	300	303	318		
R1L1	248	252	256	264	269	272	278	282	282	290	289	321	322	335		
R1L2	222	228	230	236	239	250	252	260	248	256	264	268	272	281		
R2L1	234	243	248	256	264	267	276	284	284	288	293	306	314	327		
R2L2	198	201	205	208	208	217	220	221	226	240	256	265	269	270	266	274
R3	218	224	225	230	235	243	258	258	276	293	309	326	320	347		
F																
Z	251	235	262	268	289	299	320	326	329	346	348	360	364	378		
R1	242	244	252	252	268	291	292	301	305	314	320	328	336	337	328	336
R2	274	284	291	310	318	332	346	365	370	384	399	418	252	5.5.1977 *		
L1	243	257	256	262	275	278	272	287	285	280	297	323	308	322	312	324
L2	203	210	211	216	224	229	245	228	230	238	238	246	265	268		
R1L1	203	209	209	214	222	226	230	234	280	290	236	248	245	254		
R1L2	231	237	240	244	247	266	279	261	234	240	300	312	310	319		
R2L1	215	219	219	232	233	244	263	260	224	245	260	272	262	273	270	280
R2L2	198	202	209	211	217	226	225	226	229	236	250	264	260	269*		
R3	238	242	246	250	258	266	276	277	290	298	322	336	346	360		

* dead

000066

Individual body weights 10 ppm females at week:

	80	84	88	92	96	100	104
D							
Z							
R1							
R2							
L1							
L2							
R1L1							
R1L2							
R2L1							
R2L2							
R3							
E							
Z							
R1							
R2							
L1							
L2							
R1L1							
R1L2							
R2L1							
R2L2	284	272	278	282	297	316	2.2.78*
R3							
F							
Z							
R1	343	348	349	350	352	350	346
R2							
L1	332	332	318	328	344	344	344
L2							
R1L1							
R1L2							
R2L1	294	296	280	306	297	296	311
R2L2							
R3							

* dead

000067

Individual body weights 10 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A I																
Z	94	120	147	159	169	177	187	192	196	200	204	207	214	213	208	219
R1	76	104	143	150	162	168	169	183	187	185	191	196	198	198	198	196
R2	81	108	136	152	161	160	173	178	182	188	188	193	198	195	197	215
L1	79	102	135	144	160	163	164	172	174	178	175	181	186	187	188	205
L2	86	112	138	148	159	158	175	179	182	192	196	199	200	205	204	212
R1L1	96	126	162	174	178	185	195	189	205	204	208	208	215	214	216	220
R1L2	87	104	134	144	153	154	166	174	177	204	182	180	190	188	187	190
R2L1	84	106	135	146	148	160	165	170	173	180	185	184	188	189	194	199
R2L2	80	100	131	142	150	158	164	172	171	180	185	191	198	198	198	201
R3	99	132	168	179	191	198	202	209	209	220	221	221	230	227	236	240
B I																
Z	100	124	158	172	178	189	187	198	198	206	207	212	216	224	220	245
R1	84	110	143	156	164	174	178	182	187	196	195	195	200	204	201	209
R2	91	120	161	180	188	198	208	213	213	220	228	227	230	242	242	247
L1	91	118	150	163	174	176	182	177	192	190	199	198	201	209	205	215
L2	90	116	146	163	172	179	186	188	191	190	196	199	201	207	205	208
R1L1	86	110	140	153	164	163	174	174	182	182	185	189	195	193	196	200
R1L2	84	110	139	153	165	168	171	178	182	180	183	190	190	192	192	197
R2L1	95	132	168	182	192	195	208	214	184	182	188	187	195	184	212	229
R2L2	94	118	147	158	169	174	181	188	190	190	200	205	208	209	208	223
R3	83	108	146	162	167	172	167	180	182	184	180	185	195	194	186	210
C I																
Z	88	116	146	157	164	167	179	182	187	186	191	194	195	193	196	197
R1	78	104	134	143	156	164	170	177	181	182	184	188	192	194	198	205
R2	90	116	150	172	173	187	189	206	201	208	214	216	218	226	224	229
L1	94	122	160	170	184	191	202	205	215	210	213	214	220	222	228	236
L2	82	114	143	152	164	173	179	187	191	192	191	192	188	197	198	201
R1L1	98	122	161	175	186	198	202	208	212	216	216	222	225	226	228	233
R1L2	94	118	152	163	177	181	187	194	195	198	199	201	207	206	208	213
R2L1	88	106	138	154	169	173	184	184	195	194	204	203	208	209	[illegible]	220
R2L2	98	130	171	181	198	208	217	220	222	228	230	238	233	239	238	244
R3	81	100	135	146	147	159	169	175	183	180	180	185	188	189	190	198

0000068

Individual body weights 10 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A I																
Z	221	228	231	238	238	247	251	263	287	292	292	290	302	314	318	330
R1	207	218	215	220	220	228	248	233	230	238	268	270	274	295	284	293
R2	208	212	216	198	216	217	238	232	233	244	254	234	246	238		
L1	194	194	201	203	215	213	226	221	224	238	241	252	256	265	20.6.77*	
L2	218	218	221	228	228	246	260	252	273	228	256	284	281	301	274	303
R1L1	222	230	234	240	243	247	267	246	266	290	288	302	302	313	306	300
R1L2	202	206	206	206	182	212	219	219	233	232	245	241	252	257		
R2L1	199	205	207	209	217	219	227	227	230	228	249	249	260	265		
R2L2	208	221	221	228	231	242	249	247	252	260	278	270	269	286		
R3	246	258	257	268	280	299	306	310	310	314	323	346	344	359		
B I																
Z	232	240	247	248	259	264	286	287	292	314	336	338	340	358		
R1	212	216	221	222	224	239	243	240	241	238	267	274	274	277		
R2	246	258	256	258	264	274	288	288	298	304	308	322	326	325		
L1	220	219	225	226	238	241	252	252	251	268	283	292	288	303		
L2	220	227	227	231	235	244	245	255	254	270	274	303	307	321		
R1L1	201	206	209	212	220	219	232	234	236	250	254	286	266	273		
R1L2	198	201	203	200	205	210	212	222	233	220	226	228	228	234		
R2L1	233	231	236	234	224	264	237	274	284	284	290	300	298	308		
R2L2	222	230	233	234	239	248	256	262	266	266	279	287	286	295		
R3	215	204	210	226	232	247	250	258	267	270	276	279	284	291		
C I																
Z	205	209	211	216	218	224	232	230	234	240	242	242	240	253		
R1	207	215	216	224	229	233	240	239	242	262	276	282	286	295		
R2	235	238	244	244	244	253	262	263	269	272	282	288	280	287		
L1	241	249	257	266	274	280	302	305	306	336	342	352	350	363		
L2	206	204	210	214	217	222	220	239	244	240	253	254	256	274	256	262
R1L1	229	237	240	240	248	256	259	258	260	270	273	286	290	30		
R1L2	220	219	222	228	234	243	247	242	247	262	266	274	269	298		
R2L1	221	230	230	234	238	244	253	252	260	278	300	278	289	314	285	293
R2L2	257	260	264	270	278	316	322	326	341	348	357	368	374	391		
R3	205	207	208	212		229	240	265	244	268	250	264	270	288		

* dead

690000

Individual body weights 10 ppm females at week:

	80	84	88	92	96	100	104
A I							
Z	340	330	346	345	358	362	362
R1	307	305	320	316	332	340	339
R2							
L1							
L2	296	274	286	280	308	314	312
R1L1	306	288	300	297	304	310	312
R1L2							
R2L1							
R2L2							
R3							
B I							
Z							
R1							
R2							
L1							
L2							
R1L1							
R1L2							
R2L1							
R2L2							
R3							
C I							
Z							
R1							
R2							
L1							
L2	275	270	276	284	284	278	277
R1L1							
R1L2	302	292	302	250	308	296	276
R2L1							
R2L2							
R3							

0000070

Individual body weights 10 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
DT																
Z	85	110	154	167	178	191	200	208	210	218	220	225	224	231	234	239
R1	95	122	158	180	185	202	197	201	198	204	206	210	214	218	222	227
R2	98	122	158	170	175	183	158	172	164	178	184	192	201	199	198	201
L1	86	109	129	134	147	148	155	159	160	166	170	172	170	178	178	181
L2	98	116	148	164	174	172	182	190	197	198	198	202	195	175	170	204
R1L1	85	104	136	146	156	161	165	172	169	180	178	179	186	186	184	193
R1L2	80	108	145	154	165	178	179	187	185	192	194	195	201	198	200	206
R2L1	88	112	150	161	176	186	189	196	205	208	213	215	222	219	220	228
R2L2	85	108	140	148	159	166	170	175	184	186	189	189	196	196	194	203
R3	81	102	133	141	145	152	158	164	165	170	177	176	181	184	186	190
Total	8885	11531	14854	16167	17031	17735	18345	18890	19268	19747	20029	20253	20719	20768	20867	21703
Average	89	115	149	162	170	177	183	189	193	197	200	203	207	208	209	217
Number of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

000071

Individual body weights 10 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D I Z	242	249	252	252	259	266	276	274	277	278	292	326	311	346		
R1	225	228	238	240	254	257	266	284	298	310	318	335	340	352		
R2	203	195	208	214	220	235	232	223	241	252	255	246	263	264		
L1	182	193	192	192	198	205	210	208	202	210	218	224	228	236		
L2	209	217	218	220	220	222	223	222	227	228	234	222	238	241		
R1L1	193	200	203	206	211	214	222	221	218	228	236	270	259	274	258	262
R1L2	206	208	213	220	222	241	240	252	234	246	269	270	282	279	262	265
R2L1	233	243	247	248	255	262	262	262	262	280	288	262	288	297	278	304
R2L2	203	207	210	213	218	225	227	223	228	228	243	260	238	269	242	258
R3	191	203	203	208	212	206	211	214	213	218	222	224	223	226	208	213
Total	21954	22380	22719	23069	23538	24539	24984	25223	25614	26381	27488	28433	28144	28934	6358	6580
Average	220	224	227	231	235	245	252	255	259	266	278	287	287	298	276	286
Number of animals	100	100	100	100	100	100	99	99	99	99	99	99	98	97	23	23

0000072

Individual body weights 10 ppm females at week:

	80	84	88	92	96	100	104
D I Z							
R1							
R2							
L1							
L2							
R1L1	265	280	298	287	290	290	298
R1L2	272	282	283	293	299	300	300
R2L1	309	302	298	308	306	316	302
R2L2	256	260	255	260	266	274	270
R3	224	236	226	220	222	214	24.1.78*
Total	6468	6121	6175	6152	6391	6426	5880
Aver.	294	291	294	293	304	306	309
Number of animals	22	21	21	21	21	21	19

* dead

000073

Individual body weights 50 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A																
Z	93	104	136	146	148	155	158	164	169	176	176	178	184	186	184	195
R1	90	104	136	144	147	159	161	174	176	178	184	180	186	186	192	193
R2	84	91	122	132	134	140	144	151	155	160	164	164	166	173	170	176
L1	90	97	124	136	136	148	156	165	171	178	180	186	190	195	191	205
L2	98	105	130	139	141	147	154	161	167	175	176	180	186	188	189	201
R1L1	83	91	128	142	141	152	152	161	161	170	167	172	173	178	174	184
R1L2	103	110	151	159	164	175	185	194	202	204	201	208	210	214	217	215
R2L1	80	87	126	137	137	149	150	154	160	167	168	169	176	178	178	182
R2L2	95	103	135	142	143	154	160	167	172	179	181	180	186	192	191	205
R3	90	101	134	145	146	157	161	166	170	179	178	178	184	186	185	191
B																
Z	92	103	130	142	143	156	165	169	170	180	180	184	181	192	186	190
R1	84	99	136	150	151	160	165	170	174	182	183	185	190	195	202	213
R2	88	97	131	142	146	154	162	166	170	174	177	180	186	186	186	193
L1	102	113	142	153	159	173	184	184	186	193	195	200	205	210	204	213
L2	78	91	119	126	128	141	146	155	157	168	162	174	177	182	182	190
R1L1	86	105	148	160	169	176	186	188	191	194	196	200	200	211	219	215
R1L2	83	94	123	133	134	140	146	153	162	160	165	170	172	180	182	190
R2L1	89	104	141	152	155	165	170	177	182	192	198	198	200	202	209	213
R2L2	100	115	142	150	151	162	166	168	175	179	175	180	181	184	189	193
R3	89	100	132	145	149	161	165	173	176	182	182	186	188	179	189	193
C																
Z	86	93	122	131	135	143	153	158	164	172	173	174	180	184	186	190
R1	80	91	128	141	149	158	170	176	181	188	190	192	200	205	205	213
R2	85	102	134	145	150	162	169	175	182	192	191	190	196	199	202	206
L1	76	88	120	132	134	143	150	151	156	162	160	167	168	171	171	179
L2	83	89	121	131	133	142	148	150	156	160	161	160	143	163	165	181
R1L1	96	106	141	149	152	159	165	167	173	176	183	181	185	189	184	196
R1L2	75	87	118	124	125	132	139	143	155	152	153	154	157	162	158	168
R2L1	77	9[illegible]	121	126	[illegible]8	138	146	147	153	152	157	160	160	166	163	171
R2L2	93	107	145	151	155	16[illegible]	175	176	175	184	191	192	195	197	192	204
R3	83	99	133	143	152	158	166	169	173	182	188	187	190	200	191	198

000074

Individual body weights 50 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A																
Z	195	204	201	204	218	213	221	220	223	222	228	242	251	256	266	262
R1	199	201	205	206	216	222	227	227	235	232	240	254	267	281	297	312
R2	178	182	186	186	190	203	207	209	217	210	220	236	241	249	249	254
L1	206	210	212	214	213	227	229	232	238	234	240	260	265	283	277	290
L2	245	211	209	216	218	220	230	237	240	243	248	272	281	293	295	314
R1L1	186	186	194	198	204	201	217	209	224	222	236	260	269	273	276	293
R1L2	223	232	239	230	244	244	244	252	253	252	258	268	286	294	292	301
R2L1	190	191	197	198	203	202	208	208	216	214	219	232	233	248	255	254
R2L2	203	203	207	213	216	218	239	231	236	254	260	268	278	291	291	300
R3	198	202	203	204	205	215	212	218	225	224	230	250	252	265	263	270
B																
Z	195	187	199	212	205	213	213	223	230	244	251	254	261	261	264	278
R1	221	227	229	234	237	244	249	252	260	262	268	292	280	295	284	293
R2	193	196	204	212	207	214	214	220	222	219	224	245	274	286	282	296
L1	222	223	230	240	244	248	250	252	260	262	290	300	302	313	311	317
L2	196	200	205	212	212	218	214	235	228	228	234	244	259	260	271	276
R1L1	218	224	242	234	241	251	267	282	284	294	300	310	326	338	339	354
R1L2	195	199	212	208	203	201	206	225	218	212	222	230	239	258	265	252
R2L1	214	219	224	228	230	232	233	246	240	254	258	278	278	279	292	294
R2L2	200	201	208	211	207	215	222	225	251	250	277	272	279	284	284	282
R3	214	207	212	208	206	217	221	230	230	240	258	272	245	245	261	265
C																
Z	200	198	202	208	206	214	219	223	228	228	238	254	263	271	273	281
R1	212	214	220	224	227	239	232	249	258	248	288	288	299	311	311	324
R2	214	212	219	224	224	236	230	237	243	240	276	264	276	273	274	288
L1	178	181	184	190	191	196	200	203	204	210	221	225	228	234	228	228
L2	176	171	182	196	199	190	198	198	181	188	198	214	226	227	242	1.7.77*
R1L1	196	196	202	202	207	210	214	220	219	222	221	228	229	231	241	243
R1L2	168	168	175	176	174	178	177	180	180	184	186	190	203	201	201	198
R2L1	176	176	178	180	180	178	180	190	203	196	198	210	201	206	209	209
R2L2	204	208	205	206	211	208	212	216	213	218	218	226	241	232	245	247
R3	198	204	208	208	208	211	221	225	241	232	243	245	254	260	269	272

* dead

000075

Individual body weights 50 ppm females at week:

	80	84	88	92	96	100	104	
A								
Z	278	265	268	281	295	280	290	7.2.78*
R1	307	319	325	324	332	330	338	
R2	254	260	254	266	277	264	264	
L1	299	300	310	310	311	313	320	
L2	320	326	335	324	342	282	222	7.2.78*
R1L1	287	298	297	305	320	321	332	
R1L2	310	322	328	339	350	348	342	
R2L1	262	266	268	278	286	294	295	
R2L2	300	304	313	315	325	328	336	
R3	273	285	286	298	321	318	314	
B								
Z	279	292	293	304	316	312	296	
R1	298	310	316	316	322	330	328	
R2	299	310	316	318	327	332	336	
L1	316	314	327	15.11.77*				
L2	279	280	283	292	300	304	310	
R1L1	2.8.77*							
R1L2	254	252	271	274	259	284	288	
R2L1	294	289	294	298	304	306	312	
R2L2	296	296	301	303	314	310	304	
R3	271	263	253	26.11.77*				
C								
Z	280	288	290	288	307	310	315	
R1	326	330	334	338	351	348	352	
R2	291	294	298	298	314	319	310	
L1	233	234	231	238	12.12.77*			
L2								
R1L1	240	236	239	264	264	277	272	
R1L2	204	203	205	206	215	210	215	
R2L1	210	210	216	224	212	216	226	
R2L2	253	256	254	256	261	267	266	
R3	283	286	288	295	298	312	313	

* dead

000076

Individual body weights 50 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
DZ	87	105	148	156	161	170	173	181	184	192	196	198	200	207	210	212
R1	94	112	146	156	158	168	177	179	181	190	192	196	194	202	198	207
R2	75	87	122	128	130	140	146	148	153	163	162	165	165	174	178	175
L1	92	107	137	138	142	154	157	162	167	174	175	176	178	179	182	187
L2	93	105	138	141	145	156	164	168	174	180	180	185	185	187	194	198
R1L1	82	94	123	130	132	142	147	149	158	160	163	164	166	172	151	169
R1L2	84	105	151	161	159	166	174	174	177	185	183	188	192	192	193	202
R2L1	104	120	164	176	179	184	196	204	208	216	216	215	220	226	226	230
R2L2	105	124	166	178	178	189	199	209	208	216	217	226	226	234	229	235
R3	95	113	151	161	164	175	182	185	193	196	200	198	200	204	207	212
EZ	92	110	152	163	167	178	181	184	194	204	202	208	211	213	220	225
R1	89	101	134	142	145	155	162	168	174	177	181	182	190	189	192	200
R2	86	100	139	149	152	159	164	168	176	180	184	185	188	188	194	198
L1	90	99	132	140	143	151	153	161	167	169	173	174	178	182	182	186
L2	102	119	159	171	172	183	188	191	199	205	203	206	210	213	211	218
R1L1	109	123	158	178	200	208	200	204	207	210	211	218	220	224	226	228
R1L2	107	124	169	180	186	190	204	209	216	218	222	222	228	233	234	234
R2L1	95	116	142	149	153	165	169	175	180	185	187	188	194	191	192	199
R2L2	89	105	142	150	157	167	169	182	186	196	196	204	206	216	212	223
R3	92	108	136	141	143	148	156	161	167	176	176	178	182	184	185	191
FZ	94	108	146	158	159	174	175	179	188	190	195	194	200	205	206	213
R1	78	88	118	124	127	139	145	149	158	162	163	165	173	174	175	186
R2	85	102	135	141	145	153	159	162	166	176	172	176	178	181	185	185
L1	89	105	144	156	161	176	180	185	191	200	200	204	218	219	218	219
L2	88	104	132	143	146	158	164	165	172	178	180	178	184	184	188	191
R1L1	87	93	118	132	135	143	148	152	157	161	164	166	167	168	171	176
R1L2	87	105	146	151	155	165	173	185	181	187	189	192	194	198	200	207
R2L1	90	98	154	164	164	172	175	179	186	194	194	200	200	204	207	211
R2L2	88	99	132	153	167	155	155	162	169	175	176	176	184	188	190	195
R3	88	105	144	134	157	168	173	178	181	184	184	186	189	194	198	204

000077

Individual body weights 50 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D																
Z	213	234	228	228	231	236	244	255	251	263	253	270	295	301	22.6.77*	
R1	208	215	218	218	222	230	226	238	238	242	255	270	278	287	287	294
R2	175	182	184	187	185	190	188	194	198	196	199	210	211	228	221	235
L1	187	198	203	200	200	210	208	206	205	209	215	222	236	239	241	246
L2	196	201	206	208	206	212	213	218	221	228	243	250	263	279	286	286
R1L1	175	176	184	194	203	202	208	210	205	214	214	216	210	223	225	227
R1L2	201	210	215	217	224	228	234	220	223	216	229	240	243	242	236	230
R2L1	236	238	246	245	252	254	253	263	274	279	297	312	325	341	342	353
R2L2	241	243	254	255	257	265	274	286	290	306	323	330	341	351	354	364
R3	217	222	227	236	235	242	245	253	260	254	266	300	286	306	311	325
E																
Z	219	231	235	239	243	258	272	261	266	273	294	298	301	323	321	330
R1	199	203	206	210	210	214	215	226	217	222	233	234	255	251	268	272
R2	205	210	214	216	216	222	227	235	243	244	248	270	277	289	298	308
L1	192	196	208	208	210	220	221	181	210	214	223	218	224	217	237	242
L2	221	223	233	232	240	238	243	245	246	254	255	260	286	285	288	292
R1L1	231	236	236	234	232	246	238	250	252	250	270	278	306	314	328	335
R1L2	236	238	248	246	253	256	267	281	282	294	309	325	321	336	335	348
R2L1	196	209	210	216	214	226	244	241	239	240	241	258	259	279	271	294
R2L2	226	233	243	240	240	268	273	267	280	279	295	295	321	332	326	334
R3	192	201	203	206	205	210	216	217	215	220	232	235	241	249	245	260
F																
Z	225	221	222	220	229	232	236	242	244	254	269	285	295	298	295	303
R1	190	193	201	204	205	216	213	228	224	228	242	250	269	268	269	292
R2	189	194	200	198	200	205	209	218	212	222	231	240	253	259	259	267
L1	221	230	237	242	250	244	255	255	270	278	287	300	305	315	314	320
L2	193	200	208	208	209	217	216	224	230	242	244	250	262	271	265	273
R1L1	179	180	185	190	191	192	196	200	197	198	206	215	223	221	238	245
R1L2	212	212	216	216	214	224	226	254	234	252	262	260	277	273	279	287
R2L1	218	219	226	235	235	250	259	275	297	295	303	335	331	348	351	365
R2L2	197	205	208	208	215	21[illegible]	218	223	226	228	231	240	241	256	[illegible]47	254
R3	207	213	216	218	219	220	224	234	231	234	237	248	262	268	271	272

* dead

0000078

Individual body weights 50 ppm females at week:

	80	84	88	92	96	100	104	
D								
Z								
R1	301	300	302	316	328	320	296	
R2	224	224	212	219	222	231	221	
L1	250	252	246	252	261	271	267	
L2	292	294	288	298	306	311	294	
R1L1	234	228	235	230	232	246	230	
R1L2	232	230	230	15.11.77*				
R2L1	336	20.9.77*						
R2L2	362	362	357	370	368	396	2.2.78*	
R3	324	334	337	340	364	366	384	7.2.78*
E								
Z	335	344	338	348	364	364	361	
R1	262	270	274	280	301	313	317	
R2	300	313	14.10.77*					
L1	251	256	238	266	270	264	256	
L2	302	300	299	302	316	326	336	7.2.78*
R1L1	341	350	363	352	392	392		
R1L2	351	350	356	372	6.12.77*			
R2L1	303	306	304	316	319	330	327	
R2L2	349	344	352	366	387	382	386	
R3	259	262	274	280	276	282	271	
F								
Z	303	313	314	322	326	334	336	
R1	293	294	294	296	306	312	302	
R2	272	272	268	272	286	286	283	
L1	320	329	335	343	358	375	394	3.2.78*
L2	277	270	275	292	296	299	302	
R1L1	245	260	251	256	286	274	272	
R1L2	289	288	292	296	304	290	303	
R2L1	362	382	383	396	414	4.1.78*		
R2L2	250	250	256	260	277	281	280	
R3	284	293	296	302	314	314	316	

* dead

000079

Individual body weights 50 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
A I																
Z	97	113	149	156	160	169	175	178	183	188	188	190	191	196	194	202
R1	90	105	148	168	165	177	190	193	197	210	205	208	210	224	219	223
R2	83	94	123	133	137	149	157	164	168	174	177	176	179	183	185	190
L1	80	90	112	126	129	139	149	159	163	170	172	176	176	182	182	190
L2	88	102	134	145	146	157	161	166	171	178	175	178	182	186	184	193
R1L1	79	89	118	126	130	140	143	149	154	158	159	162	162	167	164	169
R1L2	84	97	132	140	146	152	158	164	169	174	173	174	180	183	186	194
R2L1	94	108	143	154	156	166	168	176	180	188	188	190	192	194	197	205
R2L2	80	91	126	133	137	145	150	155	160	164	162	164	166	173	172	176
R3	82	94	128	143	143	152	155	160	163	170	171	174	170	175	174	182
B I																
Z	87	103	142	154	153	164	170	178	180	188	182	186	194	194	202	205
R1	92	115	148	150	155	166	172	181	186	190	187	190	194	196	200	200
R2	88	107	152	170	170	186	194	206	214	220	218	220	226	229	233	241
L1	92	112	142	156	153	167	179	176	182	186	187	190	196	204	200	209
L2	94	115	156	168	169	185	193	201	206	210	210	208	219	220	224	232
R1L1	101	98	129	155	137	145	149	156	160	168	167	170	175	178	181	221
R1L2	90	104	144	149	151	164	165	171	178	184	186	184	190	193	198	197
R2L1	86	113	151	131	165	172	177	180	188	199	195	204	210	214	213	185
R2L2	81	93	122	116	129	137	141	146	152	159	167	164	170	170	172	181
R3	90	109	150	151	162	180	181	194	196	202	209	206	211	218	218	220
C I																
Z	83	93	127	150	164	162	166	171	174	180	181	186	185	189	192	196
R1	96	114	141	155	156	164	172	177	184	192	193	208	206	208	211	217
R2	80	92	126	139	140	149	155	160	162	170	171	172	174	175	177	183
L1	90	108	145	158	158	171	175	183	188	198	194	200	202	202	204	213
L2	95	113	154	178	200	180	182	186	188	192	190	196	195	196	194	197
R1L1	80	91	126	143	139	149	159	166	168	174	173	176	179	181	183	187
R1L2	85	96	128	139	141	152	160	165	171	178	180	184	189	188	190	191
R2L1	92	105	137	144	150	162	165	170	176	178	183	188	188	194	195	195
R2L2	86	99	129	134	138	152	154	161	165	172	172	174	176	176	180	184
R3	90	102	136	146	144	159	158	164	172	176	177	178	182	183	188	190

000080

Individual body weights 50 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
A I																
Z	203	204	215	212	214	223	216	213	221	230	228	235	242	245	247	267
R1	221	227	233	236	238	245	249	260	278	276	295	310	315	324	323	331
R2	189	197	197	196	196	204	203	224	214	220	236	240	242	245	244	257
L1	191	198	204	200	210	211	214	214	217	222	227	234	252	251	253	262
L2	192	198	199	200	207	212	212	220	235	236	247	250	263	260	264	263
R1L1	170	173	182	183	185	192	192	198	198	202	205	210	221	218	231	244
R1L2	196	198	203	202	203	205	199	199	206	212	211	222	238	246	245	246
R2L1	203	203	214	214	221	222	221	224	230	226	235	240	251	253	263	258
R2L2	185	187	195	192	180	192	194	202	196	196	201	200	204	206	206	218
R3	180	183	188	190	191	196	197	199	198	204	203	210	217	225	228	226
B I																
Z	204	209	220	218	225	228	236	242	244	242	249	260	264	278	273	293
R1	206	210	216	222	219	228	220	243	243	234	254	250	266	284	287	282
R2	238	249	259	258	270	284	283	295	308	312	319	338	354	388	381	392
L1	205	211	225	216	226	238	245	239	258	260	261	282	281	309	306	315
L2	235	239	247	248	248	256	267	270	275	282	306	300	314	324	328	333
R1L1	224	232	240	240	242	252	256	261	282	272	292	310	319	331	326	329
R1L2	201	206	213	214	215	224	225	232	242	260	269	280	293	298	301	310
R2L1	184	191	197	196	203	206	213	213	218	218	221	232	242	254	253	265
R2L2	186	191	196	204	202	204	212	209	208	213	219	232	241	261	266	265
R3	229	232	240	242	246	250	253	267	268	264	282	285	301	303	295	295
C I																
Z	193	195	199	201	206	202	230	220	227	243	251	268	266	278	284	275
R1	221	231	236	242	247	250	254	276	273	274	293	308	314	326	333	340
R2	187	189	194	194	202	204	206	220	204	202	205	210	208	209	208	210
L1	208	210	222	222	225	236	240	246	251	252	258	260	263	274	264	275
L2	201	211	212	215	214	226	225	229	232	236	236	244	246	254	257	266
R1L1	19[illegible]	193	198	200	215	214	214	221	224	226	244	260	252	249	268	277
R1L2	19[illegible]	198	205	208	207	212	212	216	220	219	223	235	238	251	249	252
R2L1	198	204	212	212	214	214	218	226	225	228	231	235	238	246	244	253
R2L2	186	190	194	196	196	20[illegible]	211	213	212	224	236	250	251	265	263	265
R3	191	202	208	208	215	218	219	224	230	230	234	250	265	281	277	282

0000081

Individual body weights 50 ppm females at week:

	80	84	88	92	96	100	104	
A I								
Z	258	258	260	262	272	274	268	
R1	338	340	336	344	353	358	356	
R2	259	260	260	262	264	269	271	
L1	262	266	260	276	284	292	298	
L2	265	270	266	262	268	272	283	1.2.78*
R1L1	255	264	266	264	238	20.12.77*		
R1L2	225	238	242	249	253	265	267	
R2L1	265	263	270	270	277	280	277	
R2L2	210	206	214	216	220	224	220	
R3	235	246	242	250	262	266	257	
B I								
Z	294	303	300	302	310	313	306	
R1	295	308	313	306	312	314	318	
R2	400	404	405	416	436	436	445	
L1	328	336	14.10.77*					
L2	335	326	332	316	304	23.12.77*		
R1L1	337	334	338	344	362	358	369	
R1L2	311	318	324	332	342	346	342	
R2L1	270	281	284	286	304	324	17.1.78*	
R2L2	278	280	279	286	293	297	304	
R3	298	300	296	296	310	329	314	
C I								
Z	280	292	292	302	314	318	316	
R1	342	352	349	346	352	336	326	
R2	219	214	216	226	230	231	229	
L1	270	282	282	286	283	286	285	
L2	269	268	272	284	286	287	284	
R1L1	284	286	285	294	305	312	310	
R1L2	254	250	252	254	258	259	268	
R2L1	262	252	255	259	269	274	269	
R2L2	274	268	274	282	286	287	292	
R3	285	292	286	294	312	318	319	

* dead

000082

Individual body weights 50 ppm females at week:

	0	1	3	4	5	6	7	8	9	10	11	12	13	14	15	17
D I																
Z	92	95	143	154	155	169	172	179	186	196	195	196	200	202	204	208
R1	83	84	127	151	134	164	151	157	164	168	171	174	178	178	180	187
R2	101	108	159	136	178	189	195	205	209	212	215	216	224	225	229	235
L1	91	100	143	136	149	156	163	171	176	180	184	182	187	189	192	190
L2	82	88	126	172	135	143	150	157	161	170	170	170	176	179	180	189
R1L1	84	92	132	146	143	154	166	171	178	187	181	182	189	188	192	199
R1L2	92	101	149	157	160	170	176	180	188	196	192	196	210	214	214	229
R2L1	81	86	125	137	136	148	152	158	163	170	170	170	177	179	181	188
R2L2	95	98	145	155	156	166	174	172	182	188	188	190	195	200	204	207
R3	88	101	140	148	151	166	173	175	180	190	189	194	200	202	200	215
Total	8891	10169	13714	14694	15037	16013	16570	17111	17617	18236	18318	18568	18920	19285	19378	19987
Average	89	102	137	147	150	160	166	171	176	182	183	186	189	193	194	200
Number of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

000083

Individual body weights 50 ppm females at week:

	19	21	24	28	32	36	40	44	48	52	56	60	64	68	72	76
D I Z	213	221	226	228	232	236	239	241	244	250	254	260	266	280	282	284
R1	187	193	201	200	203	216	213	228	224	224	241	256	271	281	283	295
R2	240	248	260	260	268	268	291	299	302	304	332	350	371	374	374	378
L1	193	198	205	202	200	210	209	211	209	207	209	220	233	221	222	231
L2	193	200	211	210	216	226	242	239	254	264	275	295	311	343	22.6.77*	
R1L1	198	207	208	211	218	224	221	225	223	242	236	245	259	263	269	275
R1L2	228	232	239	242	264	256	255	257	262	292	300	320	323	338	345	357
R2L1	193	199	208	204	211	215	218	226	221	238	242	265	278	287	292	303
R2L2	208	218	224	232	235	240	243	247	253	254	264	272	301	305	309	319
R3	212	219	226	224	234	240	248	264	266	272	264	298	307	318	283	315
Total	20295	20695	21284	21450	21754	22339	22682	23226	23575	23928	24842	25918	26764	27593	27148	27598
Average	203	207	213	215	218	223	227	232	236	239	248	259	268	276	277	285
Number of animals	100	100	100	100	100	100	100	100	100	100	100	100	100	100	98	97

* dead

000084

Individual body weights 50 ppm females at week:

	80	84	88	92	96	100	104
D I							
Z	294	295	300	310	319	319	322
R1	297	302	304	304	316	330	336
R2	384	400	398	410	428	436	440
L1	228	228	232	232	236	238	230
L2							
R1L1	285	290	292	298	312	310	312
R1L2	360	372	366	372	396	412	408
R2L1	312	320	14.10.77*				
R2L2	323	334	332	26.10.77*			
R3	330	336	336	348	357	359	366
Total	27569	27592	26771	26134	26309	25601	24829
Average	287	290	291	297	306	308	307
Number of animals	96	95	92	88	86	83	81

* dead

000085 '/8

Appendix 4

Individual causes of death of rats which died spontaneously or were killed in extremis

Pathology code : see page 85

Animal house code: see page 11

Animal house code/pathology code:

Subgroup A:	1-10	101-110	201-210	301-310	401-410	501-510
Subgroup B:	11-20	111-120	211-220	311-320	411-420	511-520
	etc.	etc.				

The animals in each subgroup A: 1-10, 201-210, etc. and in each subgroup B, C, etc. are individually coded as follows:

Animal house code		Pathology code
Z	=	1, 11, etc.
R_1	=	2, 12, etc.
R_2	=	3, 13, etc.
L_1	=	4, 14, etc.
L_2	=	5, 15, etc.
R_1L_1	=	6, 16, etc.
R_1L_2	=	7, 17, etc.
R_2L_1	=	8, 18, etc.
R_2L_2	=	9, 19, etc.
R_3	=	10, 20, etc.

Individual data

Cause and time of death of animals that were killed in extremis before the terminal sacrifice period (week 105 and 106) as well as of animals that were found dead.

- Pathology code:

chloroprene concentration (ppm)	males	females
0	1 - 100	301 - 400
10	101 - 200	401 - 500
50	201 - 300	501 - 600

- The week of the experiment in which the animal died and the period (see below) in which this week fell, are listed for each animal.

- A few animals could not be examined, either because of too advanced autolysis or because of cannibalism or loss of material during processing.

- The diagnoses were based on a combination of clinical, macroscopic and microscopic pathological data.

- Abbreviations:
  suff. = suffering from (and killed in extremis for this reason)
  poss. = possibly

- periods in which the experimental period was divided:

  period 1 = week 1 - 60
  period 2 = week 61 - 72
  period 3 = week 73 - 84
  period 4 = week 85 - 96
  period 5 = week 97 - 104
  period 6 = terminal sacrifice period (week 105 and 106)

rat no.	time of death (week)	period	killed(K) or found dead (D)	MALES, CONTROLS CAUSE OF DEATH
6	94	4	K	suff. fibrosarcoma, submandibular salivary gland
10	101	5	K	suff. fibroma, subcutis
18	100	5	K	suff. respiratory disease
19	84	3	K	suff.paralysis hind legs
20	101	5	K	suff. osteosarcoma, fore leg
21	90	4	D	unknown, lost, too advanced autolysis
22	87	4	D	pneumonia and/or renal disease
28	65	2	D	haemothorax, subarachnoidal haemorrhage
29	93	4	K	suff.thrombus in left ventricle
31	88	4	D	phaeochromocytoma, adrenal
41	90	4	D	pneumonia
42	91	4	D	unknown
49	98	5	K	suff. liposarcoma in abdominal space
51	102	5	D	unknown
61	99	5	K	suff. neurological disorders and renal disease
62	94	4	K	suff. respiratory disease
64	101	5	K	suff. paralysis hind legs
67	94	4	K	suff. neurological disorders and renal and respirato
75	101	5	K	suff. fibrosarcoma, subcutis diseas
76	87	4	D	unknown
78	68	2	D	poss. encephalitis, lost, too advanced autolysis
88	92	4	K	unknown, lost for microscopy
95	71	2	K	suff. osteosarcoma,fore leg
				MALES, 10 ppm chloroprene
110	42	1	D	unknown
129	104	5	D	poss. lung haemorrhage
136	68	2	D	unknown
148	96	4	K	unknown

101-109, 111-120, 122-128, 130, 131, 133-135, 137-147, 149-164, 168-192, 197-200 died in the accident in week 72, period 2.

				MALES, 50 ppm chloroprene
202	89	4	D	renal disease
203	83	3	K	suff. squamous cell carcinoma, skin
204	72	2	D	leukemia
209	104	5	D	unknown

rat no.	period of death (week)		or found dead (D)	MALES, 50 ppm CAUSE OF DEATH
211	69	2	D	thrombus in ventricle, endocardial disease
213	93	4	K	suff. squamous cell carcinoma, skin
214	99	5	K	suff. metastazing transitional cell carcinoma, bladd
215	101	5	K	suff. pneumonia
218	83	3	K	unknown
220	78	3	D	lymphoreticular sarcoma in abdominal space
225	99	5	K	suff. hydrothorax
226	104	5	K	suff. thrombus in ventricle and hydrothorax
227	104	5	K	suff. leukemia
228	94	4	D	pituitary tumour
252	80	3	K	unknown
256	90	4	D	unknown
257	104	5	K	poss. suff. squamous cell carcinoma, skin.
264	100	5	D	haemangiosarcoma, spleen
268	99	5	K	unknown
277	99	5	D	poss. squamous cell carcinoma, skin or/and meningior brain
278	104	5	D	poss. renal or respiratory disease
279	100	5	K	suff. renal disease
284	98	5	K	suff. necrotizing pyelonephritis
				FEMALES, CONTROLS
305	95	4	K	suff. anaplastic carcinoma, uterus
308	98	5	K	suff. leimyosarcoma, uterus
310	71	2	K	suff. adenocarcinoma, mamma
313	102	5	K	suff. pituitary tumour
333	94	4	K	suff. fibroadenoma, mamma
340	95	4	K	suff. adenocarcinoma, abdominal space
341	103	5	K	suff. fibroadenoma and adenocarcinoma, mamma
342	104	5	K	suff. squamous cell carcinoma, preputial gland
343	103	5	K	suff. squamous cell carcinoma, skin
347	80	3	K	unknown
367	97	5	D	sarcoma, abdominal space
370	96	4	K	suff. adenocarcinoma, mamma
376	104	5	K	suff. fibroma, sucutis
377	95	4	D	unknown
380	92	4	D	pneumonia
385	90	4	D	unknown
390	87	4	K	suff. metastazing adenocarcinoma, mamma
392	74	3	K	suff. carcinoma, lung
398	91	4	D	unknown, lost, too advanced autolysis

rat no.	time of death (week)	period	killed(K) or found dead (D)	FEMALES, 10 ppm CAUSE OF DEATH
409	40	1	D	unknown
428	79	3	D	unknown
430	82	3	D	poss. adenocarcinoma, pituitary
435	63	2	K	poss. suff. fibroadenoma, mamma
449	100	5	K	suff. mamma tumours, lost for microscopy
453	64	2	D	unknown, lost, too advanced autolysis
464	71	2	D	poss. mamma tumour, lost due to canibalism/autolysis
500	102	5	K	poss. respiratory disease

401, 403, 404, 406-408, 410-424, 431-434, 436-448, 450, 451, 455-457, 459, 460, 463, 467-484, 486, 487, 489-495 all died in the accident in week 72, period 2.

rat no.	time of death (week)	period	killed(K) or found dead (D)	FEMALES, 50 ppm chloroprene CAUSE OF DEATH
501	104	5	K	suff. inflammation at nose
505	104	5	K	suff. poss. multiple endocrine tumours
514	92	4	K	suff. adenoma, mamma
516	77	3	K	suff. fibroadenoma, mamma
520	89	4	K	unknown
524	96	4	D	poss. squamous cell polyp, uterus
525	76	3	K	suff. fibroadenoma, mamma
531	71	2	K	suff. pyometra
537	92	4	K	suff. reticulum cell sarcoma, subcutis
538	84	3	K	suff. adenocarcinoma, pituitary
539	103	5	K	suff. fibroadenoma, mamma
540	104	5	K	suff. adenoma, mamma
543	87	4	K	suff. adenocarcinoma, mamma
545	104	5	K	suff. fibroadenoma, mamma
547	95	4	K	suff. fibroadenoma, mamma
554	104	5	K	suff. fibroadenomas, mamma
558	99	5	K	suff. fibroadenomas, mamma
565	104	5	K	suff. fibroadenoma, mamma
566	97	5	K	suff. pituitary tumour
574	87	4	K	suff. fibroadenoma, mamma
575	97	5	D	poss. necrotizing polyp, uterus
578	101	5	K	suff. fibroma, subcutis
595	71	2	K	suff. fibroadenoma, mamma
598	87	4	K	suff. fibroadenoma, mamma
599	89	4	K	suff. poss. pituitary tumour

Appendix 5

Individual data of tumours and time of death

Pathology code : see page 85

Animal house code : see page 11

Animal house code/pathology code:

Subgroup A:	1-10	101-110	201-210	301-310	401-410	501-510
Subgroup B:	11-20	111-120	211-220	311-320	411-420	511-520
	etc.	etc.				

The animals in each subgroup A: 1-10, 201-210, etc. and in each subgroup B, C, etc. are individually coded as follows:

Animal house code		Pathology code
Z	=	1, 11, etc.
R_1	=	2, 12, etc.
R_2	=	3, 13, etc.
L_1	=	4, 14, etc.
L_2	=	5, 15, etc.
R_1L_1	=	6, 16, etc.
R_1L_2	=	7, 17, etc.
R_2L_1	=	8, 18, etc.
R_2L_2	=	9, 19, etc.
R_3	=	10, 20, etc.

Individual data

Tumors and time of death

- Pathology code:

chloroprene concentration (ppm)	males	females
0	1 - 100	301 - 400
10	101 - 200	401 - 500
50	201 - 300	501 - 600

- The week of the experiment in which the animal died and the period (see below) in which this week fell, are listed for each animal as well as the number of primary tumors.

- Tumors observed macroscopically but not examined microscopically because of loss during processing are indicated as unidentified tumors lost for microscopy.

- A few animals were completely lost for examination because of too advanced autolysis and/or cannibalism or loss of material during processing.

- Abbreviations:
  M = metastasizing
  p.f.c. adenoma = parafollicular cell adenoma
  phaeochr. = phaeochromocytoma
  poss. = possibly

- For some types of tumours a gradation for size is indicated:
  1 = very small, not distorting the histological structure of the organ
  2 = moderate size, distorting the histological, but not the gross structure of the organ
  3 = large, distorting histological and gross structure of the organ

- Periods in which the experimental period was divided:
  period 1 = week 1 - 60
  period 2 = week 61 - 72
  period 3 = week 73 - 84
  period 4 = week 85 - 96
  period 5 = week 97 - 104
  period 6 = terminal sacrifice period (week 105 and 106)

Code	time of death (week)	period	no.of prim. tumors	MALES, CONTROLS, TUMORS
1	104	6	1	pancreas: island cell adenoma
2	104	6	1	pituitary: adenoma 1
3	104	6	0	
4	104	6	2	pituitary: adenoma 2; thyroid: p.f.c. adenoma 2
5	104	6	0	
6	94	4	1	submandibular sal.gland/cervical lnn.: fibrosarcom
7	104	6	2	pituitary: adenoma 1; adrenal pheaoch. 1
8	104	6	2	brain: granular cell myoblastoma; adrenal: pheaoch
9	104	6	0	
10	101	5	1	subcutis: fibroma
11	104	6	0	
12	104	6	0	
13	104	6	1	adrenal: cortical adenoma 2
14	104	6	0	
15	104	6	0	
16	104	6	0	
17	104	6	1	pituitary: adenoma 1
18	100	5	1	pituitary: adenoma 2
19	84	3	1	pituitary: adenoma 1
20	101	5	2	adrenal: pheaochr. 1; bone: osteosarcoma (fore leg)
21	90	4	-	lost
22	87	4	0	
23	104	6	1	subcutis: fibroma
24	104	6	0	
25	104	6	1	thyroid: follicular adenoma 1
26	104	6	0	
27	104	6	1	preputial gland: squamous cell carcinoma
28	65	2	0	
29	93	4	0	
30	104	6	1	mamma: adenoma
31	88	4	2	preputial gland: squamous cell carcinoma; adrenal: malignant pheaochr. 3
32	104	6	0	
33	104	6	1	thyroid: follicular adenoma 3
34	104	6	1	pituitary: adenoma 1
35	104	6	1	pituitary: adenoma 1
36	104	6	0	
37	104	6	2	thyroid: follicular adenoma 1 + p.f.c. adenoma 1

Code	Time of death (week)	period	No. of prim. tumors	MALES, CONTROLS, TUMORS
38	104	6	0	
39	104	6	0	
40	104	6	0	
41	90	4	0	
42	91	4	0	
43	104	6	0	
44	104	6	0	
45	104	6	1	pancreas: exocrine adenoma
46	104	6	0	
47	104	6	0	
48	104	6	1	adrenal: cortical adenoma 1
49	98	5	1	abdominal space: liposarcoma M
50	104	6	0	
51	102	5	0	
52	104	6	0	
53	104	6	2	pituitary: adenoma 2; mesenterial lnn.: angiosarco
54	104	6	0	
55	104	6	0	
56	104	6	0	
57	104	6	0	
58	104	6	0	
59	104	6	0	
60	104	6	0	
61	99	5	0	
62	94	4	0	
63	104	6	0	
64	101	5	2	pituitary: adenoma 1; pancreas: island cell adenoc cinoma
65	104	6	0	
66	104	6	2	thyroid: p.f.c. adenoma 1; adrenal: pheaochr. 2
67	94	4	0	
68	104	6	1	testis: Leydig cell tumor
69	104	6	2	pituitary: adenoma 1; lymphoid leukemia
70	104	6	2	pituitary: adenoma 1; thyroid: p.f.c. carcinoma 1
71	104	6	2	pituitary: adenoma 1; thyroid p.f.c. adenoma 2
72	104	6	1	thyroid: p.f.c. adenoma 2
73	104	6	2	thyroid p.f.c. adenoma 1; subcutis: fibroma
74	104	6	1	adrenal: pheaochr. 1
75	101	5	3	submandibular sal. gland: fibrosarcoma; pituitary:
76	87	4	0	adenoma 1; adrenal: pheaochr. 1

MALES, CONTROLS

Code	time of death (week)	period	no. of prim. tumors	TUMORS
77	104	6	1	adrenal: cortical adenoma 2
78	68	2	-	autolytic
79	104	6	1	adrenal: pheaochr. 3
80	104	6	2	adrenal: cortical adenoma 1; pituitary: adenoma 1
81	104	6	2	testis: Leydig cell tumor; pituitary: adenoma 1
82	104	6	0	
83	104	6	1	pituitary: adenoma 1
84	104	6	4	pituitary: adenoma 1; adrenal: pheaochr.1; thyroid: p.f.c.adenoma 1 + p.f.c.caroinoma 1
85	104	6	2	pituitary: adenoma 1; heart: haemangioendotheliosarc
86	104	6	1	brain: glia cell tumor
87	104	6	1	pituitary: adenoma 1
88	92	4	-	lost
89	104	6	0	
90	104	6	1	pituitary: adenocarcinoma 2
91	104	6	3	testes/epididymides: mesothelioma; thyroid: p.f.c. adenoma 1 + follicular adenoma 2
92	104	6	0	
93	104	6	1	thyroid: p.f.c. adenoma 1
94	104	6	0	
95	71	4	2	adrenal: cortical adenoma 1; bone: osteosarcoma M (fore leg)
96	104	6	1	pituitary: adenoma 1
97	104	6	0	
98	104	6	1	pituitary: adenoma 1
99	104	6	0	
100	104	6	1	adrenal: cortical adenoma 1

Code	time of death (week)	period	no.of prim. tumors	MALES, 10 ppm TUMORS
110	42	1	0	
121	104	6	1	thyroid: p.f.c. adenoma 2
129	104	5	0	
132	104	6	0	
136	68	2	0	
148	96	4	1	adrenal: cortical adenoma 1
165	104	6	1	adrenal: phaeochr. 2
166	104	6	0	
167	104	6	1	pituitary: adenoma 1
193	104	6	1	test is: Leydig cell tumor
194	104	6	1	adrenal: cortical adenoma 1
195	104	6	0	
196	104	6	0	

Code	time of death (week)	period	no. of prim. tumors	MALES, 50 ppm TUMORS
201	104	6	0	
202	89	4	0	
203	83	3	1	skin: squamous cell carcinoma
204	72	2	1	heamatopoietic system: lymphoid leukemia
205	104	6	1	adrenal: cortical adenoma 1
206	104	6	3	adrenal: phaeochr.1; thyroid p.f.c.adenoma 1; pituitar adenoma
207	104	6	0	
208	104	6	1	pituitary: adenoma 1
209	104	5	2	pituitary: adenoma 1; poss.preputial gland: squamous c carcino
210	104	6	3	adrenal: phaeochr.2; testes: bilateral Leydig cell tum
211	69	2	0	
212	104	6	1	adrenal: phaeochr. 3
213	93	4	2	pituitary: adenoma 1; skin: squamous cell carcinoma
214	99	5	1	bladder: transitional cell carcinoma M (with glandular and anaplastic areas)
215	101	5	0	
216	104	6	1	thyroid: p.f.c. adenoma 1
217	104	6	1	adrenal: cortical adenoma 1
218	83	3	1	pituitary: adenoma 1
219	104	6	0	
220	78	3	1	abdominal space. lymphoreticular sarcoma
221	104	6	0	
222	104	6	2	adrenal: cortical adenoma 1; thymus: reticulum cell sa coma
223	104	6	0	
224	104	6	0	
225	99	5	1	subcutis: fibroma
226	104	5	1	subcutis: fibroma
227	104	5	1	haematopoietic system: monocytic leukemia
228	94	4	2	skin,sinus maxillaris or nasal cavity:squamous cell ca noma;pituitary:poss.neurogenic tumor
229	104	6	0	
230	104	6	0	
231	104	6	1	pancreas: island cell adenoma
232	104	6	1	adrenal: cortical adenoma 1
233	104	6	1	pituitary: adenoma 1
234	104	6	0	
235	104	6	1	pituitary: adenoma 1
236	104	6	0	
237	104	6	0	
238	104	6	2	haematopoietic system: lymphocytic leukemia; adrenal: malignant phaeochr. 3
239	104	6	1	pituitary: adenoma 1

Code	of death (week)	period	prim. tumors	TUMORS
240	104	6	2	pituitary: adenoma 1; adrenal: cortical adenoma 1
241	104	6	0	
242	104	6	0	
243	104	6	1	kidney: **adenocarcinoma**
244	104	6	2	brain: meningioma; **thyroid: p.f.c. adenoma 1**
245	104	6	0	
246	104	6	1	pituitary: adenoma 1
247	104	6	0	
248	104	6	1	thyroid: follicular adenoma 2
249	104	6	1	thyroid: p.f.c. adenoma 3
250	104	6	0	
251	104	6	2	kidney : lipoma ; **subcutis: lipoma**
252	80	3	0	
253	104	6	1	pituitary: adenoma 1
254	104	6	1	adrenal: cortical adenoma 1
255	104	6	3	adrenal: phaeochr.1; thyroid: p.f.c.adenoma 1; pituita adenoma
256	96	4	0	partially lost
257	104	5	1	**skin, sinus maxillaris or nasal cavity: squamous cell c cinom**
258	104	6	2	adrenal: phaeochr. 1; thyroid: p.f.c. adenoma 1
259	104	6	0	
260	104	6	1	adrenal: phaeochr. 2
261	104	6	1	testis: Leydig cell tumor
262	104	6	0	
263	104	6	1	pituitary: adenoma 1
264	100	5	2	adrenal: cortical adenoma 1; spleen: haemangiosarcoma
265	104	6	1	pituitary: adenoma 1
266	104	6	0	
267	104	6	0	
268	99	5	2	thyroid: follicular adenoma 1; pituitary: **unidentified tumor, lost for microscopy**
269	104	6	0	
270	104	6	0	
271	104	6	0	
272	104	6	0	
273	104	6	0	
274	104	6	2	pituitary: adenoma 3; **thyroid: p.f.c. adenoma 1**
275	104	6	1	pituitary: adenoma 1
276	104	6	0	
277	99	5	2	brain: meningioma; **skin, sinus maxillaris or nasal cavity squamous cell carcinoma**
278	104	5	0	
279	100	5	0	
280	104	6	2	pituitary: adenoma 1; thyroid: p.f.c. adenoma 3

Code	time of death (week)	period	no. of prim. tumors	MALES, 50 ppm TUMORS
281	104	6	0	
282	104	6	0	
283	104	6	0	
284	98	5	0	
285	104	6	0	
286	104	6	0	
287	104	6	1	pituitary: adenoma 1
288	104	6	0	
289	104	6	0	
290	104	6	1	testis: Leydig cell tumor
291	104	6	1	thyroid: p.f.c. adenoma 1
292	104	6	1	thyroid: p.f.c. adenoma 1
293	104	6	1	pituitary: adenoma 1
294	104	6	1	pituitary: adenoma 1
295	104	6	1	pituitary: adenoma 1
296	104	6	1	thyroid: follicular adenoma 1
297	104	6	0	
298	104	6	2	pituitary: adenoma 1; thyroid: p.f.c. adenoma 2
299	104	6	0	
300	104	6	1	pancreas: multiple exocrine adenomas

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Code	time of death (week)	period	no. of prim. tumors	FEMALES, CONTROLS TUMORS
301	104	6	1	**mamma: unidentified tumour, lost for microscopy**
302	104	6	3	pituitary: adenoma 1; thyroid: p.f.c.adenoma 1; mamma:
303	104	6	2	thyroid: p.f.c. adenoma 1; mamma: fibroadenoma fibroadenoma
304	104	6	1	pituitary: adenoma 1
305	95	4	2	pituitary: adenoma 1; ovary and/or uterus: anaplastic
306	104	6	1	mamma: fibroadenoma carcino
307	104	6	2	pituitary: adenoma 2; mamma: fibroadenoma
308	98	5	1	cervix/uterus: leiomyosarcoma
309	104	6	0	
310	71	2	1	mamma: adenocarcinoma
311	104	6	1	mamma: fibroadenoma
312	104	6	2	pituitary: adenoma 1; uterus polyp
313	102	5	2	pituitary: adenoma 3; mamma: fibroadenoma
314	104	6	1	pituitary: adenoma 1
315	104	6	1	thyroid: p.f.c. adenoma 1
316	104	6	1	mamma: fibroadenoma
317	104	6	0	
318	104	6	1	mamma: fibroadenoma
319	104	6	0	
320	104	6	0	
321	104	6	3	mamma: **three fibroadenomas**
322	104	6	1	thyroid: p.f.c. adenoma 2
323	104	6	0	
324	104	6	0	
325	104	6	1	pituitary: adenoma 1
326	104	6	3	pituitary: adenoma 2; uterus: polyp; mamma: fibroadenom
327	104	6	1	thyroid: p.f.c. adenoma 1
328	104	6	0	
329	104	6	1	uterus: polyp
330	104	6	2	uterus: adenocarcinoma, polyp
331	104	6	0	
332	104	6	2	pituitary: adenoma 2; mamma: **fibroadenoma**
333	94	4	1	mamma: fibroadenoma
334	104	6	2	pituitary: adenoma 1; mamma: fibroadenoma
335	104	6	0	
336	104	6	2	pituitary: adenoma 2; mamma: **fibroadenoma**
337	104	6	0	
338	104	6	1	mamma: fibroadenoma
339	104	6	2	thyroid: p.f.c. adenoma 1; preputial gland: squamous ce carcinoma

Code	time of death (week)	period	no. of prim. tumors	TUMORS
340	95	4	2	uterus: polyp; abdominal space: adenocarcinoma M, unkno origin
341	103	5	3	thyroid: p.f.c.adenoma 1; mamma: fibroadenoma, adenocarc
342	104	5	2	preputial gland: squamous cell carcinoma; uterus: polyp, n identified microscop
343	103	5	1	skin, nasal cavity or sinus maxillaris: squamous cell carcinoma
344	104	6	0	
345	104	6	2	thyroid: p.f.c. adenoma 1; mamma: fibroadenoma
346	104	6	0	
347	80	3	0	
348	104	6	2	pituitary: adenoma 1; mamma: fibroadenoma
349	104	6	0	
350	104	6	2	pituitary: adenoma 1; adrenal: phaeochr. 1
351	104	6	1	pituitary: adenoma 1
352	104	6	0	
353	104	6	0	
354	104	6	0	
355	104	6	0	
256	104	6	0	
357	104	6	0	
358	104	6	1	thyroid: p.f.c. adenoma 1
359	104	6	0	
360	104	6	1	thyroid: p.f.c. adenoma 1
361	104	6	5	pituitary:adenoma 2;thyroid:p.f.c.adenoma 1; mamma:fibr
362	104	6	2	adenoma, adenoma, adenocarcinon uterus:polyp; subcutis:fibroma
363	104	6	1	liver: unidentified tumour, lost for microscopy
364	104	6	0	
365	104	6	2	pituitary: adenoma 3; mamma: papillary carcinoma
366	104	6	0	
367	97	5	1	abdominal space: undifferentiated sarcoma
368	104	6	1	mamma: adenoma
369	104	6	0	
370	96	4	1	mamma: adenocarcinoma/comedocarcinoma
371	104	6	3	pituitary: adenoma 1; thyroid: p.f.c.adenoma 2; mamma: adenor
372	104	6	2	mamma: fibroadenoma; hind leg: osteosarcoma
373	104	6	2	uterus: polyp; mamma: fibroadenoma
374	104	6	1	thyroid: p.f.c. adenoma 1
375	104	6	2	thyroid: p.f.c. adenoma 1; mamma: fibroadenoma
376	104	5	1	subcutis: fibroma
377	95	4	1	pituitary: adenoma 1
378	104	6	2	pituitary: adenoma 1; adrenal: phaeochr. 1

Code	time of death (week)	period	no. of prim. tumors	TUMORS
379	104	6	1	**kidney: lipoma**
380	92	4	0	
381	104	6	0	
382	104	6	0	
383	104	6	0	
384	104	6	1	thyroid: p.f.c. adenoma 2
385	90	4	1	pancreas: island cell adenoma
386	104	6	0	
387	104	6	0	
388	104	6	1	uterus: adenocarcinoma
389	104	6	0	
390	87	4	2	pituitary: adenoma 1; mamma: adenocarcinoma
391	104	6	0	
392	74	3	1	lung: anaplastic carcinoma
393	104	6	1	pituitary: adenoma 1
394	104	6	1	thymus: lymphosarcoma
395	104	6	1	**uterus: polyp**
396	104	6	0	
397	104	6	1	uterus: two polyps
398	91	4	-	partially lost and autolytic
399	104	6	3	mamma: three fibroadenomas
400	104	6	2	uterus: polyp; mamma fibroadenoma

Code	Time of death (week)	period	No. of prim. tumors	FEMALES, 10 ppm TUMORS	TOT
402	104	6	0		
405	104	6	1	pituitary: adenoma 2	
409	40	1	0		
425	104	6	1	uterus: polyp	
426	104	6	2	uterus: multiple polyps, mamma: fibroadenoma	
427	104	6	0		
428	79	3	0		
429	104	6	0		
430	82	3	2	pituitary: adenocarcinoma, mamma: adenoma	
435	63	2	1	mamma: fibroadenoma	
449	100	5	-	lost (macroscopic 2 mammatumors)	
452	104	6	0		
453	64	2	-	lost: too autolytic	
454	104	6	1	pituitary: adenoma 1	
458	104	6	2	adrenal: phaeochr. 1, mamma: fibroadenoma	
461	104	6	4	mamma: 4 fibroadenomas	
462	104	6	2	thyroid: p.f.c. adenoma 2, pituitary: adenoma 3	
464	71	2	-	lost: autolysis and cannibalism (macroscopic mammatum	
465	104	6	3	mamma: 3 fibroadenomas	
466	104	6	0		
485	104	6	0		
488	104	6	2	pituitary: adenoma 3, mamma: fibroadenoma	
496	104	6	1	uterus: polyp	
497	104	6	0		
498	104	6	0		
499	104	6	0		
500	102	5	0		

Code	time of death (week)	period	no. of prim. tumors	FEMALES, 50 ppm TUMORS
501	104	5	0	
502	104	6	0	
503	104	6	0	
504	104	6	0	
505	104	5	4	pituitary: adenoma 3; thyroid: papillary carcinoma, p.f
506	104	6	0	adenoma 1; mamma: adenoma
507	104	6	2	mamma: **fibroadenoma; brain: granular cell myoblastoma**
508	104	6	2	thyroid: p.f.c. adenoma 2; uterus: adenocarcinoma
509	104	6	1	uterus: polyp
510	104	6	1	pituitary: adenoma 3
511	104	6	2	pituitary: adenoma 3; mamma: fibroadenoma
512	104	6	1	**p.f.c. adenoma 1**
513	104	6	2	mamma: adenoma, **fibroadenoma**
514	92	4	1	mamma: adenoma
515	104	6	0	
516	77	3	1	mamma: **fibroadenoma**
517	104	6	1	uterus: polyp
518	104	6	1	kidney: lipoma
519	104	6	1	thyroid: p.f.c. adenoma 1
520	89	4	0	
521	104	6	1	thymus: epitheloid type thymoma
522	104	6	1	mamma: fibroadenoma
523	104	6	3	uterus: polyp; mamma: two fibroadenomas
524	96	4	2	thyroid: p.f.c. adenoma 1; uterus: squamous cell papill
525	76	3	1	mamma: fibroadenoma
526	104	6	1	thyroid: p.f.c. adenoma 1
527	104	6	1	uterus: polyp
528	104	6	1	thyroid: p.f.c. adenoma 1
529	104	6	1	uterus: polyp
530	104	6	1	pituitary: adenoma 2
531	71	2	0	
532	104	6	3	thyroid:p.f.c.adenoma 1;haematopoietic syst.:lymphoid
533	104	6	1	kemia;mamma: adenocarcinoma mamma: fibroadenoma
534	104	6	1	**mamma: fibroadenoma**
535	104	6	1	mamma: fibroadenoma
536	104	6	0	
537	92	4	1	**nasal cavity, sinus maxillaris or subcutis: reticulum c sarcoma**
538	84	3	3	pituitary: adenocarcinoma; mamma: adenocarcinoma; uter
539	103	5	4	mamma: four fibroadenomas

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FEMALES, 50 ppm

Code	time of death (week)	period	no. of prim. tumors	TUMORS
540	104	5	1	mamma: adenoma
541	104	6	1	thyroid: p.f.c. adenoma 1
542	104	6	0	
543	87	4	1	mamma: adenocarcinoma
544	104	6	1	preputial gland: squamous cell carcinoma
545	104	5	2	mamma: fibroadenoma; subcutis: fibroma
546	104	6	2	uterus: polyp; mamma: fibroadenoma
547	95	4	2	thyroid: follicular adenoma 1; mamma: fibroadenoma
548	104	6	1	uterus: polyp
549	104	6	4	pituitary:adenoma 1;thyroid:follicular adenoma 1; uter polyp; mamma: fibroadenoma
550	104	6	3	pituitary:adenoma 3;thyroid:follicular adenoma 1;mamma fibroaden
551	104	6	1	mamma: fibroadenoma
552	104	6	0	
553	104	6	0	
554	104	5	3	uterus: polyp; mamma: two fibroadenomas
555	104	6	0	
556	104	6	0	
557	104	6	2	uterus: polyp; mamma: fibroadenoma
558	99	5	4	mamma: two fibroadenomas;Zymbal gland: adenoma;subcuti fibroma
559	104	6	1	uterus: polyp
560	104	6	0	
561	104	6	0	
562	104	6	0	
563	104	6	1	mamma: adenoma
564	104	6	1	uterus: polyp
565	104	5	1	mamma: fibroadenoma
566	97	5	1	pituitary: adenoma 3
567	104	6	0	
568	104	6	1	thyroid: p.f.c. adenoma 1
569	104	6	1	subcutis: fibroma
570	104	6	0	
571	104	6	0	
572	104	6	1	mamma: fibroadenoma
573	104	6	3	pituitary: adenoma 2; thyroid: p.f.c. adenoma 2; mamma adenor
574	87	4	1	mamma: fibroadenoma
575	97	5	2	uterus: polyp; mamma: adenoma
576	104	6	1	mamma: fibroadenoma
577	104	6	3	thyroid: p.f.c.adenoma 3; mamma: fibroadenoma; thorac space: liposarcom

Code	time of death (week)	period	no. of prim. tumors	TUMORS
578	101	5	1	mamma: fibroadenoma
579	104	6	0	
580	104	6	1	thyroid: papillary carcinoma
581	104	6	0	
582	104	6	2	mamma: two fibroadenomas
583	104	6	1	uterus: adenocarcinoma
584	104	6	3	uterus: polyp; mamma: two fibroadenomas
585	104	6	1	thyroid: p.f.c. adenoma 2
586	104	6	1	thyroid: p.f.c. adenoma 1
587	104	6	0	
588	104	6	0	
589	104	6	2	thyroid: p.f.c. adenoma 1; mamma: fibroadenoma
590	104	6	2	pituitary: adenoma 2; mamma: fibroadenoma
591	104	6	1	pituitary: adenoma 1
592	104	6	4	thyroid:p.f.c.adenoma 1;uterus:polyp;mamma: two fibroadenoma
593	104	6	4	uterus: polyp; mamma: three fibroadenomas
594	104	6	0	
595	71	2	1	mamma: fibroadenoma
596	104	6	0	
597	104	6	4	pituitary:adenoma 1;thyroid:p.f.c.adenoma 1;mamma: two fibroadenoma
598	87	4	2	adrenal: phaeochr.1; mamma: fibroadenoma
599	89	4	1	pituitary: adenoma 3
600	104	6	2	thyroid: p.f.c. adenoma 1; mamma: fibroadenoma

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Doc I.D.	878215009	3	Old Doc I.D.	8DS	4		
Case No.(s)	OTS 8400 3A II		—		5		
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
110178		022285		N			

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Pub/Journal Name	—	9					
	—	9					
Author(s)	—	10					
Organ. Name	E I DUPONT DENEMOURS & CO INC	11					
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Contractor	—	21					
Doc Type	• R.I • U.P • H.E.A.S.D 8.D. , S.U H.S F.N	22					
Doc Title	EXAMINATION OF THE POTENTIAL MUTAGENICITY OF BETA-CHLOROPRENE USING THE MICRONUCLEUS TEST	23					

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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REPORT NO. R 5888

EXAMINATION OF THE POTENTIAL MUTAGENICITY OF β-CHLOROPRENE USING THE MICRONUCLEUS TEST

Authors : Drs M.I. Willems and H.R. Immel

At the request of: Joint Industry Committee on chloroprene

Approved by : Dr A.P. de Groot

Project No. : B78-0348

Date : November 1978

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Total or partial publication of this report without written assent is not allowed.

23a-5000-107!

SUMMARY

1. Two groups of rats, each consisting of five males and five females were exposed to atmospheres containing respectively 0 or 100 ppm β-chloroprene in air, for six hours a day, on five consecutive days.
   Body weights were recorded on days 1 and 5 of the exposure period. After the last exposure the animals were killed and bone marrow preparations were made. The ratio of poly- and normochromatic erythrocytes and the incidence of micronucleated cells per 2000 erythrocytes of each animal were recorded.

2. In test animals body weight gain was decreased compared with controls.

3. The incidence of micronucleated erythrocytes and the ratio of poly- and normochromatic erythrocytes was not affected by treatment.

4. It was concluded that the results of the present micronucleus test did not reveal any evidence of mutagenic activity of β-chloroprene.

EXAMINATION OF THE POTENTIAL MUTAGENICITY OF β-CHLOROPRENE USING THE MICRONUCLEUS TEST

1. INTRODUCTION

At the request of the "Joint Industry Committee on Chloroprene", a micronucleus test was carried out with β-chloroprene by exposing male and female rats for five consecutive days to atmospheres containing the test substance at average concentrations of 0 or 100 ppm.

2. MATERIAL AND METHOD

2.1 Animals

Randomly bred male and female Wistar rats were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. The animals were about five weeks old at the start of treatment.

All animals were housed in screen-bottomed cages, in air-conditioned rooms with automated light-darkness cycles of 12 hours and a constant temperature (24 ± 1 °C). Stock diet and tap water were constantly available, except during the exposure-periods.

2.2 Conduct of the experiment

The animals were divided according to body weight into two groups of five males and five females each, i.e. one test group and one control group. The two groups were exposed for six hours a day, on five consecutive days to atmospheres containing 0 or 100 ppm β-chloroprene in air. For details of the exposure-method see CIVO report no. R 5500.

During the exposures the animals were housed without food or water in a stainless steel/glass inhalation chamber in wire screen cages, five animals to a cage. Temperature and humidity inside the chamber was 22 ± 1 °C and 60 ± 5 per cent respectively. After each exposure the animals were returned to their living cages and provided with stock diet and tap water ad libitum.
Body weights were recorded at days 1 and 5 of the exposure-period. Immediately after the last exposure the animals were killed by decapitation. The bone marrow of the femora was flushed into centrifuge tubes containing foetal calf serum (Flow Laboratories, England) and centrifuged. The excess serum was removed. The cells were then resuspended by mixing gently with a Pasteur pipette. A drop was placed on a slide cleaned with methanol overnight and spread with a haemocytometer cover glass. Five slides were prepared of each animal. The smears were air dried, fixed in methanol and stained according to May-Grünwald Giemsa.

A total of 400 erythrocytes on each slide were examined microscopically and the number of micronucleated erythrocytes and the ratio of poly- and normochromatic erythrocytes recorded. In the tables the incidence of micronucleated erythrocytes is given per 2000 erythrocytes, the number of polychromatic erythrocytes is expressed as a % of the total erythrocyte count.

3. RESULTS

3.1 General reactions

No mortality or abnormalities of condition or behaviour, attributable to treatment, were observed in any of the animals during the exposure period.

3.2 Body weight change (table 1, appendix 1)

Body weight gain in male as well as in female test animals was less than in controls.

One male of the control group, looking ill already at the start of treatment, weighed considerably less than his group mates and was therefore excluded from the calculations of mean body weights (appendix 1, animal L_2).

3.3 Micronucleus test

Table 2 lists the group mean values and ranges of the incidences of micronucleated erythrocytes. Table 3 shows the results of a previous micronucleus test conducted with Trenimon, a known mutagen, with the same strain of rats as used in the present experiment. In addition both tables give the group mean values for the % of polychromatic erythrocytes.

The incidence of micronucleated erythrocytes and the % of polychromatic erythrocytes in the control group and the β-chloroprene treated group were comparable.
Trenimon induced a significant increase in the number of micronuclated erythrocytes and a decrease in the percentage of polychromatic erythrocytes.

4. CONCLUSION

From the present experiment it may be concluded that the micronucleus test did not reveal any evidence of mutagenic activity of β-chloroprene in male or female rats under the conditions employed in this study.

TABLE 1 - MEAN BODY WEIGHTS OF GROUPS OF FIVE ANIMALS EXPOSED FOR SIX HOURS/DAY DURING FIVE CONSECUTIVE DAYS

Group no.	ppm β-chloroprene in air	Mean body weight (g) at day 0	5	0	5
		males		females	
9964[1)]	0	134	159	109	121
9965	100	132	145	112	117

1) group mean of male control animals comprised four instead of five animals (for explanation see section 3.2).

TABLE 2 - MEAN NUMBERS OF MICRONUCLEATED ERYTHROCYTES AND % POLYCHROMATIC ERYTHROCYTES IN CONTROL AND CHLOROPRENE-TREATED RATS

Treatment	Sex	Incidence of micronucleated cells per 2000 erythrocytes per rat		% polychromatic erythrocytes
		mean	range	
ppm β-chloroprene in air				
0	♂	3.6	0 - 7	65.9
100	♂	1.2	0 - 4	66.7
0	♀	1.8	0 - 4	63.8
100	♀	1.6	0 - 3	62.8

TABLE 3 - MEAN NUMBERS OF MICRONUCLEATED ERYTHROCYTES AND % POLYCHROMATIC ERYTHROCYTES IN RATS AFTER TREATMENT WITH TRENIMON

Treatment[1)]	Sex	Incidence of micronucleated cells per 2000 erythrocytes per rat		% polychromatic erythrocytes
		mean	range	
solvent	♂	3.8	2 - 8	69
Trenimon	♂	22.6	20 - 26	42
solvent	♀	3.6	3 - 5	70
Trenimon	♀	34.4	27 - 38	46

1) Rats were, given twice i.p. 0.0625 mg Trenimon^{R} (Bayer) per kg body weight with an interval of 24 hours. Six hours later the animals were killed and bone preparations made as described in section 2. Trenimon: 2,3,5-tris-ethyleneiminobenzoquinone (1.4) was used as a solution in physiological saline.
Controls were treated in a simalar way with the vehicle only.

Appendix 1 - INDIVIDUAL BODY WEIGHTS

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Group	Animal no.	Body weight			
		Males		Females	
		Day 1	Day 5	Day 1	Day 5
9964	Z	132	156	113	125
	R_1	130	152	106	118
	R_2	136	160	111	121
	L_1	139	168	106	118
	L_2	78	111	107	123
9965	Z	133	138	106	110
	R_1	122	136	116	123
	R_2	146	160	109	118
	L_1	131	144	115	115
	L_2	130	146	113	118

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Case No.(s)	OTS 84003 A II				5		
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code •	8		
010179		022285		N			

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Pub/Journal Name	9
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Author(s)	10
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Contractor —	21
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• R.I • U.P • H.E.A.S.D 8D. , S.U H.S F.N	
Doc Title	23
EVALUATION OF LRT 314 IN THE SALMONELLA /MICROSOME MUTAGENICITY TEST BY ATMOSPHERIC EXPOSURE	

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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REPORT NO. R 5957

Title: Evaluation of LRT 314 in the Salmonella/microsome mutagenicity test by atmospheric exposure

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At the request of: The Joint Industry Committee on Chloroprene

Approved by: Dr. A.P. de Groot

Project no. B78/64-3

Date: January 1979

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Gehele of gedeeltelijke publikatie van dit rapport is zonder schriftelijke toestemming verboden.

SUMMARY

1. The mutagenic activity of LRT 314 was examined in the Salmonella/microsome mutagenicity test by atmospheric exposure, using a set of five histidine requiring mutants of S. typhimurium (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) and liver homogenate of Aroclor-induced rats.

2. Atmospheric exposure of the bacteria to the test material induced an increase in the number of his^+ revertants with strains TA 1535 and TA 100. Only with TA 100 the increase in the number of revertants was clearly dose-related.

3. It was concluded that LRT 314 showed mutagenic activity with strains TA 1535 and TA 100.

4. A comparison of the mutagenic activity of LRT 314 with that of β-chloroprene suggested LRT 314 being a more potent mutagen with TA 100 than β-chloroprene under the test conditions used in the present experiment.

Evaluation of LRT 314 in the Salmonella/microsome mutagenicity test by atmospheric exposure

1. INTRODUCTION

In a previous Ames test, performed to compare the mutagenic response of the microorganisms to β-chloroprene with five of its dimers, β-chloroprene appeared positive but none of the dimers showed any mutagenic activity (cf. CIVO Report No. R 5712). The treatment of the bacteria with β-chloroprene and the dimers was, however, not fully comparable: β-chloroprene was used as vapour and examined by atmospheric exposure of the bacteria, whereas the dimers were used as liquids and incorporated as such into the top agar with the bacteria.

Since the dimers are relatively volatile substances one might assume that part of the amount applied escaped by evaporation before and during the contact with the test organisms.

Therefore another test was conducted with one of the dimers (LRT 314) in which atmospheric exposure instead of plate incorporation was applied.

2. MATERIAL AND METHODS

2.1. Test materials

The sample of LRT 314 was taken from the series of samples of chloroprene dimers which have been examined in the previous Ames assay mentioned above and stored at 5°C. For identification and description see CIVO Report No. R 5712. The β-chloroprene used in this study was taken from a sample freshly supplied by Bayer AG, Dormagen, Western Germany.

2.2. Bacterial strains

The Salmonella typhimurium mutants used in this study, viz. S. typh. TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were provided by Dr. B.N. Ames, Berkeley, California, USA. They are stored as frozen cultures at -80°C. To obtain fresh cultures for mutagenesis testing, nutrient broth is inoculated with the frozen culture and

2.5. Mutagenesis assays

The procedure used in this mutagenic assay has been described in detail by Ames et al. (1975). Briefly the procedure was as follows: to 2 ml molten soft agar with biotine and a trace of histidine were added 0.1 ml of a fully grown culture of one of the tester strains and 0.5 ml S-9 mix if indicated. The ingredients were thoroughly mixed and immediately poured onto minimal glucose agar plates. The plates for the determination of the total number of colony forming units per plate were prepared in a similar way, but with 0.1 ml of an appropriately diluted bacterial culture and a larger amount of histidine to allow growth of all bacteria. The petri dishes were placed in 10 l desiccators, that were closed and partly evacuated for two minutes. A measured volume of the liquid to be tested was introduced into the desiccator via a sterilized rubber cap and allowed to vaporize for one minute. Then air was allowed to enter until atmospheric pressure was reached and the desiccators were incubated at 37°C. Desiccators containing the control plates were treated in a similar way, except that no test substance was introduced via the cap. After an incubation period of 48 hours the various test atmospheres were removed by passing air through the desiccaters. Then the bacterial colonies were counted and the background lawn of bacterial growth examined. The determinations were made in triplicate (unless otherwise indicated) and appropriate controls were included in each assay.

3. RESULTS

LRT 314 was examined in various independent experiments numbered 1 up to 5. Levels of 250, 500 and 1000 µl of the test liquid per desiccator appeared clearly toxic to the bacteria (experiment 1, results not presented in the tables). In experiments 2 up to 5 there were no signs that chemical toxicity

grown up overnight with shaking at 37°C. The reversion properties of the strains were checked, using the mutagens 2-aminoanthracene and 9-aminoacridine (cf. table 1). In addition, the strains were checked for histidine requirement, for sensitivity to crystal violet and deoxycholate as well as for resistance to ampicilline.

2.3. Chemicals

Nicotinamide adenine dinucleotide phosphate disodium salt (NADP) and D-glucose-6-phosphate disodium salt (G-6-P) were obtained from Boehringer, Mannheim, Germany; 4-aminobiphenyl (4-ABP) from Aldrich-Europe, Beerse, Belgium; Aroclor 1254, from Monsanto Chemical Company Co., St. Louis, USA; 2-aminoanthracene from ICI, Alderley Park, UK and dimethylsulphoxide (DMSO) from Merck-Schuchardt, Munich, Germany.

2.4. Liver homogenate, S-9, S-9 mix

The liver homogenate used in this study was prepared from two male rats (weighing about 200 g each), induced once with 500 mg Aroclor 1254/kg body weight i.p., 7 days before sacrifice. Aroclor was suspended in soya bean oil to a concentration of 200 mg/ml. After homogenizing in 3 volumes 0.1 M phosphate buffer pH 7.4 and centrifugation at 9000 g, the supernatant of the liver homogenate (=S-9) was stored at -80°C.

Immediately before use the S-9 mix was prepared by mixing the thawed S-9 fraction with a NADPH generating system. The final concentrations of the cofactors in the S-9 mix were: 4 mM NADP, 7 mM $MgSO_4$, 4mM G-6-P in 0.05 M phosphate buffer pH 7.4. The metabolic activation properties of the liver homogenate were checked with S. typh. TA 98 and 4-ABP. The amount of S-9 used througout this study was 50 µl S-9/0.5 ml S-9 mix/plate.

interfered with the mutagenicity testing: background lawn of bacterial growth in control and test plates was comparable. Moreover exposure of strain TA 100 to 760 µl β-chloroprene or 100 µl LRT 314 did not adversely affect the total number of colony forming units per plate (cf. table 2, experiment 5).

In experiments 2 and 3 the number of revertants with TA 1535 were slightly higher in the test plates than in the control plates, but there was no indication of a dose-response relationship (cf. table 2). Because of the somewhat inconsistent and not easily interpretable results obtained in these experiments, the assay was repeated with all tester strains (experiment 4, table 2).

From the latter experiment it appeared that the number of revertants with TA 100 increased with increasing dose levels. The numbers of revertants with TA 1535 were increased, as in the former experiments, whithout showing a dose-response relationship.

The results obtained with TA 100 in experiment 4 could be confirmed in experiment 5 (cf. table 3). Comparison of the effect of β-chloroprene and LRT 314 on TA 100 revealed that 50 µl LRT 314 induced more revertants than did 760 µl β-chloroprene. Seven hundred and sixty µl β-chloroprene in a 10 l desiccator is comparable to a concentration of 20,000 ppm as used in the previous Ames test with β-chloroprene (CIVO Report No. R 5712).

The use of glass instead of plastic plates did not prevent the induction of revertant colonies.

4. DISCUSSION AND CONCLUSION

Exposure of TA 1535 to LRT 314 resulted in increased numbers of revertants but there was no dose-related response relationship in any of the tests performed. With TA 100 LRT 314 showed distinct mutagenic activity: increasing levels of the test substance induced a considerable dose-related increase in the number of revertants in two out of three separate tests. In view of the

similarity of TA 100 and TA 1535 (both strains detect base pair substitions), and the distinct positive effect obtained with TA 100 in experiments 4 and 5 the results found with TA 1535 in each of three separate tests are considered indications of a mutagenic effect, in spite of the absence of a dose-related response. No explanation can be given for the negative result obtained with TA 100 in experiment 2.

Comparison of the mutagenic activity of β-chloroprene (as described in CIVO-report No. R 5712) with that of LRT 314 obtained in the present study suggest that LRT 314 is more active than β-chloroprene.

Although the concentration of β-chloroprene applied in the present experiment 5 was the same as that used in the experiment described in report No. R 5712 the mutagenic activity was less.

5. LITERATURE

Ames, B.N., J.Mc Cann and E. Yamasaki (1975)
Methods for detecting carcinogens and mutagens with the Salmonella/microsome mutagenicity test
Mutation Research 31/6, 347-364

CIVO-report No. R 5712 (1978)
Evaluation of β-chloroprene and five dimers in the Salmonella/microsome mutagenicity test.

Table 1. Response of the tester strains to standard mutagens

treatment	µl S-9/ plate	number of his^+ revertants (mean of two plates) with				
		TA 1535	TA 1537	TA 1538	TA 98	TA 100
1 µg 2-aminoanthracene per plate	0	43	-')	22	32	155
	50	310	-	1410	1800	7620
33 µg 9-aminoacredine per plate	0	-	369	-	-	-
	50	-	100	-	-	-
0.1 ml DMSO	0	36	8	18	32	175
	50	16	18	49	64	131

') - = not determined

Table 2. Evaluation of LRT 314 in the Salmonella microsome mutagenicity test by atmospheric exposure

µl LRT 314/ desiccator	µl S-9/ plate	mean numbers of his$^+$ revertants[1] ±SD with				
		TA 1535	TA 1537	TA 1538	TA 98	TA 100
		experiment 2 (22.3.78)				
0	0	16±4	12±6	10±2	25±2	57±9
50	0	13±2	16±2	18±2	20±6	39±9
100	0	34±4	15±3	22±10	21±6	66±5
250	0	15±9	17±1	5±2	28±4	39±4
0	50	15±2	29±3	15±5	25±1	64±19
50	50	18±9	26±6	18±3	23±2	52±6
100	50	35±5	28±9	24±2	26±3	72±15
250	50	26±1	29±5	26±11	23±3	55±2
		experiment 3[1] (4.4.78)				
0	0	12±6		5±2		
25	0	43±7		9±2		
50	0	41±10		9±2		
75	0	36±3		8±3		
0	50	15±4		7±2		
25	50	65±9		8±2		
50	50	49±4		13±4		
75	50	47±8		9±5		
		experiment 4 (5.10.78)				
0	0	78±10	15±2	22±5	44±3	157±10
25	0	84±20	10±1	20±6	33±2	207±41
50	0	166±30	9±1	18±2	45±6	438±35
100	0	126±18	6±4	20±5	50±20	622±20

[1]) mean of five instead of three plates

[2]) means surpassing twice the control value are underlined.

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Table 3. LRT 314 and β-chloroprene, comparison of mutagenic- and growth inhibiting effect on strain TA 100, using glass- and plastic petri plates (experiment 5: 13-10-78)

treatment	plastic		glass	
	revertants/plate (mean S.D.)	CFU')/plate	revertants/plate (mean S.D.)	CFU')/plate
control	149±34	158	136±18	182
50 µl LRT 314/desiccator	406±84	179	331±53	169
100 µl LRT/314/desiccator	777±165	181	472±48	188
760 µl β-chloroprene/desiccator (± 20.000 ppm)	241±32	194	225±15	182

CFU = colony forming units

The number of CFU was determined by plating a $1/10^6$ diluted bacterial culture in top agar with extra histidine.

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CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) • OTS 0206752	1	No. of Pages	2
Doc I.D 8782150011	3	Old Doc I.D. 8DS	4
Case No.(s) OTS 84003 A II			5
Date Produced (6) 120178	6	Date Rec'd (6) 022285	7
Conf. Code • N	8		

Check One: ☐ Publication ☐ Internally Generated ☒ Externally Generated

Pub/Journal Name	9
	9
Author(s)	10
Organ. Name E I DUPONT DENEMOURS & CO INC	11
Dept/Div	12
P.O. Box 13 Street No./Name	14
City WILMINGTON 15 State DE 16 Zip 19898 17 Country	18
MID No. (7) 19 D & B NO. (11)	20
Contractor	21
Doc Type •R.I•U.P•HEASD 8D, SU HS FN	22
Doc Title REPRODUCTION STUDY WITH BETA-CHLOROPRENE VAPOUR IN RATS (PRELIMINARY REPORT)	23

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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REPORT NO. R 5859

REPRODUCTION STUDY WITH β-CHLOROPRENE VAPOUR IN RATS (PRELIMINARY REPORT)

Author	: Drs. L.M. Appelman
At the request of	: The Joint Industry Committee on Chloroprene
Project no.	: B77/2650
Date	: December 1978
Approved by	: Dr. V.J. Feron
Start of study	: 23 December 1977
Termination of study	: 2 August 1978
Study director	: Drs. P.G.J. Reuzel

All raw data and this report are stored in the archives of the department of Biological Toxicology under the reference "Joint Industry Committee on chloroprene".

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Total or partial publication of this report without written consent is not allowed.

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SUMMARY

1. A reproduction study with β-chloroprene vapour was carried out, in which two successive generations of rats (F_0- and F_1-generation) were exposed to atmospheres containing 0, 10, 33 and 100 ppm β-chloroprene. The F_0-generation males and females were exposed 6 hours/day, 5 days/week for a period of 13 weeks, and thereafter, mated with untreated females and males respectively. F_1-generation rats were exposed 6 hours/day, 5 days/week for a 10-week period.

2. The actual mean concentrations of β-chloroprene in the test-atmospheres were 0, 9.80, 32.60 and 100.01 ppm for the F_0-generation and 0, 9.85, 32.99 and 100.58 ppm for the F_1-generation.

3. Fertility of females, number of young born per litter, general condition, appearance, male/female ratio, and mortality of the young were not adversely affected by the exposure to β-chloroprene. There was no indication of increased intra-uterine mortality.

4. Growth retardation was observed in the F_0-generation at the high-dose level and in the F_1-generation rats at the mid- and high-dose levels.

5. Gross examination at autopsy did not reveal any treatment-related abnormalities.

REPRODUCTION STUDY WITH β-CHLOROPRENE VAPOUR IN RATS

1. INTRODUCTION

At the request of "The Joint Industry Committee on Chloroprene" an inhalation toxicity study in rats over two successive generations was carried out with β-chloroprene.
The test animals were exposed to atmospheres containing 0,10,33 or 100 ppm β-chloroprene.

2. EXPERIMENTAL

2.1 Material

Twelve bottles, each containing 200 ml freshly purified β-chloroprene, were received weekly from Bayer AG, Dormagen, Western-Germany.

2.2 Animals

One hundred male and 100 female SPF reared rats (cpb:wu, Wistar random) were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands on December 13, 1977. They weighed about 50 g on arrival. The animals were allocated according to body weight and sex to four groups, 1 control group and 3 test groups, each consisting of 25 males and 25 females.

2.3 Diet

After the daily exposure to chloroprene the F_0-animals were provided ad libitum with a pelleted rat diet (Muracon I, Trouw & Co. N.V., Putten, The Netherlands) and with unfluoridized bottled water. The F_1-animals received the Institute's stock diet for rats and unfluoridized bottled tap water ad libitum. The nutrient composition and the levels of the various contaminants, as determined periodically in batches of the Institute's stock diet, are shown in appendices 1,2 and 3. During exposures neither food nor water was available to the animals.

2.4 Exposure chambers

The chambers used have a capacity of 2.5 m^3 and are constructed of stainless steel with a glass-door which allows observation of the animals during the exposures. The chambers have a cylindrical form with a conical top and bottom. Inside they are fitted with a heptagonal frame, which is rotatable around the vertical axis of the chamber.
Forty two tinned, wire mesh cages with a floor surface of 930 cm^2 each, were suspended over seven vertical rows with 6 layers each. The position of the rows within the chambers was changed daily in a fixed order.
The chambers are illuminated externally by normal laboratory lighting.
The light cycle was 12 hours light and 12 hours darkness.

The chambers were maintained at a negative pressure of 1-2 mm H_2O relatively to the room, to prevent leakage of the test-atmospheres into the room.
The main airflow was drawn from the inhalation room and had, when it entered the chamber, a temperature of 22 ± 1 °C and a relative humidity of 45-60%. Before entering the inhalation chamber the main airflow was mixed with a nitrogen flow which was laden with the test material.
In the chamber the airflow is spred equally by an air disseminator which is located at the top. The flow rate through the chamber was approximately 40 m^3/h during exposures, and 60 m^3/h during the non-exposure periods.

2.5 Accomodation

The animals stayed in the exposure chambers, also during the non-exposure periods. During the mating period the animals were housed in stainless steel cages (one male and one female in each cage) which were suspended in an open rack in an animal room.
The temperature and the relative humidity were controlled at 22 ± 1 °C and 45-60% respectively.

2.6 Generation of the test atmosphere

ß-chloroprene was evaporated by passing a small filtered and dried nitrogen flow through a glass evaporator, filled with a suitable quantity of ß-chloroprene and kept at 0 °C.
The ß-chloroprene-saturated nitrogen flow was fed into the inlet system of the inhalation chamber and mixed with the air flow entering the inhalation chamber in such a way that the desired concentration was obtained.

2.7 Dose levels

Groups of rats were exposed, 6 hours/day, 5 days a week, to 0, 10, 33 or 100 ppm ß-chloroprene. The exposure period was 13 weeks for the F_0-generation and about 10 weeks for the F_1-generation.

Group no.	number of animals				nominal concentration of ß-chloroprene in ppm
	males		females		
	F_0	F_1	F_0	F_1	
9854	25	40	25	40	0
9855	25	40	25	40	10
9856	25	40	25	40	33
9857	25	40	25	40	100

2.8 Analysis

Analyses of the test atmospheres were performed by gaschromatography: the gaschromatograph used being an Intersmat GC 120, fitted with a flame ionization detector, operating at the following conditions:

Column : 300 cm x 3 mm internal diameter, packed with Carbowax 20M

Temperature : Injection port 105 °C
Detector 105 °C
Oven 65 °C

Carriergas : Nitrogen, with a flow rate of 65 ml/min

The gaschromatograph was calibrated with a standard solution of β-chloroprene in carbondisulfide, which was diluted to obtain the β-chloroprene concentration desired.
Peak surfaces, obtained by injecting samples of the dilutions of the β-chloroprene standard solution, were compared with those of samples of the test atmosphere.

Samples from each test atmosphere were taken automatically with a sample loop, which was calibrated by comparing peak surfaces of atmosphere samples taken with a gas-tight syringe with peak surfaces of simultaneously taken loop-samples.

2.9 Design and conduct of the study

2.9.1 The F_0-generation

Exposure period of the F_0-generation

The exposure of the F_0-generation was started on December 23, 1977. The last exposure day was March 17, 1978.

Condition and behaviour

The general condition and behaviour of all rats were checked regularly; all signs of ill health or reactions to treatment were recorded.

Body weights

Individual body weights were measured weekly.

Autopsy

After weaning their litters the females were killed and the uterus was treated in an ammonium sulfide solution for staining and counting the implantation sites. After the mating period (see 2.9.2) the males were killed and the testicles were collected and fixed in a 4% aqueous neutralized formaldehyde solution.

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2.9.2 The mating period

After the exposure period of 13 weeks the rats were mated for 20 days: The males of the 3 exposure groups and of the control group were mated with females of the stock colony (1 male x 1 female). The females of the 3 exposure groups and of the control group were mated with males of the stock colony (1 female x 1 male) (see also figure 1).
After the mating period the females were caged individually until their litters had been weaned. Records were made of the number of pups in each litter at birth and the total number of survivors at day 1, 3, 14 and 28. The total weight of the litter was recorded at day 1, 3, 14 and 28. At the same time the male/female ratio was determined.
Litters containing more than 8 siblings were randomly culled to eight at day 4, in order to equalize the stress of lactations among the dams.

2.9.3 The F_1-generation

Exposure period of the F_1-generation

From the F_1-litters, 20 males and 20 females were selected randomly from each mating group one week after weaning, making a total of 160 males and 160 females. These animals were exposed for a period of 10 weeks. The exposure of the F_1-litters was started on May 22, 1978. The last exposure was on August 2, 1978.

Condition and behaviour

The general condition, behaviour and possible signs of intoxication were checked daily; all signs of ill health or reactions to treatment were recorded.

Body weights

Individual body weights were recorded weekly.

Haematology

Blood samples were collected from the tip of the tail of 15 rats/sex/group at an age of 4 weeks. All samples were examined for haemoglobin concentration (Hb) by the cyan-methaemoglobin method using Zap-Oglobin® of Coulter Electronics ltd.

Autopsy

Ten rats/sex/group were killed at the end of the exposure period and autopsied. The liver, lungs, ovaries and testes were weighed and fixed in a 4% aqueous neutralized formaldehyde solution.

2.10 Statistical analysis

Statistical analyses of body weight changes and organ-to-body weight ratios were carried out using the Student's t-test; the haematological data were evaluated statistically by means of the test of Wilcoxon.

3. RESULTS

3.1 Exposure levels

The mean actual concentrations of β-chloroprene, to which the rats in the different groups were exposed, are summarized in table 1.
From this table it can be seen that the actual levels were very close to the design concentrations.

3.2 The F_0-generation

3.2.1 Condition and behaviour

The general condition and behaviour of the rats in the different groups did not show any abnormality, which could be ascribed to treatment with the test substance.

3.2.2 Body weights

The mean body weights are summarized in table 2. In the top dose group slight growth retardation was observed in males during the first 8 weeks and in females throughout the study. In the low and mid dose groups the mean body-weights sometimes differed statistically significantly from those of the controls, but there was no evidence of a constant pattern. Moreover, the mean body weights of the controls showed some irregularities, especially from week 9 till week 12. Inspection of the individual body weight figures learned that these irregularities were most propably due to occasional shortage of feed in several cages, which was almost surely caused by providing an insufficient daily portion of pellets.

3.2.3 Reproductive performance

The results of the breedings are summarized in the tables 3 and 4.
The percentage of females which casted a litter was high in all groups.
There were no consistent decreases in fertility observed in any of the groups.
The number of young born per litter did not show treatment related differences between the various test groups and controls.
The ratio of males/females showed some variation amongst groups, but there was no evidence of a dose-related response.
The mean body weights of the young descended from treated females and untreated males were significantly lower in the top-dose group than in the control group both at birth and during lactation.
The mortality figures varied somewhat among the groups, but there was no indication of a dose-related response.
The resorption quotiënts were similar in all groups and did not indicate any increase in foetal mortality as a result of exposure to β-chloroprene vapour.

3.3 The F_1-generation

3.3.1 Condition and behaviour

The general condition and behaviour of the young did not show any abnormality, which could be ascribed to the treatment.

3.3.2 Body weights

Growth retardation occurred at the 33 and 100 ppm levels in both sexes, the differences with the controls being statistically insignificant at 33 ppm in male descendants from treated females and untreated males (table 5).

3.3.3 Haematology

Haemoglobin levels were similar in all groups (table 6).

3.4 Alopecia

The incidence of focal alopecia is presented in table 7.
This table shows that alopecia was seen in a limited number of rats of each of the groups, the control group included. There was no evidence of chloroprene exposure inducing this skin lesion or enhancing its degree or incidence.

3.5 Autopsy

Gross examination at autopsy of the F_0 and F_1-generation rats did not reveal any abnormality that could be attributed to the test material.

Table 1. Mean concentrations of β-chloroprene, the standard deviations and the standard errors of the mean.

nominal concentration of β-chloroprene in ppm		actual concentration of β-chloroprene in ppm		standard deviation		standard error of mean	
F_0	F_1	F_0	F_1	F_0	F_1	F_0	F_1
0	0	0	0	0	0	0	0
10	10	9.80	9.85	0.68	0.56	0.08	0.08
33	33	32.60	32.99	1.50	2.16	0.19	0.30
100	100	100.01	100.58	5.48	4.82	0.69	0.67

Table 2. Mean body weights of F_0-generation rats

β-chloroprene concentration in ppm	mean body weights (in g) at the end of week:													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
							males							
0	92	110	146	183	198	223	251	277	295	280	304	300	311	332
10	94	108	144	182	208	238	249	276	292	292	298	298	300	324
33	92	107	142	178	204	222	245	264	275*	282	293	285	292	313
100	93	112	141	173*	188	219	237*	258*	269**	275	287*	295	301	317
							females							
0	85	92	112	129	139	151	161	172	178	178	186	189	194	200
10	85	93	111	129	139	150	155*	166*	172	175	179*	178**	186*	193*
33	84	93	111	128	139	148	158	166*	171*	173	180	176***	185*	193*
100	82	92	106**	119***	131***	143*	150***	158***	165***	169*	177**	177**	182**	187***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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Table 3. Reproductive performance

Litters of females, which were exposed to ß-chloroprene

ß-chloroprene concentration in ppm	females		mean litter size at birth		male/female ratio
	no. mated	% with litters	dead	alive	
0	25	100	0.04	10.5	1.00
10	25	100	0.04	10.9	1.38
33	25	100	0.60[1)]	9.6	1.13
100	25	96	0.16	9.8	1.13

percentage males at birth	percentage females at birth	mean body weight of young at day			
		1	3	14	28
50	50	6.2	8.1	27.4	68.6
58	42	6.1	7.8	26.9	66.2
53	47	6.3	8.2	27.0	66.4
53	47	6.0*	7.9	25.7**	64.5**

% mortality of young during lactation at day				resorption quotient[2)]
1	3	4-14	4-28	
0.8	1.1	0	0.5	1.06
0.7	0.7	0	0.5	1.06
7.4	8.3	0	0	1.08
2.1	2.1	0	0	1.08

1) From one litter 9 of 10 young were dead at birth

2) Number of implantation sites/number of young born

*P < 0.05; **P < 0.01;*** P < 0.001

Table 4. Reproductive performance

Litters of females, which were not exposed to β-chloropr[illegible]e

β-chloroprene concentration in ppm	females		mean litter size at birth		male/female ratio
	no. mated	% with litters	dead	alive	
0	25	100	0	11.[illegible]	0.85
10	25	92	0	10.2	1.17
33	25	96	0.25	11.0	0.85
[illegible]0	25	100	0.08	11.2	1.13

percentage males at birth	percentage females at birth	mean body weight of young at day			
		[illegible]	3	14	28
46	54	6.0	7.9	28.2	68.6
54	46	6.2	8.0	28.2	69.3
46	54	6.0	7.8	27.8	67.3
53	47	6.0	7.8	28.2	67.4

% mortality of young during lactation at day				resorption[1] quotient
1	3	4-14	4-28	
1.8	3.3	0	0	1.07
0.4	0.4	0.6	0.6	1.08
3.0	3.0	0	0	1.10
1.8	1.8	0	0	1.06

1) Number of implantation sites/number of young born

Table 5. Mean body weights of F_1-generation rats

β-chloroprene concentration in ppm	mean body weights (in g) at the end of week:										
	0	1	2	3	4	5	6	7	8	9	10
descendants from treated females and untreated males											
males											
0	123	153	194	224	243	266	283	291	300	312	328
10	125	159	202	235	261*	280	296	309	313	324	336
33	121	148	186	217	237	257	271	277	291	302	314
100	119	142**	178***	206**	227*	248*	263*	278	288	298	305*
females											
0	104	123	139	152	164	173	179	182	186	190	196
10	104	122	139	153	164	173	180	185	187	191	191
33	104	117*	133*	146*	155**	166*	172*	178	181	186	191
100	99	110***	127***	141***	151***	161***	167**	175*	178**	182*	186**
descendants from untreated females and treated males											
males											
0	119	161	200	232	259	280	296	307	320	330	342
10	127	165	204	235	263	288	302	314	320	333	346
33	122	147*	183**	211***	233***	255***	267***	274***	286***	299***	310**
100	129*	151*	187*	211***	229***	252***	264***	274***	290***	300***	306**
females											
0	106	126	142	157	169	179	187	192	195	199	204
10	108	125	142	155	167	182	183	188	189	194	199
33	104	118**	135*	149*	158***	167**	173***	178**	182**	187**	194*
100	105	118**	133***	146***	154***	161***	167***	178***	182***	186***	190**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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Table 6. Mean haemoglobin values of F_1-generation rats

β-chloroprene concentration in ppm	Descendants from treated females and untreated males		Descendants from untreated females and treated males	
	haemoglobin (mmol/l)		haemoglobin (mmol/l)	
	males	females	males	females
0	7.2	7.4	7.3	7.4
10	7.0	7.2	7.4	7.5
33	7.0	7.6	7.3	7.3
100	7.3	7.2	7.3	7.5

Table 7. Incidence of focal alopecia.

β-chloroprene concentration in ppm	F_0-generation		Descendants from females, exposed to β-chloroprene		Descendants from females, not exposed to β-chloroprene	
	♂♂ males	♀♀ females	♂♂ males	♀♀ females	♂♂ males	♀♀ females
0	2	2	0	0	0	0
10	0	3[1]	0	0	0	8[4]
33	0	2[1]	0	0	0	0
100	2[2]	2	0	9[3]	2	0

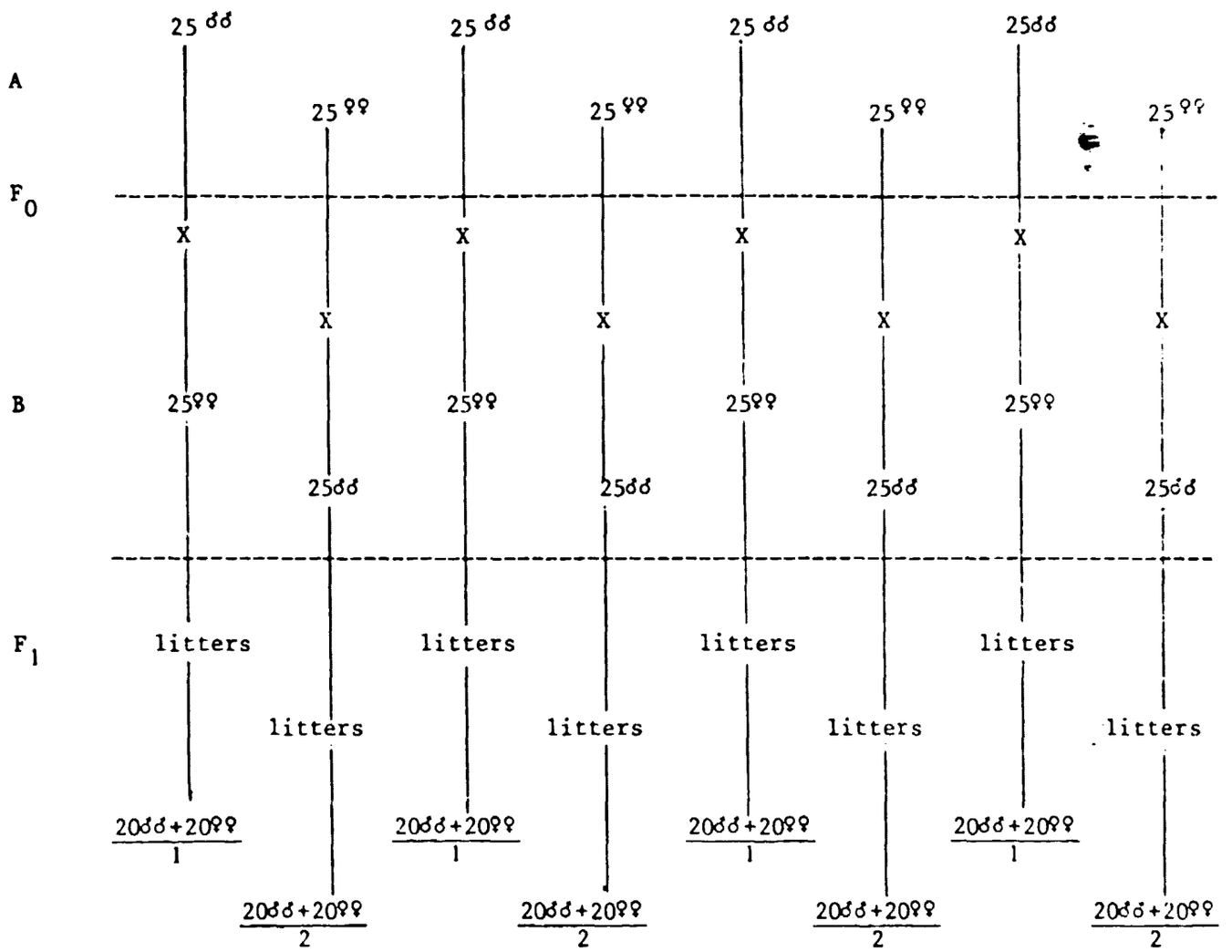
1) Two in one cage of four.

2) Two males in one cage of three

3) Four of the nine were housed in one cage of 5 and three of the nine in one cage of four.

4) Four animals were housed in one cage of 5, and the other four were also housed in one cage of four.

fig. 1: Mating diagram



1 F_1-generation of treated males and untreated females

2 F_1-generation of untreated males and treated females

A Animals exposed to different concentrations of β-chloroprene

B Animals of the stock-colony

SUMMARY

1. An 18-month inhalation carcinogenicity study with chloroprene was performed in hamsters. Three groups of 100 male and 100 female hamsters each were exposed to 0 (control group), 10 or 50 ppm chloroprene, 6 hours/day, 5 days/week, for a period of 18 months.

2. No abnormalities were observed in behaviour.

3. Mortality was lower in both test groups than in the controls.

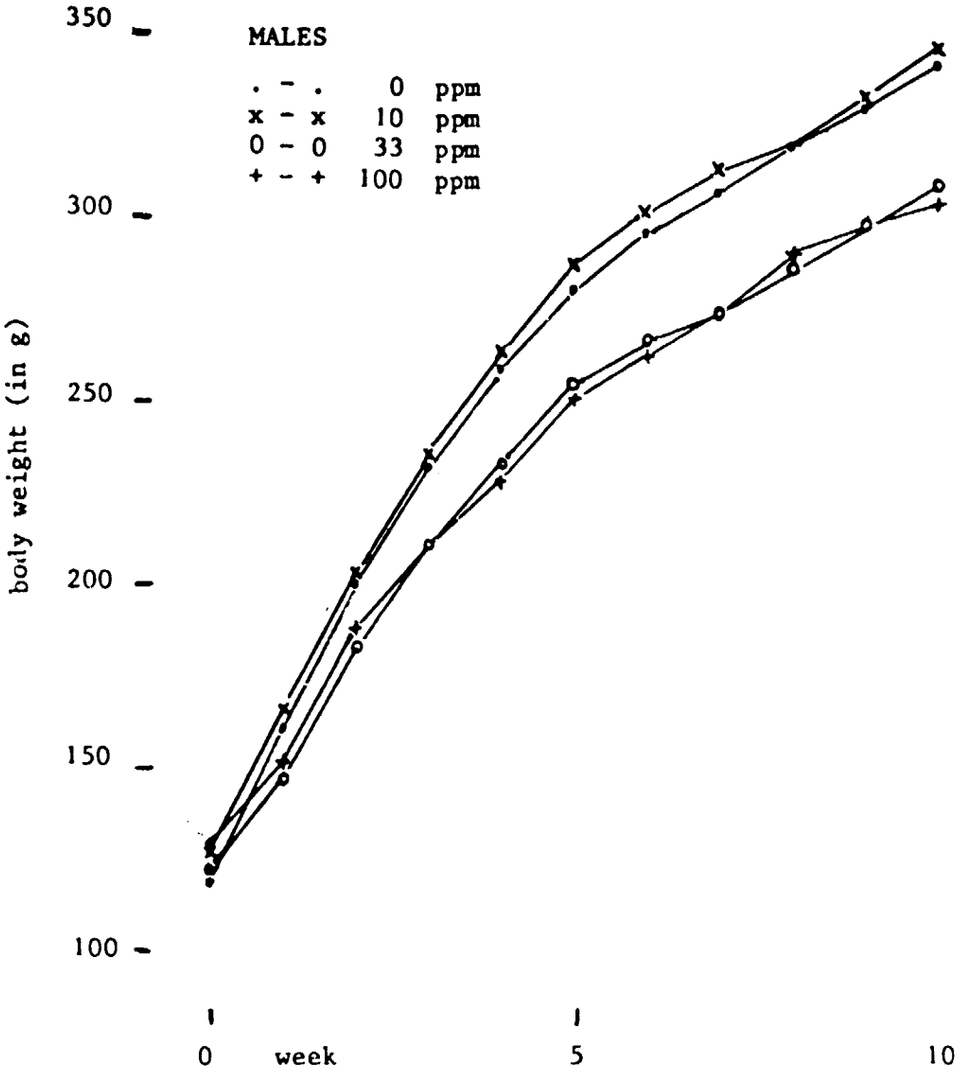
4. Growth retardation was observed in males and females of the high-level group.

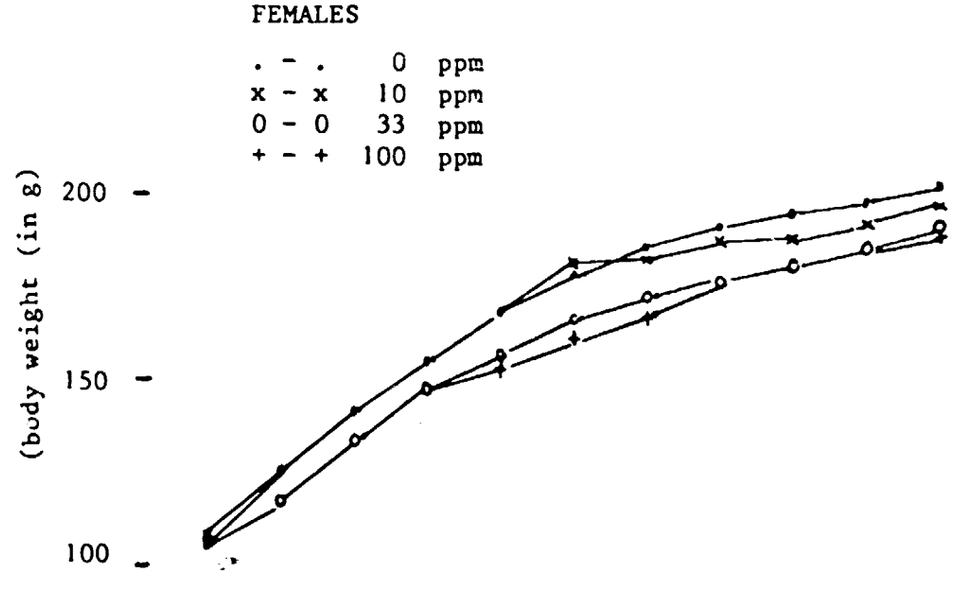
5. A slight reduction in amyloidosis was observed in hamsters of the high-level group.

6. At the 10 ppm level no adverse effect was found.

7. No indications were found for carcinogenic properties of chloroprene up to an exposure level of 50 ppm.

fig. 3: Mean body weights of the F_1-generation rats descended from females which were not exposed to β-chloroprene.





Appendix 1

NUTRIENT COMPOSITION OF BASAL DIET FOR RATS

Analyses of a 1000 kg batch produced in March 1978

moisture	12.0 %	iron	235 ppm
crude protein (N x 6.25)	20.1 %	manganese	84 ppm
crude fat	7.0 %	copper	17 ppm
calcium	0.86 %	zinc	49 ppm
phosphorus	0.71 %	cobalt	0.8 ppm
magnesium	0.14 %	chromium	1.5 ppm
		selenium	0.14 ppm

vitamin A	13.000 I.U./kg	vitamin B_1	8.5 mg/kg
vitamin D	3.800 I.U./kg	vitamin B_2	7.2 mg/kg
vitamin E	77.6 mg/kg	vitamin B_6	5.1 mg/kg
carotene	0.2 mg/kg	niacin	63 mg/kg
vitamin K_3	< 1 mg/kg	pantothenic acid	13 mg/kg
		folic acid	1.0 mg/kg
		choline	1500 mg/kg
		biotin	0.3 mg/kg
		vitamin B_{12}	18 µg/kg

	g/100 g	g/16 g N		g/100 g	g/16 g N
isoleucine	0.89	4.4	valine	1.10	5.5
leucine	1.64	8.2	arginine	1.18	5.9
lysine	1.06	5.3	histidine	0.45	2.2
methionine	0.40	2.0	alanine	1.12	5.6
cystine	0.35	1.7	aspartic acid	1.85	9.2
phenylalanine	0.90	4.5	glutamic acid	3.68	18.3
tyrosine	0.68	3.4	glycine	1.03	5.1
threonine	0.80	4.0	proline	1.32	6.6
tryptophan	0.21	1.0	serine	1.06	5.3

Appendix 2

CONTAMINANTS IN BASAL DIET FOR RATS

Sample of 1000 kg batch produced on March 10, 1978

lead		3.8 mg/kg
cadmium		0.07 mg/kg
mercury		0.02 mg/kg
tin		3 mg/kg
arsenic		0.1 mg/kg
selenium		0.14 mg/kg
organo-P-compounds		not detectable*)
malathion		0.02 mg/kg
organo-Cl-compounds		not detectable
dithiocarbamates		not detectable
aflatoxin B_1		not detectable
oestrogenic activity (Tiecco test)		negative
3,4-benzpyrene		2.8 µg/kg
3,4-benzfluoranthene		3.3 µg/kg
indeno-1,2,3,c,d-pyrene		2.8 µg/kg
1,2-benzfluoranthene		1.7 µg/kg
fluoranthene		25 µg/kg
K-nitrate		314 mg/kg
Na-nitrite		2 mg/kg
nitrosodimetylamine		3.2 µg/kg
nitrosopyrrolidine		1.2 µg/kg
other nitrosamines		not detectable
urease activity		0.05 Δ pH
trypsin inhibitor		0.3 % inhibition
haemagglutinins	positive dilution	1 : 20
	negative dilution	1 : 50

*) see list of compounds detected and lower detection limits

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Appendix 3

CONTAMINANT ANALYSES

Compounds detected and lower detection limits

Organochlorine pesticides and PCB's (in mg/kg)

HCB	0.005	aldrin	0.01
α-HCH	0.01	dieldrin	0.01
β-HCH	0.02	endrin	0.02
γ-HCH, lindane	0.01	methoxychlor	0.05
heptachlor	0.01	p,p'-DDE	0.02
heptachlorepoxide	0.02	p,p'-TDE	0.03
α-chlordane	0.02	o,p'-DDT	0.03
γ-chlordane	0.02	p,p'-DDT	0.04
		PCB's	0.30

Organophosphorus pesticides (in mg/kg)

chlorpyrifos	0.01	malathion	0.01
diazinon	0.01	mevinfos	0.01
dichlorvos	0.01	parathion	0.01
fenithrothion	0.01	sulfotep	0.01

Dithiocarbamates (in mg carbondisulphide/kg)

maneb	0.5	ziram	0.5
zineb	0.5	mancozeb	0.5
nabam	0.5	thiram	0.5
ferbam	0.5		

Aflatoxin-B_1 0.01 mg/kg

Polycyclic aromatic hydrocarbons (in µg/kg)

3,4-benzpyrene	0.2
3,4-benzfluoranthene	0.2
indeno-1,2,3,c,d-pyrene	0.4
1,2-benzfluoranthene	0.4
fluoranthene	1.0

Nitroso compounds (in µg/kg)

nitrosodimethylamine	0.1
nitrosodiethylamine	0.2
nitrosomethylpropylamine	0.4
nitrosomethylbytylamine	0.2
nitrospyrrolidine	0.6
nitrosopiperidine	0.3
nitrosomorpholine	3

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MID No. (7) — 19 D & B NO. (11) —	20
Contractor —	21
Doc Type • RI • UP • HE A.SD 8.D. S.U H.S F.N	22
Doc Title	23 18-MONTH INHALATION CARCINOGENICITY STUDY OF BETA-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

1A

3/1 BS VS.

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REPORT NO. R 6330

AN 18-MONTH INHALATION CARCINOGENICITY STUDY OF β-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

(Draft)

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At the request of : Joint Industry Committee on Chloroprene

Date : January 1980

Project number : B76/4975

Approved by : Dr. V.J. Feron

Start of exposures : September 30, 1976

Termination of exposures : March 29, 1978

Study director : Drs. P.G.J. Reuzel

All raw data and the final report are filed in the archives of the Department of Biological Toxicology under the reference "Joint Industry Committee on Chloroprene".

Gehele of gedeeltelijke publikatie van dit rapport is zonder schriftelijke toestemming verboden.
Total or partial publication of this report without written assent is not allowed.

23e-5000-1075

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SUMMARY

1. An 18-month inhalation carcinogenicity study with chloroprene was performed in hamsters. Three groups of 100 male and 100 female hamsters each were exposed to 0 (control group), 10 or 50 ppm chloroprene, 6 hours/day, 5 days/week, for a period of 18 months.

2. No abnormalities were observed in behaviour.

3. Mortality was lower in both test groups than in the controls.

4. Growth retardation was observed in males and females of the high-level group.

5. A slight reduction in amyloidosis was observed in hamsters of the high-level group.

6. At the 10 ppm level no adverse effect was found.

7. No indications were found for carcinogenic properties of chloroprene up to an exposure level of 50 ppm.

AN 18-MONTH INHALATION CARCINOGENICITY STUDY OF β-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

1. INTRODUCTION

At the request of the Joint Industry Committee on Chloroprene, a chronic (18-month) inhalation study with β-chloroprene was performed in hamsters. The exposure levels were selected on the basis of the results of a previous 4-week inhalation study with chloroprene in hamsters, in which compound-related effects were found at levels of 627, 162 and 39 ppm (Reuzel et al. 1976). The effects were, mortality, nose and eye irritation, incontinence of urine and faeces, microscopical changes in the respiratory tract and the liver and changes in relative weights of some organs. Since the effects observed at 39 ppm consisted of slightly abnormal behaviour and a minimal degree of nose and eye irritation, 50 ppm was selected as high-dose level for the present study, expecting that this dose would induce mild, but distinct toxicity. The low exposure level was fixed at 10 ppm.

2. PROCEDURES

2.1 Material and methods

Twelve bottles, each containing 200 ml freshly purified 1,3-butadiene, 2-chloro, synonym: β-chloroprene (further indicated as chloroprene), were received weekly from Bayer AG, Dormagen, W.-Germany, and stored at -20°C.

To generate the various test atmospheres, sufficient quantities of the test material were delivered to fritted glass vaporization vessels kept at 0°C. A measured, dried and filtered nitrogen flow was passed through the vessels. Each of the vapour/nitrogen mixtures thus produced was led into the inlet piece of an inhalation chamber, where it was mixed with the main air flow. Teflon and stainless steel transport tubes were used.

2.2 Analyses

Samples of the test atmospheres were taken from each inhalation chamber during the exposures (average three samples/hour) by means of a sample loop and then injected into an Intersmat IG 120 gas-chromatograph fitted with a flame ionisation detector. The following operating conditions were established:

Column:		300 x 4 mm parked with Carbowax 20M/chromosorb PAW
Temperature:	a. Injection port	95° C
	b. Oven	65° C
	c. Detector	150° C

Exposure chamber concentrations of chloroprene were established by comparing the peak areas produced by samples of the atmosphere with those produced by a standard chloroprene solution. The sample loop was calibrated by comparing peak surfaces of atmosphere samples taken with a gas-tight syringe with peak surfaces of simultaneaously taken loop-samples.

2.3 Animals

Three hundred male and three hundred female Syrian golden hamsters were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, in September 1976. On arrival, they weighed 35-55 g and were four weeks old. After an acclimatization period of two weeks the animals were allocated equally, according to body weight and sex, to three groups of 100 males and 100 females each.

2.4 Exposure chambers

The chambers used had a capacity of 2.5 m^3 and were constructed of stainless steel with a glass door, which allows observation of the animals during the exposures. The chambers had a cylindrical form with a conical top and bottom. Inside they were fitted with a heptagonal frame, which was rotatable around the vertical axis of the chamber.

Forty two wire mesh cages, each with a floor surface of 930 cm^2, were suspended in the rotatable frame in seven vertical rows and six horizontal layers. The position of the rows was changed each day in a fixed order. The position of the animals in the layers was changed once every two months. The chambers were illuminated externally by normal laboratory TL-lighting, resulting in an intensity of light inside the chamber varying from 1 - 7 lumen, dependent on the position of the cage in the chamber. The light cycle was 12 hours light and 12 hours dark (light from 06.00 a.m. to 06.00 p.m.).

To prevent leakage of the test atmospheres into the room, the chambers were maintained at a negative pressure of 1-2 mm H_2O, relative to the room. The main airflow was drawn from the room, entered the chambers at the top, and had - when entering the inhalation chambers - a temperature of 21 $\pm$ 2° C and a relative humidity of 45 - 60 per cent. Before entering the inhalation chambers the main airflow was mixed with a nitrogen flow which was laden with the test material. In the chambers the airflow was equally spread by an air disseminator which was located in the top of the upper cone of the chambers.

The flow rate through the chambers was approximately 40 m^3/h during the exposures and 60 m^3/h thereafter.

2.5 Maintenance

All animals were housed in the inhalation chambers during 24 hours a day. For each group all conditions were the same, except for the concentration of the test material in the atmosphere during the exposures. Five males or five females were housed in each cage.

Neither food nor water was present in the inhalation chambers during the exposures. During the non-exposure periods the hamsters were provided with bottled unfluoridated tap water and a pelleted basal diet (Muracon I, Trouw & Co. N.V., Amsterdam - Putten, The Netherlands). The levels of contaminants in tap water were determined periodically (Appendix I).

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2.6 Exposures

Groups of 100 hamsters of each sex were exposed to one of three different atmospheres, for 6 hours a day, 5 days a week during a period of 18 months. The nominal concentrations were 0, 10 and 50 ppm chloroprene. The exposures were generally started between 08.30 and 10.30 a.m. On public holidays the animals were not exposed.

2.7 Conduct of the study

2.7.1 Condition and behaviour

All animals were checked daily and all changes were recorded. Special attention was paid to the occurence of tumours. During the last seven months of the test period all animals were palpated monthly for the presence of tumours. Time of appearance, size, location and progression were recorded. Moribund animals were killed to avoid spontaneous death and subsequent loss by autolysis or cannibalism.

2.7.2 Body weights

All animals were weighed immediately before the first exposure, weekly during the first 8 weeks and then at 4-week intervals. Animals loosing more than 10 per cent weight between two weighings were observed daily, until their weight gain was normal or they were sacrificed.

2.7.3 Pathology

After the 18-month exposure period the animals were sacrificed on 13 successive working days, in such a way that, on the average, time of sacrifice was approximately the same for each group. The hamsters were killed by exsanguination from the abdominal aorta under ether anaesthesia. A post-mortem examination was conducted during which the tissues were examined for gross abnormalities. The following organs were weighed *):

spleen	testes
adrenals	ovaries
kidneys	brain
liver	pituitary
heart	lungs with trachea and larynx

*) Organs of animals which died spontaneously or were killed in extremis were not weighed

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Samples of the organs weighed and of those mentioned below were preserved in a 4 per cent aqueous neutral phosphate-buffered formaldehyde solution. The lungs were fixed (after weighing) by intratracheal infusion of the fixative under 10 cm water pressure

skin	thymus
external auditory canal with ceruminal (Zymbal's) glands	uterus
skeletal muscle	nose (four transverse sections)
sciatic nerve	eyes (with attached optic nerve)
mammary glands	sternum (bone marrow)
seminal vesicles	oesophagus
prostate	thyroid (with parathyroid)
epididymides	stomach
caecum	duodenum
colon	jejunum
pancreas	ileum
lymph nodes (axillary, cervical and mesenteric)	adipose tissue
urinary bladder	submaxillary salivary glands
spinal cord	exorbital lachrymal glands
femur (with knee joint)	parotid salivary glands
all gross lesions	sublingual salivary glands

The organs and tissues to be examined microscopically were processed to paraffin wax, sectioned at 5 µm and stained with haematoxilin and eosin. Nose, sternum and femur were decalcified prior to processing to paraffin wax. Transverse and longitudinal sections of the trachea including the bifurcation and longitudinal sections of the larynx and of each of the pulmonary lobes were prepared at three different levels.

Microscopic examination was carried out

a) of all organs and tissues preserved from all control and all high level hamsters and
b) of the liver, spleen, pituitary, thyroid, adrenals and of all grossly visible tumours or tumour-like lesions from hamsters of the low-level group.

Attention was paid to detection of possible metastases from malignant tumours.

2.7.4 Statistical analyses

Statistical analyses of body weight changes and organ-to-body weight ratios were carried out using Student's t-test. The Chi-square test was used for analysing differences in the incidence of alopecia, of mortality and of pathological changes, including tumours.

3. RESULTS

3.1 Exposure period

The nominal exposure period extended for 18 months, from 30 September 1976 to 30 March 1978. The subsequent autopsies were carried out over a period of 17 days, during which the exposures were continued, so that the last exposure day was 17 April, 1978. The nominal exposure period comprised 385 planned exposure days. As a result of maintenance and repair work to the inhalation chambers on one of the planned exposure days exposure could not take place: therefore the actual number of exposure days was 384 for both test groups.

3.2 Chloroprene concentrations (table 1)

The actual mean concentrations of chloroprene in the test atmospheres during all the test period were 10.0 and 49.7 ppm for the low- and high-level group respectively.

3.3 Condition and behaviour (table 2)

No abnormalities were observed in behaviour in any of the groups. Alopecia was only occasionally seen in each of the groups during the first 64 weeks of the study. During the next weeks increasing numbers of hamsters were seen with more or less diffuse alopecia of back and sacral region. In week 68 and at the end of the experimental period all animals were examined for alopecia. Because of the considerable differences in mortality between the groups,not only the number of hamsters showing alopecia was given, but also the incidences of the lesions expressed as percentages of the number of animals examined (table 2).

From this table it appears that the percentages of animals affected varied considerably both at week 68 and at the end of the study. Moreover, the differences between the test groups and the control group could not be confirmed by the Chi-square test. Therefore, chloroprene exposure was not considered to play a role in the development of the alopecia observed.

3.4 Mortality (table 3)

The death rate was negatively correlated with the concentration of chloroprene. In the high level group mortality started at a considerably later point of time and was clearly lower than in the controls, the differences being more pronounced in females than in males. In the low level group the differences with the controls were less pronounced and only statistically significant in females towards the end of the study.

3.5 Body weights (table 4; figures 1 and 2)

Slight growth depression was observed in hamsters of the high level group throughout the study. Body weight of the low level hamsters were similar to or differed only very slightly from those of the controls. A wave like course of the body weight curves (figures 1 and 2) occurred in all groups. This phenomenon is quite normal in hamsters, and is very probably controlled by seasonal factors.

3.6 Organ weights (table 5)

Relative brain weight was statistically significantly higher in hamsters of the high level group than in the controls. Relative lung weight was slightly, but statistically significantly higher in males of the high level group than in the controls. A few isolated statistically significant changes of relative organ weights were: low relative weights of kidneys, liver and adrenals in males of the low-level group and a low relative spleen weight in males of the high-level group.

3.7 Pathology

Macroscopic examination (table 6)

Macroscopic examination at autopsy revealed a slight, dose-related decrease in incidence of pale adrenals in males. No other remarkable differences in gross changes were noticed between the various groups.

Microscopic examination (table 7 and 8)

a) Non-neoplastic lesions

The site, type and incidence of non-neoplastic changes observed in hamsters of the control and high-dose group are presented in table 7. The incidence of generalized amyloidosis was slightly lower in the high-dose group than in controls. Amyloid deposits in the liver, kidneys, spleen and adrenals were less frequently observed in high-dose animals than in controls, the differences often being statistically significant.

Several other differences between control and high-dose groups were statistically significant. These differences, however, were small and the alterations in question were of a minor degree, and are common in aged Syrian golden hamsters of the strain used. Therefore, the differences are considered not to be related to chloroprene exposure but rather to the large variability in incidence of this type of lesions.

b) Neoplastic lesions

The site, type and incidence of the neoplastic lesions found in the various groups are presented in table 8. The incidence of cystadenomatous polyps of the gall-bladder was slightly elevated in the males of the low-dose group. All other tumours observed were about equally distributed among test and control groups, or occurred only once or twice. Moreover, they are all common neoplastic lesions as is shown in recent papers on the background pathology in the strain of hamsters used (Pour et al. 1976, a, b, c and d).

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4. DISCUSSION AND CONCLUSIONS

During the previous 4-week range-finding inhalation study with chloroprene in hamsters severe toxic effects were found at levels of 627 and 162 ppm in air (Reuzel et al. 1976). The changes observed at a level of 39 ppm were only very slight and consisted of minimal irritation of the mucous membranes of the nasal cavity, transient restlessness and some eye irritation. On the basis of these results it was expected that in a chronic inhalation study with hamsters 50 ppm chloroprene would be an effect level, but would not induce marked toxicity. This expection was confirmed by the present chronic study from which it appeared that at the 50 ppm level a slight reduction in body weight gain occurred.

Though in the 4-week study with hamsters (Reuzel et al. 1976) one animal exposed to 162 ppm chloroprene showed severe alopecia and moreover, hair loss as a result of chloroprene exposure has been observed in rats (Reuzel et al. 1979) and also in men (Nyström, 1948; Ritter and Carter, 1948; Swartz,1945), the results of the present study do provide evidence of chloroprene inducing or enhancing the formation of alopecia in hamsters.

The increase in relative brain weight in the high-level group was not accompanied by treatment-related histopathological changes in this organ. Since it is known that in rats an inverse relationship exists between body weight and relative brain weight (Feron et al. 1973),it is most likely that the increase in relative brain weight in high-level hamsters is the result of the relatively low body weight of these animals rather than the expression of an effect of chloroprene on this organ.

A few isolated statistically significant changes in relative organ weight in no case were accompanied by compound-related histopathological changes. They were, therefore, regarded as fortuitous findings unrelated to chloroprene.exposure.

The pathological examinations revealed some indications that high-level hamsters were less affected by generalized amyloidosis than the controls. This finding may have some relation to the better survival of the high-dose animals as compared to the controls. In the 4-week study with chloroprene (Reuzel et al. 1979) of the lining epithelium of the nasal cavity was found in some hamsters exposed to 39 or 162 ppm of the test substance. In

the present study there was no evidence of chloroprene exposure affecting the epithelium of the nasal mucosa.

In conclusion, the present study in hamsters provides evidence that exposure to 50 ppm chloroprene resulted in:

a. slight depression of body weight gain;
b. better survival;
c. slight reduction of amyloidosis

At the level of 10 ppm no effect of chloroprene was detected.

There was no evidence of chloroprene p ocessing carcinogenic activity in Syrian golden hamsters.

5. REFERENCES

1. Feron, V.J., A.P. de Groot, M.T. Spanjers and H.P. Til
   An evalution of the criterion "organ weight" under conditions of growth retardation
   Food Cosmet. Toxicol. 11 (1973) 85-94

2. Nyström, A.E.
   Health hazards in the chloroprene rubber industry and their prevention
   Acta Med. Scand. 132 (1948) Suppl. 219, 1-125

3. Pour, P., N. Kmoch, E. Greiser, U. Mohr, J. Althoff and A. Cardesa
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 a.) 931-935
   I. Incidence and sites

4. Pour, P., U. Mohr, A. Cardesa, J. Althoff and N. Kmoch
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 b.) 937-948
   II. Respiratory tract and digestive system

5. Pour, P., U. Mohr, J. Althoff, A. Cardesa and N. Kmoch
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   J. Nat. Cancer Inst., 56 (1976 c.) 949-961
   III. Urogenital system and endocrine glands

6. Pour, P., U. Mohr, J. Althoff, A. Cardesa and N. Kmoch
   J. Nat. Cancer Inst., 56 (1976 d.) 963-974
   Spontaneous tumors and common diseases in two colonies of Syrian hamsters
   IV. Vascular and lymphatic systems and lesions of other sites

7. Reuzel, P.G.J., W. Tichelman, E.R. Immel and V.J. Feron
   Sub-acute (4-week) inhalation toxicity study with β-chloroprene in hamsters
   Central Institute for Nutrition and Food Research TNO
   Report number R 5085

8. Reuzel, P.G.J., M.C. Bosland, H.C. Dreef van der Meulen and V.J. Feron
   A (2-year) inhalation carcinogenicity study of chloroprene in rats
   Central Institute for Nutrition and Food Research TNO
   1980 Report number R 6077

9. Ritter, W.L. and A.S. Carter
   Hair loss in neoprene manufacture
   J. Ind. Hyg. Toxicol. 30 (1948) 192-195

10. Schwartz, L.
    Skin hazards in the manufacture and processing of synthetic rubber
    J. Am. Med. Assoc. 127 (1945) 389-391

We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedure herein described, and that this report represents a true and accurate record of the results obtained.

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Table 1. Actual mean chloroprene concentrations, their standard deviation and standard error of the mean during 18 months

Group	Mean concentration of chloroprene					
	in ppm			in mg/m^3 air		
	Mean	SD[1)]	SEM[2)]	Mean	SD	SEM
Low dose	10.0	0.3	0.0	36.7	1.2	0.1
High dose	49.3	1.3	0.1	182.4	4.9	0.2

[1)] SD = Standard deviation

[2)] SEM= Standard error of the mean

Table 2. Degree and incidence of alopecia

Degree of alopecia	Number of hamsters with alopecia					
	males			females		
	chloroprene concentration (in ppm)			chloroprene concentration (in ppm)		
	0	10	50	0	10	50
	week 68					
a. slight	23	16	26	32	45	33
b. moderate	5	2	4	7	3	18
c. severe	1	0	0	0	0	1
total incidence	29	18	30	39	48	52
	at terminal sacrifice (week 79-81)[1]					
total incidence	32	20	28	17	16	24
	Incidence of alopecia expressed as percentage of the number of animals examined					
	week 68					
a. slight	25	17	27	41	54	35
b. moderate	5	2	4	9	4	19
c. severe	1	0	0	0	0	1
total incidence	32	19	31	49	58	55
	at terminal sacrifice (week 79-81)					
total incidence	37	22	31	27	21	41

1) The degree of alopecia was not recorded at autopsy

Table 3. Cumulative mortality in groups initially consisting of 100 male and 100 female hamsters

Chloroprene concentration (in ppm)	Total number of deaths at the end of week																	
	4	8	12	16	20	28	36	40	44	48	52	56	60	64	68	72	76	7
Males																		
Control	0	1	5	6	6	6	6	7	7	7	7	7	8	8	9	10	11	1
10	1	2	3	3	3	3	3	3	3	4	4	4	4	4	5	5	8	
50	0	0	0*	1	1	2	2	2	2	2	3	3	3	3	3	6	7	7
Females																		
Control	0	0	0	1	2	3	3	3	3	4	7	10	13	16	21	30	37	37
10	0	0	0	0	0	0	2	4	7	8	9	10	11	12	17	20	23*	2
50	0	0	0	0	0	0	0	0	0	0*	1*	3*	4*	5*	6**	11***	17*	2

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$

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Table 4. Mean body weights of groups initially consisting of 100 male and 100 female hamsters

Chloroprene concentration (in ppm)	Mean body weights (in g) at the end of week													
	0	1	4	8	12	17	24	32	40	48	56	64	72	78
Males														
0	83	91	103	109	111	112	107	105	104	108	108	90	94	101
10	83	92	104	109	111	109	107	102	101	105	107	98***	101**	101
50	83	89	101	107	106*	106**	99***	98**	97***	99***	99**	91	91	94***
Females														
0	84	97	112	120	124	130	127	117	115	117	117	102	95	99
10	85	94	110	120	127	131	128	115	110	111**	112*	102	95	98
50	85	91***	104**	111***	111***	116***	110***	99***	100***	106***	99***	90***	86***	90***

*$P \leq 0.05$; **$P \leq 0.01$; ***$P < 0.001$

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Table 5. Mean body weights (g), mean relative organ weights (g/100 g body weight) and the standard error of the mean of groups initially consisting of 100 female hamsters

group no.	β-chloroprene ppm	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	pituitary	adrenals	lungs	number of survivors
					MALES							
8168	0	101. (2.)	.523 (.008)	1.25 (.02)	5.11 (.11)	.197 (.005)	1.10 (.02)	2.30 (.05)	.0068 (.0003)	.0311 (.0012)	.85 (.01)	86
8169	10	101. (2.)	.507 (.006)	1.17** (.02)	4.75* (.09)	.190 (.007)	1.11 (.02)	2.21 (.05)	.0067 (.0003)	.0279* (.0008)	.84 (.01)	92
8170	50	93.*** (1.)	.526 (.006)	1.22 (.02)	4.91 (.11)	.174** (.005)	1.19*** (.02)	2.36 (.05)	.0078 (.0005)	.0294 (.0009)	.90** (.01)	92
					FEMALES							
8168	0	99 (2.)	.628 (.013)	1.48 (.03)	6.73 (.19)	.253 (.010)	1.13 (.02)	.036 (.001)	.0094 (.0004)	.0340 (.0011)	1.01 (.02)	60
8169	10	98 (2.)	.595 (.011)	1.50 (.03)	6.54 (.17)	.269 (.011)	1.16 (.02)	.036 (.001)	.0084 (.0004)	.0356 (.0016)	.97 (.02)	74
8170	50	90.*** (2.)	.625 (.011)	1.50 (.02)	6.37 (.18)	.286 (.049)	1.24*** (.02)	.042 (.004)	.0100 (.0009)	.0383 (.0038)	1.01 (.02)	72

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

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Table 6. Site, type and incidence of gross observations in survivors and non-survivors in the various groups

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
Initial number of animals	100	100	100	100	100	100
Effective number of animals	100	97	97	94	93	97
Mode of death:						
a) found dead	12	5	8	32	21	21
b) killed in extremis	2	2	1	5	5	6
c) scheduled sacrifice	86	93	91	63	74	73
Autolysis/Cannibalism:						
a) slightly autolytic	4	1	2	4	0	0
b) partially lost	8	3	3	27	14	17
c) completely lost	0	3	3	6	7	3
SKIN						
1. Scale	4	1	5	3	2	6
2. Alopecia	32	20	31	21	19	30
3. Abscess	1	0	0	0	0	0
4. Edema formation	0	0	0	0	0	1
AXILLARY LYMPH NODES						
1. Enlarged	2	0	2	0	0	1
PRAEPUTIAL / CLITORIAL GLANDS						
1. Enlarged	1	0	0	0	1	0
MAMMARY GLANDS						
1. Tumour or suspected of tumour	0	0	0	0	0	1
2. Evidence of secretory activity	0	0	0	2	3	1
ABDOMINAL CAVITY						
1. Tumour or suspected of tumour	0	0	0	0	0	1
2. Ascites	0	0	0	1	0	3
3. Haemorrhagic fluid	0	0	0	4	1	2
4. Peritonitis	2	0	1	0	0	0
SPLEEN						
1. Tumour or suspected of tumour	0	1	3	0	0	1
2. Splenomegaly	0	1	0	8	4	4
3. Atrophy	2	0	0	2	1	4
4. Granular surface	0	0	0	0	0	1
5. Pale	1	0	1	0	0	0
6. Enlarged	1	0	1	0	0	0

Table 6. Continued 1

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
7. Cyst	0	0	1	0	0	0
ADRENALS						
1. Tumour or suspected of tumour	8	4	5	3	7	5
2. Small - unilateral	0	1	1	0	0	1
- bilateral	1	1*	1**	0	0	1
3. Discoloured - pale	11	3	0	4	8	3
- dark	3	3*	0	7	6	2
4. Spotted	9	2	7	2	5	1
KIDNEYS						
1. Tumour or suspected of tumour	2	1	0	0	0	3
2. Granular surface	6	2	10	27	33	27
3. Discoloured - pale	16	9	10	36	31	30
- dark	0	1	0	0	2	0
- greenish	3	4	2	18	17	12
4. Cyst(s)	1	0	0	1	0	1
5. Enlarged	2	0	0	0	0	0
STOMACH						
1. Tumour or suspected of tumour	1	1	4	1	1	0
2. Thickened cardia	2	0	0	0	0	0
3. Haemorrhage / erosion	1	1	0	2	2	0
4. Trichobezoar	3	2	2	1	0	0
5. Diverticulum	0	0	0	0	2	0
SMALL INTESTINES						
1. Prominent Peyer's patches	0	1	0	0	0	0
2. Enlarged/swollen	0	2	0	2	1	0
3. Haemorrhagic fluid	0	0	0	0	1	0
4. Dark mucosa	1	0	0	0	0	0
5. Evidence of inflammation	0	0	0	0	1	0
COECUM						
1. Enlarged/swollen	1	0	0	3	2	3
2. Thickened wall	1	0	0	0	0	0
COLON						
1. Tumour or suspected of tumour	0	0	0	1	0	0

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Table 6. Continued 2

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
PANCREAS						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Dark	1	0	0	3	2	0
3. Hyaline aspect	0	0	0	1	0	0
4. Cyst	0	0	0	0	0	1
MESENTERIC LYMPH NODES						
1. Enlarged	1	1	2	3	1	1
2. Small	0	1	0	0	0	0
3. Greenish	0	0	0	0	1	1
URINARY BLADDER						
1. Proteïnaceous plug	1	0	0	0	0	0
SEMINAL VESICLES						
1. Evidence of inflammation	1	1	1			
2. Hyaline	59	57	61			
3. Cyst	0	1	0			
4. Enlarged	13	14	13			
5. Atrophy	8	4	7			
TESTES						
1. Atrophy - unilateral	9	9	10			
- bilateral	5	4	12			
2. Abscess	3	1	2			
3. Unilateral agenesis	5	3	4			
EPIDIDYMIDES						
1. Abscess	19	5**	11			
OVARIES						
1. Tumour or suspected of tumour				0	0	1
2. Cyst(s)				1	3	0
3. Enlarged				1	0	0
4. Haemorrhages				0	0	1

Table 6. Continued 3

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
LIVER						
1. Tumour or suspected of tumour	0	2	1	1	0	1
2. Prominent lobular pattern	11	5	6	5	6	11
3. Swollen	4	2	1	3	0	4
4. Discoloured - pale	2	1	6	4	0	5
- dark	2	2	1	0	4*	4*
- greenish	3	1	2	0	2	0
- yellow	0	0	0	0	0	1
5. Cysts	60	70	69	72	86	79
6. Small surface lesions (rosette, constriction)	0	0	2	2	1	0
7. White spots	1	0	0	1	0	0
8. Small right lateral lobe	0	1	0	0	0	0
GALL BLADDER						
1. Tumour or suspected of tumour	0	0	0	0	1	0
2. Enlarged	0	0	0	1	0	0
THORACIC CAVITY						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Hydrothorax	0	0	0	2	0	0
3. Haemothorax	0	1	0	4	0*	1
HEART						
1. Enlarged atrium (thrombus)	1	1	0	9	1**	1**
LUNGS						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Evidence of chronic respiratory disease	0	0	0	1	1	0
3. Spongy	1	1	0	9	6	7
4. Spotted	11	18	20	12	22	13
5. Atelectasis	0	0	0	1	2	0
6. Haemorrhage	1	0	0	3	0	1
7. Pleuritis	0	1	0	1	0	0
CERVICAL LYMPH NODES						
1. Enlarged	0	0	0	1	0	0

Table 6. Continued 4

Site and type of observations	Incidence of observations					
	Males			Females		
	Chloroprene concentration (ppm)			Chloroprene concentration (ppm)		
	0	10	50	0	10	50
SUBMAXILLARY SALIVARY GLANDS						
1. Tumour or suspected of tumour	0	0	1	0	0	0
2. Edema	1	0	0	1	0	0
EXORBITAL LACHRYMAL GLANDS						
1. Discoloured - pale	1	0	0	1	0	0
- dark	1	0	0	0	0	0
THYROID						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Pale	0	0	0	0	0	1
EYES						
1. Pale	1	0	0	0	0	0
BRAIN						
1. Pale	0	0	0	0	0	1
2. Increase in cerebro-spinal fluid	1	1	0	0	0	0
PITUITARY						
1. Tumour or suspected of tumour	0	0	0	1	0	0
2. Discoloured - pale	2	1	0	0	1	0
- dark	0	0	1	0	0	0
MISCELLANEOUS						
1. Small injuries in hind limb	0	3	4*	0	0	1
2. Cheek-pouch: evidence of inflammation	0	0	0	0	0	1

* $P < 0.05$, ** $P < 0.01$.

Table 7. Site, type and incidence of non-neoplastic histopathological changes in animals of the control and the high-dose group[1)]

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
Initial number of animals	100	100	100	100
KIDNEYS	(92)	(95)	(84)	(88)
1. Some focal infiltrates of mononuclear inflammatory cells	9	4	30	12***
2. Tubular nephrosis: - slight	63	62	20	25
- moderate	18	7*	37	43
- marked	4	3	26	19
3. Degenerated glomeruli: - a few	34	37	19	19
- several	8	10	33	35
- many	2	2	29	32
4. Some calcareous deposits in the inter-cortico-medullary layer and/or papilla	22	22	59	50
5. Dilatation of tubules: - slight	17	6*	24	27
- moderate to marked	10	5	23	21
6. Casts within tubules: - a few	20	12	40	32**
- several to many	5	4	0	8
7. Slight urothelial hyperplasia in the renal pelvis	0	2**	2	3**
8. Slight to moderate amyloidosis	14	3	44	27
9. Moderate urothelial degeneration	0	1	0	0
10. Moderate accumulation of proteinaceous droplets within tubular epithelium	0	0	2	0
11. Small cyst	0	0	1	0
12. Cortical infarct	0	0	0	1
13. Slight accumulation of yellow pigment in glomeruli	0	0	0	1
LIVER	(99)	(96)	(90)	(92)
1. A few periportal infiltrates of mono-nuclear inflammatory cells	13	9	18	7*
2. Slight periportal fibrosis	0	2	0	0
3. Slight to moderate vacuolation of liver cells: - centrilobular	2	0	1	0*
- focal	0	1	7	1
- diffuse	0	0	3	0
4. Slight focal necrosis	3	1	7	10
5. Proliferation of bile-ducts:				
- slight	39	31**	63	61
- moderate to marked	15	4*	20	24

Table 7. Continued 1

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
6. Foci of cellular alteration:				
- clear cell foci: - a few/small	2	3	22	20
- several/large	0	0	8	11
- one or a few basophilic foci	0	0	0	1
- one or a few eosinophilic foci	0	0	2	0
- one or a few mixed cell foci	0	0	0	2
7. Some RES-cell aggregates accompanied by necrotic hepatocytes	47	45	23	36
8. Moderate to marked centrilobular degeneration	0	0	2	2
9. Amyloidosis: - slight	9	6	41	29
- moderate to marked	7	1	15	16
10. Slight to moderate accumulation of yellow-brown pigment	32	27	83	81
11. Cyst(s) lined with flattened epithelium	47	53	73	71
12. Cyst(s) lined with cuboidal or cylindrical epithelium and showing papilliform outgrowths	0	0	1	1
13. Cholangiomatosis	3	8	5	2
14. A few-several infiltrates of polymorpho-nuclear inflammatory cells	1	3	1	0
15. A few giant cells	0	1	0	0
16. Abscess	0	1	0	0
17. Some extra-medullary haematopoiesis	1	1	1	0
18. Granuloma	2	0	0	0
19. Slight nuclear polymorphism	1	0	1	0
20. Moderate congestion	0	0	1	0
21. Slight focal calcification	0	0	1	2
GALLBLADDER	(84)	(80)	(67)	(72)
1. Cyst(s) - small	8	3	5	5
- medium to large	3	1	0	1
2. Cystic hyperplasia of epithelium:				
- slight	4	0	0	1
- moderate to marked	2	2	2	5
SPLEEN	(94)	(94)	(84)	(85)
1. Slight fibrosis of red pulp	3	1	2	1
2. Slight/moderate increased amount of yellow-brown pigment in red pulp	17	20	8	5
3. Increased extramedullary haematopoiesis:				
- slight	12	3	8	5
- moderate to marked	1	0	5	5
4. Depletion of the white pulp:				
- slight	9	9	21	12
- moderate to marked	13	9	16	14

Table 7. Continued 2.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
5. Slight to moderate atrophy	2	3	2	1
6. Amyloidosis: - slight	2	4	14	9
- moderate	0	1	20	15
- marked	1	1	10	10
7. (Haemo) cyst(s)	0	4*	0	0
8. Abscess	0	1	0	0
PANCREAS	(89)	(92)	(84)	(78)
1. Some focal infiltrates of mononuclear inflammatory cells	3	4	2	1
2. Slight periarteritis	0	0	1	0
3. Slight focal vacuolation of exocrine cells	0	1	0	0
4. Slight focal loss of zymogen granules	0	2	1	1
5. Slight change of acini into (dilated) duct-like structures	3	11*	13	15
6. Degeneration of exocrine part: - focal, slight to moderate	4	3	9	4
- severe fatty atrophy	1	0	1	0
7. Cyst(s)	0	0	0	1
8. Granuloma	1	0	0	0
LUNGS	(85)	(96)	(89)	(93)
1. Slight focal infiltrates of mononuclear inflammatory cells	2	2	7	0**
2. Pneumonitis: - focal	0	2	4	5
- massive	1	0	0	0
3. Severe pleuritis	0	0	1	0
4. Thrombi in bloodvessels	0	1	0	0
5. Some foci of alveolar macrophages	3	1	1	3
6. Heart failure lung: - slight to moderate	8	5	5	7**
- marked	2	1	14	4
7. Slight focal epithelialization	0	0	1	2
8. A few small calcareous deposits in alveolar tissue	10	10	18	29
9. Granuloma	1	2	0	0
10. Slight focal oedema	0	0	1	0
11. Slight focal proliferation of mucoid cells in bronch(iol)i	1	0	0	0

Table 7. Continued 3.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
TRACHEA	(84)	(94)	(83)	(86)
LARYNX	(73)	(71)	(68)	(79)
1. Some subepithelial infiltrates of mononuclear inflammatory cells	2	0	0	2
2. Slight focal epithelial hyperplasia	1	1	1	0
3. Slight laryngitis	1	0	0	0
HEART	(95)	(96)	(87)	(93)
1. Some focal infiltrates of mononuclear inflammatory cells	9	6	2	6
2. Slight periarteritis	1	0	0	0
3. Slight myocardial fibrosis	0	0	3	3
4. Slight valvular oedema and fibrosis	0	0	1	1
5. Slight to moderate focal myocardial degeneration	5	4	11	7
6. Thrombus in atria and/or ventricles	4	3	29	22
7. Slight focal endocarditis	1	0	0	0
8. Slight focal epicarditis	0	0	1	0
9. Slight accumulation of yellow-brown pigment	1	0	2	0
10. Ectopic ossification	0	0	2	2
11. Slight focal necrosis	0	0	0	1
12. A few cholesterol clefts	0	0	0	1
STOMACH	(85)	(89)	(75)	(82)
1. Some focal infiltrates of mononuclear inflammatory cells	7	2	1	0
2. Large ulcer	1	0	0	0
3. Slight focal degeneration of fundic glands	2	1	0	0
4. A few cystic,dilated fundic glands	1	4	10	10
5. Slight hyperkeratosis of the forestomach	1	1	1	0
6. A few calcareous deposits in fundic glands	0	1	0	0
7. Slight focal necrosis	0	0	1	0
SKELETAL MUSCLE	(93)	(95)	(90)	(90)
1. Some focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Slight focal degeneration	1	0	0	0

Table 7. Continued 4.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
TESTES	(95)	(94)		
1. Abscess and/or granuloma	4	3		
2. Obstructive dilated seminiferous tubules	0	3		
3. Mineralization of seminiferous tubules	1	1		
4. Atrophy of seminiferous tubules: - a few	19	16		
- several	12	10		
- many	8	7		
5. Complete atrophy: - unilateral	3	3		
- bilateral	2	3		
6. A few multinucleated giant cells	1	4		
7. Slight to moderate proliferation of interstitial cells	0	2		
8. Unilateral agenesis	2	3		
9. Some focal infiltrates of mononuclear and/or polymorphonuclear inflammatory cells	0	3		
10. Slight accumulation of yellow-brown pigment	0	1		
11. Degeneration and desquamation of germinal epithelium	3	0		
EPIDIDYMIDES	(94)	(91)		
1. Ducts filled with cellular debris: - a few	2	6		
- several to many	7	11		
2. Epididymitis: - slight	6	2		
- moderate to marked	7	8		
3. Abscess(es)	12	7		
4. Granuloma(s)	7	2		
5. Large, obstructively dilated ducts	8	4		
6. Unilateral agenesis	2	3		
7. Mineralization within ducts	3	1		
COAGULATING GLANDS	(91)	(89)		
1. Moderate to marked adenitis	3	0		
2. Many glands containing cellular débris	1	0		
3. Slight mineralization of glands	2	2		

Table 7. Continued 5.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
SEMINAL VESICLES	(94)	(91)		
1. Moderate to marked vesiculitis	3	1		
2. Moderate, focal degeneration of glandular epithelium	1	0		
3. A few to several glands containing cellular débris	0	4*		
PROSTATE	(91)	(85)		
1. Slight to moderate prostatitis	4	2		
2. A few to several glands containing granular material and/or cellular débris	17	10		
3. Slight mineralization within glands	6	8		
4. A few cholesterol clefts	1	0		
UTERUS			(79)	(86)
1. A few cystic,dilated glands, often containing cellular débris			39	28*
2. Endometritis			1	0
3. Dilated uterine lumen			1	0
MAMMARY GLANDS	(0)	(0)	(3)	(1)
AORTA	(91)	(84)	(86)	(90)
1. Thrombus	0	0	1	0
OESOPHAGUS	(87)	(91)	(79)	(86)
PHARYNX[2)]	—	—	—	—
1. Subepithelial abscess	0	0	0	1
2. Focal pharyngitis	0	0	1	0
3. Focal degeneration of epithelium	0	0	1	0
CHEEK POUCH[2)]	—	—	—	—
1. Focal metaplasia of epithelium	0	0	0	1
SMALL INTESTINES	(89)	(95)	(73)	(81)
1. Slight hyperplasia of patches of Peyer	4	0*	0	0
2. A few focal infiltrates of mononuclear inflammatory cells	1	0	0	0
COECUM	(89)	(95)	(72)	(81)

Table 7. Continued 6.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
COLON	(89)	(95)	(72)	(81)
1. Several cystic,dilated crypts	0	0	0	1
URINARY BLADDER	(92)	(92)	(77)	(86)
1. Proteinaceous plug	8	3	0	0
SPINAL CORD	(81)	(79)	(67)	(77)
SCIATIC NERVE	(62)	(72)	(64)	(70)
BRAIN	(93)	(94)	(79)	(86)
1. Focal calcareous deposits	0	0	4	7
2. Slight focal degeneration	0	0	1	0
3. Slight focal meningitis	0	0	1	0
PITUITARY	(62)	(70)	(48)	(57)
1. Cyst in pars distalis	0	1	0	0
2. A few focal infiltrates of mononuclear inflammatory cells	0	0	1	0
3. Moderate focal calcification	0	0	0	1
THYROID	(76)	(75)	(75)	(80)
1. Some focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Activated appearance	2	0	0	0
3. Slight accumulation of yellow-brown pigment	0	0	0	1
4. Amyloidosis: - slight	0	0	18	14
- moderate to marked	0	1	14	9
5. Slight focal proliferation of parafollicular cells	2	2	0	1
6. A few cystic,dilated follicles, often containing cellular débris	14	12	22	35
7. Slight to moderate focal degeneration	2	8	9	10
PARATHYROID [2]	—	—	—	—
1. Slight hyperplasia	0	1	0	0
2. Cyst	0	0	0	1

Table 7. Continued 7.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
ADRENALS	(93)	(93)	(88)	(91)
1. Unilateral atrophy	1	0	0	0
2. (Haemo)cyst(s)	2	0	7	10
3. Slightly increased vacuolization of cortical cells	2	0	1	0
4. Slight extramedullary haematopoiesis	5	3	0	0
5. Slight to moderate focal degeneration of cortical cells	4	5	8	19*
6. Amyloidosis: - slight	9	9**	6	17
- moderate	16	3	34	37*
- marked	5	2	43	29
7. Focal hyperplasia of the zona glomerulosa: - slight	30	32	13	6
- moderate	6	1	1	0
8. Slight accumulation of yellow-brown pigment	11	4	1	1
OVARIES			(74)	(85)
1. Follicular cyst			1	0
2. Haemorrhages			1	2
3. Focal granulosa and theca cell hyperplasia			1	0
PAROTID SALIVARY GLANDS	(91)	(90)	(77)	(88)
1. A few focal infiltrates of mononuclear inflammatory cells	0	0	2	0
2. Slight focal degeneration of glandular epithelium	0	0	3	0
3. Slight focal proliferation of ductules	0	0	2	0
4. Slight mucoid metaplasia	4	6	1	0
SUBMAXILLARY SALIVARY GLANDS	(93)	(91)	(80)	(92)
1. A few focal infiltrates of inflammatory cells	3	2	3	4
2. Slight focal degeneration of glandular epithelium	1	0	3	0
3. Slight focal proliferation of ductules	0	0	1	0
4. Abscess	0	1	0	0
SUBLINGUAL SALIVARY GLANDS	(68)	(62)	(60)	(70)
1. A few focal infiltrates of mononuclear inflammatory cells	0	0	0	1

Table 7. Continued 8.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
EXTRA ORBITAL LACHRYMAL GLAND	(96)	(89)	(77)	(85)
1. A few focal infiltrates of mononuclear inflammatory cells	0	2	5	0*
2. Slight dacryoadenitis	0	4*	5	4
3. Slight focal degeneration of glandular epithelium	2	2	0	0
EYES	(94)	(91)	(79)	(89)
1. Slight keratitis and/or conjunctivitis	3	6	2	2
THYMUS	(2)	(19)	(11)	(3)
1. Small colloid filled cysts	0	3	3	1
2. Slight proliferation of epitheloid cells	0	1	0	1
3. Involution: - slight to moderate	0	5	4	0
- marked	2	12	7	3
MESENTERIC LYMPH NODES	(74)	(71)	(68)	(66)
1. Slight accumulation of yellow-brown pigment	0	0	0	1
2. Dilated sinuses	0	0	1	0
3. Slight/moderate lymphoid hyperplasia	2	3	4	1
4. Slight/moderate sinushistiocytosis	22	17	22	19
5. A few amyloid deposits	1	0	0	0
6. Moderate atrophy	1	0	0	0
MEDIASTINAL LYMPH NODES	(24)	(16)	(17)	(25)
1. Slight accumulation of yellow-brown pigment	1	0	0	1
AXILLARY LYMPH NODES	(69)	(68)	(54)	(66)
1. Some focal fibrosis	0	2	0	0
2. Slight/moderate lymphoid hyperplasia	6	2	2	2
3. Slight accumulation of yellow-brown pigment	4	1	1	1

Table 7. Continued 9.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
CERVICAL LYMPH NODES	(70)	(75)	(67)	(73)
1. Slight-moderate accumulation of yellow-brown pigment	10	8	5	1
2. Lymphoid hyperplasia: - slight	11	21*	19	18
- moderate/marked	12	4	8	7
3. Slight erythrophagia	1	0	2	0
4. Cyst(s)	0	2	0	0
5. Dilated sinuses	0	0	0	1
6. Slight focal fibrosis	0	2	0	0
7. Marked lymphadenitis	0	0	0	1
SKIN/SUBCUTIS	(97)	(90)	(83)	(90)
1. Slight hyperkeratosis	0	0	5	0*
STERNUM	(78)	(91)	(85)	(85)
1. Increased ratio myeloid tissue/fatty tissue	0	1	0	0
ABDOMINAL WALL	(64)	(63)	(56)	(49)
1. Scar tissue	1	0	0	0
2. Peritonitis	0	0	0	1
ZYMBAL GLAND/EAR DUCTS	(77)	(85)	(65)	(75)
1. A few focal infiltrates of mononuclear inflammatory cells	1	0	0	0
2. Slight adenitis	0	0	0	1
DEPOT FAT	(77)	(61)	(60)	(69)
1. Focal fat necrosis	0	0	4	3
NOSE	(93)	(95)	(77)	(89)
1. The precense within conchae of exudate containing cell débris and/or inflammatory cells	15	20	30	30
2. Focal rhinitis	6	1	1	0
3. Slight sinusitis	0	0	2	0
4. A few subepithelial infiltrates of mononuclear inflammatory cells	1	0	0	0
5. Slight focal hyperplasia of goblet cells	1	0	0	0
6. Slight/moderate periodontitis	3	2	1	3
7. Slight/moderate odontodystrophy	3	2	1	1
8. Haemorrhage	0	0	0	1

Table 7. Continued 10.

Site and type of changes	Incidence of changes			
	Males		Females	
	Chloroprene concentration (ppm)		Chloroprene concentration (ppm)	
	0	50	0	50
FEMUR/KNEE JOINT	(84)	(71)	(70)	(81)
1. Callus	0	0	0	0
2. Slight/moderate increase ratio myeloid tissue/fatty tissue	0	1	5	3
3. Focal osteodystrophy	0	0	0	1

1) The number of animals from which the organ(s) menioned was (were) examined is given in brackets. Some animals or organs were nct examined because of too advanced autolysis or because of loss of autopsy material or cannibalism.

2) Occasional observations only are presented. Not all organs or sites were scored.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; X^2-test.

Table 8. INCIDENCE, SITE AND TYPE OF TUMOURS IN SURVIVORS AND NON-SURVIVORS AFTER AN EXPERIMENTAL PERIOD OF 18 MONTHS

Site and type of tumours	Incidence of tumours					
	MALES			FEMALES		
	chloroprene (in ppm)			chloroprene (in ppm)		
	0	10	50	0	10	50
Initial number of animals	100	100	100	100	100	100
Effective number of animals[1)]	100	97	97	94	93	97
Number of tumour-bearing animals[2)]	14	17	20	10	11	15
Total number of primary tumours[2)]	15	18	23	11	11	18
KIDNEYS						
1. Cortical adenocarcinoma	2	0	0	0	0	0
LIVER						
1. Neoplastic (hepatocellular) nodule	0	1	0	0	0	0
2. Unidentified tumour-like lesion[3)]	0	1	0	0	0	1
GALL BLADDER						
1. Cystadenomatous polyp.	1	6*	1	1	2	3
PANCREAS						
1. Islet cell adenoma	1	0	2	0	0	0
2. Islet cell adenocarcinoma	0	0	0	1	0	1
STOMACH						
1. Papilloma	0	0	2	0	0	0
2. Unidentified papilloma-like lesion[3)]	1	1	1	1	2	0
TESTES						
1. Leydig cell tumour	1	0	0			
COLON						
1. Adenomatous polyp	0	0	0	1	0	0
PITUITARY						
1. Adenoma	0	0	1	2	0	0
THYROID						
1. Parafollicular cell adenoma	2	0	0	0	2	1
2. Cystadenoma	1	0	0	0	0	0
3. Papillary adenoma	0	1	1	1	0	2
4. Follicular adenoma	2	1	0	1	2	1
PARATHYROID						
1. Adenoma	0	0	0	0	1	0

Site and type of tumours	Incidence of tumours					
	MALES			FEMALES		
	chloroprene concentration (in ppm)			chloroprene concentration (in ppm)		
	0	10	50	0	10	50
ADRENALS						
1. Cortical adenoma	4	1	10	0	0	3
2. Cortical carcinoma	0	1	0	1	0	1
3. Phaeochromocytoma	0	4*	2	0	0	0
4. Malignant phaeochromocytoma	0	0	2	0	0	0
OVARIES						
1. Granulosa-theca cell tumour				0	2	1
PAROTID SALIVARY GLANDS						
1. Adenoma	0	0	0	0	0	*
SKIN						
1. Unidentified tumour-like lesion[3)]	0	1	0	0	0	0
ZYMBAL GLAND						
1. Sebaceous adenoma	0	0	1	0	0	0
DEPOT FAT						
1. Lipoma	0	0	0	0	0	1
NOSE						
1. Adenoma of Bowman 's glands	0	0	0	1	0	0
2. Adenocarcinoma of Bowman 's glands	0	0	0	0	0	1
BONE (RIBS)						
1. Osteosarcoma	0	0	0	1	0	0
ABDOMINAL CAVITY						
1. Reticulum cell sarcoma	1	0	0	0	0	0

1) a few animals were not examined because of cannibalism or loss of autopsy material

2) some animals bore more than one tumour

3) macroscopically observed tumour-like lesion, but lost for microscopy

* $P<0.05$, χ^2 - test

Figure 1 Body weight curves of male hamsters

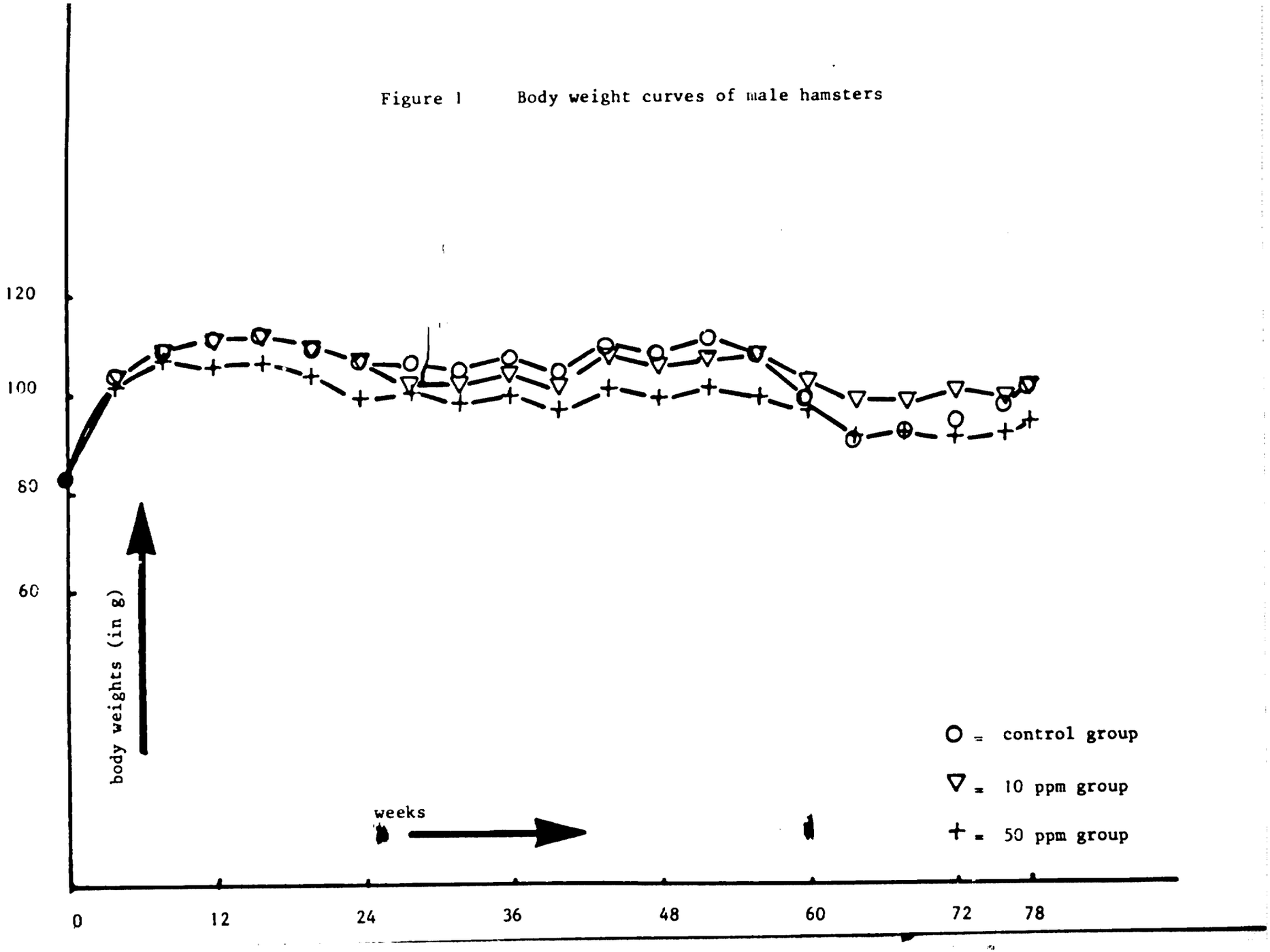

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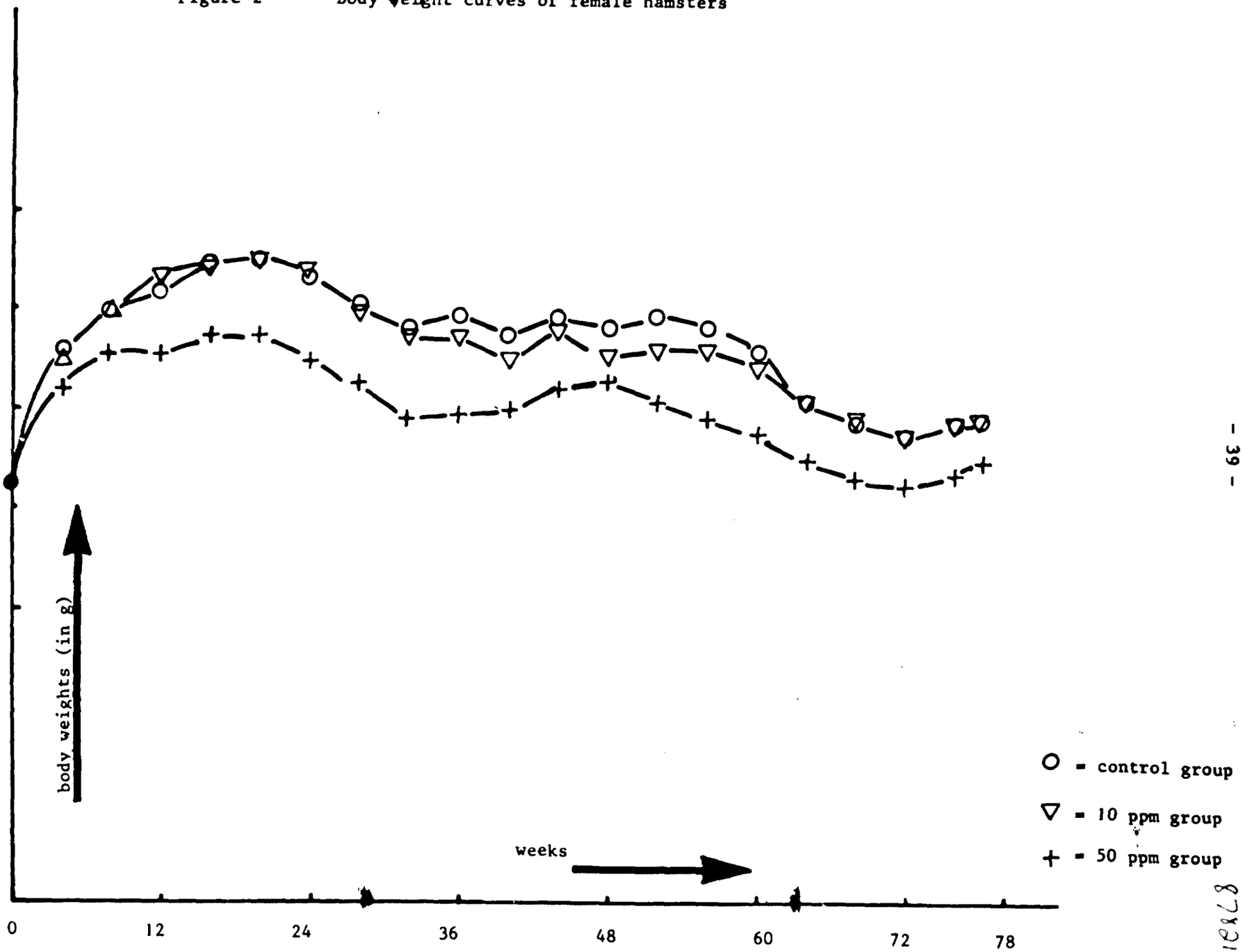
Figure 2 Body weight curves of female hamsters



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18 – MONTH INHALATION CARCINOGENICITY STUDY OF BETA–CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS APPENDICES

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

1A

3/11/85 VS.

CENTRAAL INSTITUUT VOOR VOEDINGSONDERZOEK TNO

CENTRAL INSTITUTE FOR NUTRITION AND FOOD RESEARCH

Utrechtseweg 48
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878215013

REPORT NO. 6328

AN 18-MONTH INHALATION CARCINOGENICITY STUDY OF β-CHLOROPRENE IN SYRIAN GOLDEN HAMSTERS

APPENDICES

INDIVIDUAL DATA

* no page 9

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Gehele of gedeeltelijke publikatie van dit rapport is zonder schriftelijke toestemming verboden.

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Appendix 1

Contaminants in tap water

CONTAMINANT ANALYSES

Compounds detected and lower detection limits

Organochlorine pesticides and PCB's (in mg/kg)

HCB	0.005	aldrin	0.01
α-HCH	0.01	dieldrin	0.01
β-HCH	0.02	endrin	0.02
γ-HCH, lindane	0.01	methoxychlor	0.05
heptachlor	0.01	p,p'-DDE	0.02
heptachlorepoxide	0.02	p,p'-TDE	0.03
α-chlordane	0.02	o,p'-DDT	0.03
γ-chlordane	0.02	p,p'-DDT	0.04
		PCB's	0.30

Organophosphorus pesticides (in mg/kg)

chlorpyrifos	0.01	malathion	0.01
diazinon	0.01	mevinfos	0.01
dichlorvos	0.01	parathion	0.01
fenithrothion	0.01	sulfotep	0.01

Dithiocarbamates (in mg carbondisulphide/kg)

maneb	0.5	ziram	0.5
zineb	0.5	mancozeb	0.5
nabam	0.5	thiram	0.5
ferbam	0.5		

Aflatoxin-B_1 0.01 mg/kg

Polycyclic aromatic hydrocarbons (in µg/kg)

3,4-benzpyrene	0.2
3,4-benzfluoranthene	0.2
indeno-1,2,3,c,d-pyrene	0.4
1,2-benzfluoranthene	0.4
fluoranthene	1.0

Nitroso compounds (in µg/kg)

nitrosodimethylamine	0.1
nitrosodiethylamine	0.2
nitrosomethylpropylamine	0.4
nitrosomethylbytylamine	0.2
nitrospyrrolidine	0.6
nitrosopiperidine	0.3
nitrosomorpholine	3

CONTAMINANTS IN TAP WATER

sample of January 1978

		ppb
lead	<	1
cadmium	<	1
mercury	<	1
arsenic	<	20
selenium	<	1
copper		10 [1]
chromium	<	10 [1]
zinc		10 [1]
cyanide		0.8

		ppb
dichloromethane	<	5
1,1-dichloroethane		-
chloroform		1
1,1,1-trichloroethane	<	1
tetrachloromethane	<	1
trichloroethene	<	1
tetrachloroethene	<	1
unknown	c.	1

		ppt
3,4-benzpyrene	<	5
3,4-benzfluoranthene	<	5
indeno (1,2,3-c,d) pyrene	<	5
1,2-benzfluoranthene	<	5
fluoranthene	<	10

		ppb
HCB	<	0.01
α-HCH	<	0.01
lindane	<	0.01
heptachlor	<	0.01
heptachl. epoxide	<	0.02
aldrin	<	0.02
dieldrin	<	0.03
α-chlordane	<	0.03
γ-chlordane	<	0.03
endrin	<	0.05
pp DDE	<	0.03
op DDT	<	0.06
pp TDE	<	0.06
pp DDT	<	0.10
β-HCH	<	0.03
PCB's	<	0.5

E. coli	in 50 ml	n.d. [2]
Lancefield D streptococci	in 10 ml	n.d.
coliforms	in 50 ml	n.d.

[1]) sample of October 1977

[2]) n.d. = not detectable

Appendix 2

Individual body weights

Coding system (animal house code)

Each group of 100 males or 100 females was subdivided into 10 subgroups coded A, B, C, D, E, F, G, H, K and L.

Each subgroup consisted of 10 animals coded (ear clip code) Z, R_1, R_2, L_1, L_2, R_1L_1, R_1L_2, R_2L_1, R_2L_2 and R_3.

Individual body weights Control group Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A																
Z	65	69	83	90	90	91	93	95	91	100	106	112	115	120	128	132
R1	73	78	86	97	100	107	111	111	109	114	112	119	107	114	112	108
R2	77	76	81	85	95	100	107	106	112	110	108	106	104	103	100	92
L1	85	85	104	115	119	125	123	120	119	117	111	108	106	102	101	100
L2	82	89	113	128	138	145	151	150	152	158	157	160	160	157	156	154
R1L1	83	94	100	106	111	113	118	122	125	128	129	130	131	125	121	116
R1L2	93	100	111	98	98	98	99	100	100	106	104	112	112	115	116	116
R2L1	98	108	111	118	122	119	119	117	118	118	120	123	124	128	128	126
R2L2	93	101	98	119	124	127	127	127	118	112	109	99	91	87	83	84
R3	96	100	90	89	94	95	97	97	102	104	103	106	105	107	110	114
B																
Z	61	70	76	82	86	90	91	90	90	98	98	104	105	110	110	108
R1	74	87	90	97	106	104	105	105	117	110	116	98	95	97	132	130
R2	78	92	88	94	102	107	109	109	104	100	101	118	123	131	94	92
L1	75	82	83	93	102	110	117	118	123	124	123	118	110	105	104	100
L2	81	97	97	106	109	112	114	117	116	117	124	124	130	137	139	140
R1L1	81	82	81	81	81	85	81	86	86	89	93	96	94	97	100	98
R1L2	79	93	93	96	101	99	101	97	98	102	104	112	107	110	112	109
R2L1	85	94	97	96	101	103	105	105	106	106	108	110	102	103	99	96
R2L2	90	88	90	92	94	95	95	97	99	102	103	106	111	103	117	118
R3	98	99	97	95	95	97	99	97	99	100	102	109	108	109	111	110
C																
Z	69	73	74	78	82	87	87	86	90	94	97	102	103	109	113	112
R1	76	93	93	105	111	119	126	131	137	144	147	154	150	151	150	148
R2	79	88	104	112	128	139	143	142	144	144	136	132	129	128	127	126
L1	79	86	90	93	102	109	109	113	117	122	121	123	119	117	108	96
L2	79	86	95	99	101	102	97	95	88	94	97	96	94	95	93	96
R1L1	83	87	93	100	107	114	117	119	121	126	127	126	123	121	117	114
R1L2	87	84	87	89	94	95	98	97	100	104	106	108	107	110	113	110
R2L1	94	87	83	86	89	87	89	90	90	94	95	99	98	102	104	106
R2L2	89	83	82	88	89	88	89	89	92	96	99	102	101	105	104	104
R3	95	91	97	98	99	101	104	103	106	110	110	112	95	83	87	92

Individual body weights — Control — Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	134	135	132	130	124	128	125	114	110	108	104	105	108	107	109	109
R1	107	109	106	104	99	104	102	96	96	99	102	104	107	106	110	106
R2	90	88	88	96	84	88	87	84	84	85	89	92	94	89	91	92
L1	99	98	100	102	99	104	97	98	97	100	106	104	107	105	107	107
L2	154	152	148	150	144	149	144	140	136	144	144	140	142	136	131	134
R1L1	114	107	109	108	104	107	115	106	105	108	105	106	107	109	108	108
R1L2	116	113	113	110	100	102	100	94	95	97	93	96	98	99	100	96
R2L1	123	122	121	118	112	115	112	105	102	100	99	106	111	104	115	111
R2L2	83	86	90	90	89	96	99	94	99	98	101	101	103	100	106	103
R3	113	114	113	112	112	114	118	112	110	110	99	96	99	99	102	99
B																
Z	98	100	100	98	94	93	95	89	89	90	82	82	94	90	89	89
R1	134	136	135	134	130	136	127	118	117	113	116	114	121	113	121	120
R2	94	95	97	100	95	102	103	102	107	110	109	122	127	121	122	125
L1	99	101	103	102	100	104	107	104	108	113	118	120	120	114	118	118
L2	142	148	154	156	152	158	155	148	149	145	138	144	143	141	148	148
R1L1	98	99	98	98	94	98	96	94	95	93	87	86	91	85	91	94
R1L2	118	113	114	114	112	113	114	112	108	106	99	92	102	100	104	105
R2L1	93	90	92	90	86	93	94	95	96	97	96	97	97	99	105	103
R2L2	112	116	115	118	115	118	115	115	111	108	99	106	112	110	112	111
R3	110	111	109	108	101	104	105	99	96	92	77	84	84	82	82	87
C																
Z	110	115	114	112	106	106	105	97	97	99	95	94	93	91	97	100
R1	142	138	134	132	125	128	126	125	132	134	129	122	116	111	118	117
R2	130	131	135	136	132	132	135	129	125	127	123	127	126	121	126	126
L1	97	99	97	98	95	96	95	92	95	95	93	92	94	89	89	93
L2	95	95	95	98	94	99	100	98	101	103	100	100	98	93	99	101
R1L1	116	114	112	112	107	112	116	115	115	116	118	112	109	109	113	107
R1L2	113	113	114	114	108	111	112	109	109	111	114	115	114	112	116	107
R2L1	106	107	108	108	103	114	104	101	103	101	94	94	94	92	95	97
R2L2	100	102	103	100	92	90	91	86	86	85	87	90	85	*13/6	77	-
R3	94	96	92	86	80	82	81	79	81	84	83	90	95	91	98	94

*DEAD

600009

Individual body weights — Control — Males

Week	52	56	60	64	68	72	76	78
A								
Z	113	114	98	89	96	99	102	105
R1	113	111	100	91	92	96	99	99
R2	97	92	76	70	65	65	71	77
L1	110	108	89	81	81	94	93	97
L2	140	141	122	107	113	119	127	132
R1L1	112	112	101	96	93	89	94	99
R1L2	101	98	84	79	81	78	82	97
R2L1	114	114	101	94	99	99	103	113
R2L2	105	104	88	86	86	82	80	87
R3	107	110	99	91	98	92	95	100
B								
Z	79	72	*14/11	77	-	-	-	-
R1	128	131	125	109	112	114	112	115
R2	125	111	115	118	115	115	107	106
L1	124	118	110	113	118	111	105	106
L2	150	165	149	139	133	131	128	127
R1L1	97	98	85	73	71	75	82	88
R1L2	108	111	99	89	81	79	80	81
R2L1	103	100	88	84	79	88	92	93
R2L2	111	112	96	88	95	99	107	108
R3	83	75	70	64	63	61	65	+25/3.78
C								
Z	103	102	94	85	88	90	95	94
R1	119	114	100	96	97	96	96	94
R2	125	120	104	99	102	110	116	119
L1	94	94	85	81	87	88	90	88
L2	103	94	85	79	81	88	88	90
R1L1	111	118	124	114	108	107	109	111
R1L2	125	116	110	108	109	109	114	118
R2L1	96	95	88	81	83	89	94	97
R2L2	-	-	-	-	-	-	-	-
R3	101	96	84	76	77	82	91	95

*DEAD

000010

Individual body weights — Control group — Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	77	93	97	94	103	104	101	94	88	82	76	80	76	79	78	77
R1	74	93	93	103	113	115	105	109	108	104	100	99	94	98	100	99
R2	74	89	99	110	119	124	127	127	125	128	128	125	123	123	123	122
L1	79	98	89	96	98	99	99	99	99	97	96	96	92	93	97	93
L2	88	87	95	105	113	111	119	111	110	102	95	90	89	93	92	94
R1L1	90	97	107	114	121	127	130	130	128	130	128	127	123	123	122	118
R1L2	84	90	96	101	105	108	108	106	104	102	102	103	99	100	94	96
R2L1	95	98	95	100	99	103	101	102	100	104	107	115	115	119	116	112
R2L2	91	92	93	95	97	100	108	107	108	112	114	118	120	123	116	117
R3	103	98	107	103	104	105	105	105	105	108	113	119	121	123	118	122
E																
Z	66	86	96	107	119	129	128	132	134	134	132	129	120	120	118	117
R1	68	82	79	80	83	83	84	83	86	86	86	91	92	94	100	100
R2	79	98	99	107	114	119	119	117	116	118	119	115	109	102	92	90
L1	69	88	88	90	90	98	100	102	104	110	110	115	117	120	122	126
L2	80	87	86	90	95	103	100	103	105	110	113	118	114	114	112	110
R1L1	95	103	107	110	108	107	109	115	120	126	130	138	140	144	138	146
R1L2	95	112	113	123	133	138	143	147	150	146	142	144	146	146	138	142
R2L1	92	87	87	89	90	92	95	101	101	101	111	114	118	122	121	124
R2L2	91	92	89	91	95	97	101	105	110	112	116	120	122	127	122	126
R3	111	122	126	133	138	142	141	140	138	139	148	145	144	143	138	136
F																
Z	58	79	83	87	96	101	103	103	102	103	102	97	96	92	88	89
R1	71	86	96	101	108	109	107	108	105	112	114	115	115	117	118	126
R2	71	82	89	92	98	103	106	108	108	116	118	118	119	116	114	116
L1	77	82	83	86	94	100	105	107	109	107	113	114	108	103	100	99
L2	74	78	76	78	81	89	89	93	95	101	107	109	111	116	114	114
R1L1	84	93	97	103	110	108	106	105	106	107	110	112	113	113	123	120
R1L2	89	81	81	83	83	84	85	85	86	90	92	96	98	103	108	107
R2L1	99	100	95	101	107	110	113	116	117	120	124	128	127	128	111	116
R2L2	80	92	93	97	101	101	103	104	104	105	108	110	111	112	114	116
R3	92	96	94	95	100	100	100	99	100	100	103	106	105	107	104	104

00001 11

Individual body weights — Control group — Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
D																
Z	80	80	84	82	82	84	88	79	84	84	88	88	89	87	92	89
R1	99	101	100	100	99	104	104	97	100	101	103	104	104	99	109	105
R2	120	121	121	122	119	124	124	124	117	119	121	124	125	122	126	124
L1	96	102	98	102	101	106	112	101	103	105	110	108	113	111	117	114
L2	96	95	99	98	98	104	105	95	99	99	103	102	107	102	107	107
R1L1	116	116	112	118	110	112	119	114	113	111	110	112	113	111	112	113
R1L2	94	95	94	92	89	91	91	90	91	90	89	88	91	88	90	92
R2L1	110	107	106	100	96	100	97	92	87	81	94	67	76	77	87	88
R2L2	118	117	113	112	105	108	104	99	100	102	106	108	115	114	120	116
R3	122	122	122	118	112	114	111	106	104	99	91	96	101	101	109	108
E																
Z	123	116	125	100	111	114	115	109	110	110	110	108	106	104	108	106
R1	99	99	99	99	89	93	90	81	77	76	76	84	89	89	91	95
R2	82	83	89	92	90	94	97	90	93	93	95	96	99	93	100	103
L1	130	129	129	130	125	130	134	122	121	121	114	112	113	117	120	120
L2	110	106	104	114	98	96	101	95	97	97	102	100	101	97	102	104
R1L1	148	151	148	148	145	150	145	140	141	138	130	136	138	134	135	132
R1L2	145	149	149	152	148	152	155	149	151	152	152	148	147	144	131	135
R2L1	130	131	133	132	129	130	130	122	125	124	116	110	107	102	105	106
R2L2	128	137	140	136	134	136	137	130	132	129	124	116	113	114	118	113
R3	136	139	144	146	144	147	150	145	147	150	152	150	149	147	149	145
F																
Z	85	86	88	88	88	91	100	98	99	103	102	102	103	96	103	100
R1	124	125	126	122	104	107	114	115	118	113	109	116	115	112	118	118
R2	116	115	115	112	121	126	128	129	113	114	115	114	110	104	111	110
L1	98	101	101	100	98	104	105	106	106	106	104	102	100	97	96	98
L2	114	114	113	112	106	106	108	108	107	105	105	100	101	98	104	106
R1L1	120	115	113	110	105	106	101	98	97	89	86	92	98	93	101	98
R1L2	111	110	113	114	106	108	110	109	108	109	97	97	96	97	101	102
R2L1	120	115	113	112	112	118	117	115	112	112	117	118	111	110	115	105
R2L2	118	116	122	121	116	120	120	116	109	101	87	90	94	94	106	100
R3	114	99	101	101	94	96	99	96	94	95	94	98	103	102	104	99

000012

Individual body weights — Control group — Males

Week	52	56	60	64	68	72	76	78
D								
Z	91	88	80	72	77	78	84	84
R1	109	106	94	86	91	96	104	103
R2	127	120	108	98	101	107	115	117
L1	115	110	106	102	106	111	112	119
L2	107	108	86	89	96	99	104	109
R1L1	125	124	114	95	93	81	88	95
R1L2	93	90	89	77	78	75	72	79
R2L1	93	90	78	63	61	64	66	68
R2L2	119	119	110	98	102	104	110	114
R3	110	114	110	96	90	100	105	105
E								
Z	104	92	84	79	89	92	96	*4/4.78
R1	100	94	82	76	73	78	79	86
R2	103	94	84	79	74	74	75	86
L1	119	119	108	94	98	99	99	107
L2	108	110	102	85	87	95	92	99
R1L1	138	128	128	128	128	127	127	135
R1L2	146	144	144	128	108	102	*3/3-78 -	
R2L1	119	120	116	108	108	107	108	106
R2L2	120	126	128	127	121	121	117	116
R3	147	148	144	140	134	137	137	138
F								
Z	106	104	90	79	79	82	87	92
R1	121	116	114	101	100	104	105	111
R2	108	104	96	88	83	89	86	82
L1	100	96	86	77	77	85	85	88
L2	111	118	104	86	98	100	100	105
R1L1	97	116	102	81	84	88	93	92
R1L2	104	106	93	81	81	90	97	99
R2L1	118	92	86	91	88	93	100	104
R2L2	107	100	83	71	76	80	86	89
R3	102	93	87	79	81	83	82	85

* DEAD

0000013

Individual body weights Control group Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G																
Z	47	51	60	66	68	73	70	74	74	84	90	92	92	95	88	86
R1	69	85	95	103	113	118	120	117	118	122	118	116	108	99	93	90
R2	69	77	86	94	101	107	111	111	110	114	114	114	110	107	100	100
L1	80	88	96	104	109	118	115	114	113	116	122	121	115	117	106	106
L2	80	96	105	110	115	122	122	119	116	116	116	118	112	116	110	112
R1L1	73	83	82	84	86	91	94	96	96	98	4/12* 76		-	-	-	-
R1L2	79	93	99	103	109	115	119	117	116	116	106	102	95	93	128	84
R2L1	81	100	109	112	117	120	121	124	125	126	122	121	125	125	128	132
R2L2	82	88	88	94	96	97	97	92	86	29/11* 76		-	-	-	-	-
R3	88	93	99	101	107	113	120	121	25/11* .76		-	-	-	-	-	-
H																
Z	61	71	71	72	77	78	78	81	78	80	83	80	82	86	75	89
R1	75	86	90	95	97	98	101	99	100	104	108	106	116	121	122	124
R2	77	98	100	98	102	102	97	101	100	105	106	106	112	112	118	114
L1	79	86	93	98	107	110	113	115	117	118	119	110	114	111	110	108
L2	75	85	87	89	93	94	93	93	92	98	100	98	110	105	106	110
R1L1	92	93	95	96	98	102	103	104	106	105	112	115	112	111	108	108
R1L2	95	89	90	93	94	95	99	100	100	2/12* .76		-	-	-	-	-
R2L1	91	104	101	102	106	107	110	110	110	110	119	118	91	22/12* .76		-
R2L2	85	97	101	105	106	118	115	113	108	107	101	92	27/12* .76		-	-
R3	100	110	119	121	126	128	129	128	130	124	118	114	106	102	95	98
K																
Z	68	78	90	97	104	112	113	115	116	116	118	118	102	110	118	112
R1	75	79	80	82	84	87	84	85	89	92	96	94	86	96	98	98
R2	78	89	92	93	92	94	95	97	100	104	110	110	101	114	120	120
L1	77	89	91	97	107	107	112	116	120	124	126	127	116	122	117	110
L2	86	96	100	97	116	124	126	129	134	134	131	128	118	126	123	122
R1L1	87	91	92	92	93	95	97	96	96	98	102	106	108	115	119	136
R1L2	83	88	88	95	97	99	101	105	106	108	115	116	124	124	134	136
R2L1	89	92	93	100	101	107	109	113	114	124	132	132	129	132	131	130
R2L2	99	103	108	113	116	118	116	117	113	114	102	93	93	93	96	98
R3	107	111	113	122	126	131	131	129	126	125	126	120	118	122	131	134

* dead

Individual body weights — Control group — Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
G																
Z	89	86	90	88	81	86	82	79	77	73	75	78	83	81	86	87
R1	92	90	95	94	92	96	98	94	96	95	96	94	98	98	103	102
R2	100	97	99	100	94	103	107	100	105	104	105	106	109	113	116	116
L1	104	103	106	104	102	106	105	102	103	101	104	102	103	104	108	105
L2	109	108	109	110	112	114	115	113	113	113	119	110	117	110	113	109
R1L1	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R1L2	86	86	83	88	88	93	96	94	97	98	103	104	106	101	111	108
R2L1	130	130	133	134	126	124	123	118	109	110	107	110	115	114	120	119
R2L2	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R3	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H																
Z	90	89	91	89	88	94	90	88	89	92	91	82	81	78	82	82
R1	128	132	134	135	134	137	139	137	136	136	137	128	119	114	116	115
R2	120	115	115	112	106	109	104	100	99	102	101	106	106	101	105	106
L1	108	107	107	107	102	110	110	112	110	115	119	120	112	106	108	103
L2	112	112	111	110	106	114	114	112	111	113	112	115	115	106	109	107
R1L1	106	106	103	100	94	93	94	87	86	86	92	94	97	96	96	100
R1L2	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R2L1	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R2L2	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R3	96	94	96	96	89	94	98	92	94	96	94	93	93	96	99	98
K																
Z	111	110	110	108	105	108	110	107	106	104	105	88	69	76	86	80
R1	100	98	97	100	96	98	97	95	91	89	85	82	86	87	92	90
R2	122	119	121	118	114	116	115	108	103	97	86	84	95	92	96	95
L1	108	104	102	99	98	100	115	100	102	100	99	103	105	102	112	108
L2	120	122	120	122	113	120	120	116	115	116	113	112	112	109	112	113
R1L1	124	125	126	126	120	121	118	116	113	113	112	112	119	115	119	120
R1L2	135	136	138	134	132	124	124	118	109	104	100	101	107	106	114	112
R2L1	126	124	117	115	110	115	120	121	123	125	121	125	128	126	128	126
R2L2	102	106	107	110	106	110	115	115	116	119	121	116	114	112	113	108
R3	140	140	142	143	142	142	145	146	145	147	145	132	133	128	131	130

0000015

Individual body weights — Control group — Males

Week	52	56	60	64	68	72	76	78
G								
Z	88	82	82	75	74	70	73	75
R1	110	104	91	78	80	89	95	95
R2	116	114	106	90	91	98	99	102
L1	119	99	88	83	91	94	95	97
L2	114	101	91	85	96	99	104	107
R1L1	-	-	-	-	-	-	-	-
R1L2	107	103	99	92	92	93	96	104
R2L1	123	122	118	106	103	106	112	114
R2L2	-	-	-	-	-	-	-	-
R3	-	-	-	-	-	-	-	-
H								
Z	85	84	74	64	66	68	72	*5/4.78
R1	120	106	104	84	91	96	99	102
R2	113	100	90	78	84	83	77	72
L1	112	104	96	84	87	90	93	97
L2	115	110	102	91	92	96	96	103
R1L1	101	94	86	80	76	85	93	98
R1L2	-	-	-	-	-	-	-	-
R2L1	-	-	-	-	-	-	-	-
R2L2	-	-	-	-	-	-	-	-
R3	97	98	90	88	91	93	96	98
K								
Z	84	76	78	68	*4/1.78	-	-	-
R1	93	95	88	72	76	86	90	85
R2	97	96	80	76	72	74	81	81
L1	112	104	98	86	98	100	106	107
L2	112	106	98	92	101	103	108	108
R1L1	122	128	122	109	108	105	108	113
R1L2	119	117	104	90	93	102	108	112
R2L1	128	118	108	100	105	109	111	117
R2L2	112	106	94	82	81	77	79	82
R3	130	132	124	117	118	114	112	121

000016

Individual body weights — Control group — Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L																
Z	75	81	105	115	122	127	125	125	126	130	132	130	128	120	115	110
R1	79	102	105	119	127	135	135	135	134	133	130	128	124	123	124	126
R2	75	83	80	83	81	83	83	83	86	90	93	95	97	99	105	112
L1	81	99	103	106	111	117	118	117	112	110	110	106	106	102	104	102
L2	83	96	93	99	107	113	113	116	118	124	126	128	126	123	125	120
R1L1	85	96	94	94	99	103	100	100	102	107	108	110	107	109	110	108
R1L2	83	91	92	94	99	105	107	105	104	106	108	112	107	110	110	110
R2L1	99	105	108	111	119	123	125	125	123	125	126	124	117	117	115	112
R2L2	93	97	100	101	104	109	107	110	110	112	115	115	112	113	114	110
R3	104	102	104	103	107	111	113	112	112	114	116	115	111	115	117	120

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L																
Z	108	103	108	108	104	104	111	107	111	114	119	120	123	116	120	123
R1	126	129	127	128	128	126	127	119	121	122	121	120	119	115	121	116
R2	108	110	111	109	106	106	113	99	95	93	81	83	85	84	87	89
L1	104	106	108	106	106	108	113	109	109	109	110	112	110	105	114	112
L2	116	111	113	112	108	107	110	108	106	108	108	110	105	104	110	111
R1L1	110	105	106	104	100	98	102	94	98	97	96	98	104	103	108	107
R1L2	116	116	125	126	121	128	124	119	113	112	110	100	105	101	106	107
R2L1	110	116	118	124	119	126	125	121	123	123	125	122	122	120	122	119
R2L2	114	111	115	116	112	114	115	109	110	110	110	108	113	108	112	109
R3	121	123	129	128	132	137	131	127	123	125	118	99	106	106	109	110

Week	52	56	60	64	68	72	76	78
L								
Z	120	114	98	92	100	108	113	117
R1	118	112	101	99	98	108	110	113
R2	95	90	84	78	77	75	72	76
L1	114	108	101	86	90	96	100	109
L2	101	112	106	87	83	93	94	96
R1L1	113	110	95	83	92	97	98	102
R1L2	111	102	104	88	82	84	87	101
R2L1	122	126	117	98	94	104	106	111
R2L2	115	113	106	100	103	105	104	110
R3	105	100	92	87	87	*20/1.78		-

000017

Individual body weights 10 ppm

Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A																
Z	56	63	63	64	68	66	67	65	65	67	70	75	77	80	80	78
R1	71	84	98	111	117	123	129	126	132	132	126	120	115	114	116	114
R2	82	93	90	98	104	105	109	109	115	116	121	120	116	113	111	112
L1	89	91	97	106	115	123	121	120	123	123	125	119	114	110	107	104
L2	89	100	108	108	110	106	104	102	102	105	113	114	122	123	123	120
R1L1	88	104	105	104	109	112	108	109	111	95	95	95	115	114	112	112
R1L2	80	80	86	85	87	89	89	90	90	93	91	94	99	102	103	104
R2L1	95	97	102	90	103	102	103	106	102	112	115	113	93	92	92	94
R2L2	94	104	118	118	123	125	123	122	125	121	118	115	119	115	112	112
R3	107	116	116	113	118	120	122	122	122	123	130	131	129	131	131	130
B																
Z	55	67	71	73	77	77	81	81	81	82	86	88	89	89	90	91
R1	67	82	94	103	114	122	123	125	133	128	136	128	128	120	120	118
R2	74	81	91	96	105	112	113	113	113	110	115	110	116	100	100	99
L1	75	79	77	87	86	89	86	90	91	91	99	100	104	104	108	109
L2	82	78	80	82	78	80	82	83	87	87	94	96	96	95	94	93
R1L1	75	97	96	107	111	117	118	117	118	119	116	111	105	102	102	100
R1L2	79	93	76	76	81	81	83	93	86	96	98	94	94	94	100	109
R2L1	86	99	99	101	104	105	107	108	110	111	110	113	116	116	119	117
R2L2	79	78	77	96	99	95	95	83	93	89	90	93	97	97	100	98
R3	88	96	99	96	101	102	104	103	107	107	109	108	109	108	106	108
C																
Z	65	81	89	89	91	92	97	95	100	100	102	115	100	104	106	111
R1	74	89	97	97	97	101	100	101	103	106	108	111	109	114	117	128
R2	78	96	98	104	111	116	120	117	117	122	122	122	116	126	127	130
L1	79	91	89	90	98	101	99	102	107	113	113	115	111	102	108	108
L2	81	89	93	88	91	93	99	105	98	101	98	114	96	100	106	110
R1L1	85	100	108	101	105	107	107	108	113	112	114	120	115	120	122	123
R1L2	91	101	108	113	116	118	119	118	115	112	107	104	103	106	105	110
R2L1	90	96	96	93	98	100	105	101	104	101	106	107	114	108	112	118
R2L2	88	81	84	80	83	87	88	91	94	95	97	99	100	102	104	106
R3	101	100	107	102	104	106	111	116	116	116	122	123	129	128	130	133

0010018

Individual body weights 10 ppm Male

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	86	72	72	75	70	70	69	68	64	60	55	58	64	55	70	70
R1	114	116	118	114	115	116	116	116	117	114	116	111	114	100	116	112
R2	111	108	105	109	108	107	112	113	118	112	112	114	116	89	118	113
L1	110	104	106	102	102	102	101	103	101	100	95	96	93	81	100	96
L2	117	116	112	112	110	111	108	101	98	93	91	100	108	96	113	110
R1L1	112	112	129	117	115	117	112	110	107	93	81	88	89	94	103	93
R1L2	105	106	106	105	103	102	99	98	91	85	89	87	91	88	94	103
R2L1	96	95	89	95	94	94	98	97	94	101	91	92	97	93	96	97
R2L2	114	110	111	112	110	108	107	107	109	104	104	106	106	103	108	108
R3	130	125	123	120	113	110	105	104	97	94	100	108	111	107	112	111
B																
Z	90	85	83	83	82	82	80	81	76	72	70	74	78	83	87	86
R1	115	113	108	110	108	109	106	105	100	97	95	99	98	98	101	102
R2	96	96	98	94	90	91	90	90	86	82	82	88	87	86	91	88
L1	106	105	107	106	105	103	102	94	87	83	72	84	87	88	95	92
L2	88	86	81	77	73	72	70	67	65	64	59	63	71	73	80	82
R1L1	100	101	99	105	104	105	105	105	107	106	103	106	110	106	109	107
R1L2	100	100	103	102	104	100	97	96	97	95	82	84	86	90	90	93
R2L1	116	118	115	118	110	110	104	96	94	89	89	96	107	107	112	111
R2L2	104	102	98	105	99	104	101	101	100	99	97	96	95	96	99	98
R3	104	104	103	104	100	99	99	100	100	97	95	96	105	106	110	106
C																
Z	112	108	115	117	118	121	123	121	119	120	122	123	114	107	113	109
R1	133	136	140	147	149	150	151	152	151	147	143	138	128	123	129	130
R2	132	134	134	132	139	140	142	145	143	141	146	144	136	128	132	128
L1	105	101	102	106	108	109	112	112	118	118	117	122	117	109	117	113
L2	109	110	112	116	115	114	110	103	104	101	95	98	104	101	102	101
R1L1	128	120	119	121	120	120	119	114	115	119	121	122	114	108	119	112
R1L2	112	113	106	114	102	106	106	104	109	123	122	124	118	111	117	114
R2L1	111	109	108	114	115	117	116	119	124	102	102	97	95	92	97	94
R2L2	109	103	98	104	116	119	120	122	100	100	102	99	97	97	103	100
R3	131	136	129	134	130	134	131	132	136	122	129	128	125	119	123	119

610019

Individual body weights 10 ppm — Males

Week	52	56	60	64	68	72	76	78
A								
Z	72	69	68	65	65	61	64	62
R1	122	117	111	94	97	100	103	103
R2	114	117	104	94	96	95	97	98
L1	98	95	90	84	78	82	84	85
L2	114	113	108	104	106	109	109	109
R1L1	106	109	100	98	99	98	101	103
R1L2	96	96	92	90	90	89	92	96
R2L1	100	100	94	112	*27/12.78	-	-	-
R2L2	110	110	106	105	107	106	106	108
R3	120	114	98	88	91	90	96	99
B								
Z	86	87	84	80	81	81	83	83
R1	102	105	102	96	92	94	98	98
R2	90	88	80	70	90	73	73	76
L1	92	94	84	75	68	66	77	76
L2	80	76	70	64	63	63	67	68
R1L1	108	106	102	100	99	105	101	105
R1L2	92	103	87	82	85	85	84	95
R2L1	112	115	100	98	98	99	101	102
R2L2	100	91	101	100	95	98	98	98
R3	96	88	89	95	87	88	96	98
C								
Z	106	107	114	115	119	117	114	116
R1	130	137	141	134	132	133	133	135
R2	128	129	120	126	128	128	127	128
L1	114	112	110	114	106	103	98	99
L2	102	99	112	118	115	109	103	103
R1L1	116	119	111	108	107	109	110	110
R1L2	114	115	108	102	103	108	106	108
R2L1	98	97	92	90	89	90	92	93
R2L2	102	105	102	89	84	86	91	92
R3	120	120	115	106	101	96	91	95

*dead

000020

Individual body weights 10 ppm

Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	68	98	105	110	119	119	120	115	118	118	118	109	107	103	101	101
R1	72	94	90	92	95	99	103	100	95	97	97	99	102	103	105	105
R2	81	89	83	*20/10.76		-	-	-	-	-	-	-	-	-	-	-
L1	80	91	96	96	102	104	107	106	106	100	97	92	90	97	84	87
L2	93	116	113	104	129	130	130	127	125	125	121	115	118	119	117	122
R1L1	87	93	110	109	105	113	118	116	111	111	109	103	103	110	108	112
R1L2	91	96	91	86	93	86	93	91	90	93	93	98	111	105	102	106
R2L1	100	109	105	112	119	120	125	127	123	123	119	116	126	118	112	106
R2L2	99	92	90	93	100	95	101	102	100	106	106	109	111	116	116	115
R3	84	88	93	95	96	96	100	96	93	91	95	94	95	86	80	82
E																
Z	57	79	90	98	106	112	117	121	119	119	121	112	122	118	115	116
R1	78	92	95	98	107	111	110	107	108	107	104	94	111	104	104	107
R2	78	95	106	108	113	113	118	117	114	114	113	103	110	109	110	114
L1	81	92	94	99	105	110	115	117	113	114	120	110	116	103	114	114
L2	64	81	85	87	90	96	99	105	103	107	113	109	121	125	124	133
R1L1	77	90	89	85	89	88	93	92	90	94	96	96	100	103	100	104
R1L2	90	94	99	100	102	106	109	109	111	113	114	115	119	120	118	124
R2L1	87	84	88	89	92	91	93	95	92	97	97	103	107	105	104	108
R2L2	88	90	92	91	93	95	97	99	96	84	93	100	104	106	106	108
R3	99	93	98	98	97	97	100	103	22/11*.76		-	-	-	-	-	-
F																
Z	59	81	91	100	110	118	129	128	133	134	134	129	127	121	122	123
R1	72	84	93	105	117	123	129	129	129	125	124	117	114	109	112	118
R2	89	105	108	105	104	101	101	99	98	101	103	110	113	115	116	123
L1	76	87	97	97	99	101	103	101	98	95	89	85	80	79	80	82
L2	76	91	101	101	108	115	123	126	126	131	128	124	120	116	111	107
R1L1	97	101	92	94	95	94	99	98	93	93	88	90	87	82	84	88
R1L2	82	95	92	94	101	102	111	114	110	110	105	105	107	110	102	106
R2L1	92	89	97	89	93	89	95	101	95	101	99	102	109	108	108	118
R2L2	94	110	108	112	115	113	120	117	116	118	110	109	114	104	101	102
R3	81	98	106	109	113	114	129	134	127	133	129	131	125	124	122	119

*dead

000021

Individual body weights 10 ppm

Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
D																
Z	100	100	100	101	102	102	105	105	106	116	113	110	119	116	113	109
R1	106	107	108	104	102	104	102	98	96	95	102	109	99	102	105	101
R2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L1	87	88	91	92	94	94	99	100	98	99	86	93	111	111	115	115
L2	123	122	117	120	119	118	118	116	114	116	117	123	119	119	122	120
R1L1	114	114	120	114	110	114	110	111	113	111	106	103	107	102	110	103
R1L2	105	102	100	98	93	92	87	83	86	84	78	80	87	88	92	89
R2L1	112	101	101	102	99	101	98	102	99	99	96	94	98	97	105	103
R2L2	116	112	110	108	105	104	99	97	96	92	91	92	98	97	103	101
R3	76	77	79	80	80	80	83	83	84	85	85	84	87	87	93	91
E																
Z	116	120	118	122	122	118	118	115	109	109	106	104	105	101	104	102
R1	112	106	106	107	104	104	105	103	104	102	103	100	103	102	105	100
R2	115	116	118	117	115	116	117	116	114	114	111	108	113	110	112	108
L1	113	112	112	114	118	118	116	116	115	112	109	106	115	108	109	108
L2	128	126	122	122	116	115	111	113	106	100	98	96	99	100	102	96
R1L1	108	108	106	112	102	106	105	104	101	101	91	82	85	86	93	95
R1L2	126	125	128	124	123	126	120	118	112	108	92	80	82	85	94	7/8*.77
R2L1	103	102	100	98	91	92	88	84	81	81	79	82	88	85	89	88
R2L2	107	107	107	106	100	102	100	97	93	90	85	86	91	89	98	96
R3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F																
Z	120	121	118	121	121	125	122	121	122	120	116	112	113	110	120	119
R1	120	119	120	124	126	124	127	122	129	124	126	121	121	115	128	119
R2	116	112	109	106	101	99	96	93	91	91	92	84	89	91	104	101
L1	84	85	85	87	86	88	90	91	88	87	82	90	87	84	90	89
L2	108	106	104	110	111	111	113	111	113	114	113	111	110	105	111	114
R1L1	85	85	89	90	91	88	86	87	89	83	89	96	83	75	102	90
R1L2	104	106	104	108	106	107	104	104	104	103	100	102	97	94	102	101
R2L1	118	118	119	118	117	116	116	114	112	107	103	83	97	98	83	100
R2L2	105	104	105	106	103	104	105	100	103	103	100	101	101	102	103	105
R3	118	115	116	120	115	114	112	113	110	110	112	108	106	105	111	119

*dead

0000022

Individual body weights 10 ppm

Males

Week	52	56	60	64	68	72	76	78
D								
Z	108	105	91	86	94	96	97	99
R1	106	105	101	96	97	100	101	100
R2	-	-	-	-	-	-	-	-
L1	118	119	116	108	114	116	118	120
L2	117	113	108	108	117	118	122	117
R1L1	102	105	94	86	89	92	92	95
R1L2	94	94	89	85	87	90	92	98
R2L1	104	104	90	88	94	94	95	96
R2L2	104	101	94	90	86	88	89	95
R3	91	94	87	85	84	90	91	93
E								
Z	104	107	101	96	100	102	98	102
R1	96	103	96	94	92	95	95	100
R2	107	106	101	94	93	94	91	92
L1	106	107	100	94	98	97	93	92
L2	98	95	92	92	94	93	89	91
R1L1	96	99	97	94	92	90	93	93
R1L2	-	-	-	-	-	-	-	-
R2L1	94	95	86	82	82	85	87	89
R2L2	99	102	95	92	90	87	86	90
R3	-	-	-	-	-	-	-	-
F								
Z	122	108	94	94	97	96	101	100
R1	124	123	114	100	104	103	24/2*.78	
R2	107	107	99	90	88	89	98	102
L1	96	93	92	88	87	84	88	87
L2	116	114	110	103	101	98	105	106
R1L1	82	85	82	80	88	90	13/3*.78	
R1L2	104	101	94	92	90	93	98	100
R2L1	106	106	103	98	96	97	97	104
R2L2	106	106	100	86	97	104	106	110
R3	112	111	107	106	107	108	109	111

*dead

0000023

Individual body weights 10 ppm

Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C																
Z	51	55	65	68	73	72	76	77	77	77	79	80	81	79	80	78
R1	69	84	91	93	97	101	100	99	99	101	105	102	108	110	110	108
R2	75	91	99	102	105	107	110	112	113	111	115	113	119	116	116	113
L1	78	80	85	84	89	96	99	97	95	95	91	93	92	82	76	72
L2	74	80	86	85	86	89	90	90	93	91	92	93	96	95	96	94
R1L1	79	83	91	93	97	100	101	106	106	109	112	115	117	118	118	122
R1L2	88	90	100	98	101	103	105	105	106	107	111	112	115	112	112	117
R2L1	96	108	115	117	120	123	129	129	132	131	132	133	137	136	139	135
R2L2	88	87	93	91	98	101	101	107	109	109	110	109	111	109	105	104
R3	101	116	114	117	126	118	119	116	106	102	98	97	107	103	108	108
H																
Z	67	73	82	90	99	105	107	109	116	115	113	113	109	110	106	105
R1	67	74	76	77	82	84	85	85	87	89	90	92	95	92	98	95
R2	71	82	93	98	108	110	109	107	106	103	100	96	94	98	90	90
L1	90	96	96	93	102	99	99	100	100	107	106	106	105	108	107	107
L2	84	96	102	107	113	121	119	119	119	115	116	116	117	120	120	119
R1L1	75	96	102	104	113	117	122	121	125	121	130	126	125	123	124	127
R1L2	92	108	115	118	128	130	132	127	124	119	109	110	107	113	110	108
R2L1	80	94	99	100	102	107	111	119	114	113	114	130	128	134	128	130
R2L2	96	118	128	133	136	139	134	136	124	120	125	124	115	113	118	120
R3	92	105	105	107	112	111	113	114	113	111	118	118	121	124	122	123
K																
Z	68	85	95	108	120	127	134	140	141	145	146	144	144	140	140	138
R1	77	90	98	99	101	104	105	108	106	109	114	116	120	125	126	130
R2	76	87	92	97	107	119	119	125	127	130	133	135	133	131	130	130
L1	80	78	79	81	85	92	98	107	101	99	103	104	107	105	94	87
L2	83	90	92	92	101	93	96	103	97	107	101	106	108	108	108	128
R1L1	90	104	120	117	127	133	143	146	151	150	141	140	147	135	122	115
R1L2	93	105	108	107	118	125	138	133	136	138	155	153	136	135	130	126
R2L1	93	107	114	113	120	125	127	126	127	123	130	124	120	115	110	110
R2L2	86	101	106	100	121	125	131	133	133	133	132	111	*23/12.76		-	-
R3	109	126	131	128	136	141	139	135	134	130	135	132	129	130	126	123

* DEAD

000024

Individual body weights 10 ppm Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
C																
Z	82	82	81	82	78	79	73	73	71	66	68	70	73	71	71	64
R1	110	110	110	114	111	113	116	114	112	110	104	105	106	102	107	103
R2	115	112	111	110	107	108	106	109	116	104	103	105	105	104	109	103
L1	74	74	77	78	82	80	82	85	83	80	83	82	85	85	88	86
L2	96	94	91	93	90	92	90	89	88	80	71	76	79	79	86	80
R1L1	123	118	117	116	114	114	110	110	109	105	102	110	106	104	107	104
R1L2	122	122	125	126	124	125	123	122	109	110	105	110	117	115	110	111
R2L1	138	134	128	126	121	120	116	118	117	118	114	115	113	111	114	106
R2L2	104	96	95	92	90	90	92	93	94	93	95	98	99	94	99	94
R3	112	108	112	114	116	115	120	120	114	110	115	116	121	112	123	108
H																
Z	102	101	99	101	100	105	100	97	100	99	97	101	99	97	103	100
R1	97	95	98	97	92	88	84	79	76	71	74	76	86	81	92	90
R2	92	90	92	96	95	95	96	94	97	97	96	96	101	96	105	101
L1	108	109	111	110	109	112	110	108	109	106	101	94	105	101	108	108
L2	121	119	121	118	118	119	116	113	117	114	113	116	120	111	120	118
R1L1	129	130	133	128	130	132	128	129	129	123	125	126	127	120	133	129
R1L2	108	105	107	108	108	106	112	109	108	110	109	110	116	113	120	117
R2L1	129	127	128	126	128	124	127	125	121	119	117	114	121	114	121	120
R2L2	121	124	116	124	125	123	125	123	128	119	124	122	126	119	127	121
R3	124	124	120	120	121	117	120	116	116	108	104	106	113	112	120	116
K																
Z	140	141	140	144	143	151	154	152	149	152	153	154	138	132	136	135
R1	134	134	137	140	146	148	153	144	145	145	146	146	132	123	124	122
R2	127	130	131	132	136	138	138	137	137	135	138	139	127	121	127	128
L1	83	86	92	98	103	110	116	116	117	120	125	124	111	104	109	113
L2	119	122	119	117	120	124	127	129	125	126	126	130	125	117	120	118
R1L1	112	109	105	108	106	108	106	108	114	108	106	104	105	103	111	110
R1L2	126	116	112	118	119	118	116	115	114	119	117	114	117	116	121	119
R2L1	106	105	101	106	106	104	103	102	101	104	103	101	105	101	110	104
R2L2	+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R3	120	120	115	124	119	119	118	120	120	116	113	110	113	109	115	109

Individual body weights: 10 ppm — Males

Week	52	56	60	64	68	72	76	78
G								
Z	65	68	63	63	64	60	59	57
R1	108	113	106	98	99	103	105	107
R2	98	99	98	97	101	100	101	101
L1	92	85	78	80	99	88	86	91
L2	84	87	84	84	86	86	85	89
R1L1	108	105	104	94	89	92	95	100
R1L2	112	119	122	117	114	120	120	121
R2L1	106	103	95	90	83	83	80	93
R2L2	94	93	88	89	84	84	82	87
R3	108	109	108	106	109	107	109	117
H								
Z	100	99	92	86	80	77	82	86
R1	92	93	88	76	73	69	72	76
R2	102	104	92	88	89	89	90	94
L1	108	112	110	102	102	102	101	108
L2	120	120	116	110	109	108	107	112
R1L1	134	136	138	134	131	136	131	129
R1L2	124	121	116	108	101	97	97	98
R2L1	126	126	121	110	103	99	102	95
R2L2	130	127	129	122	117	118	114	111
R3	120	124	112	102	102	104	110	108
K								
Z	132	130	132	136	134	132	130	130
R1	128	128	129	135	129	126	124	126
R2	132	127	128	136	132	127	123	120
L1	116	116	116	120	109	112	116	116
L2	120	120	126	132	127	125	119	115
R1L1	112	110	102	102	103	101	106	108
R1L2	118	124	116	110	114	113	116	120
R2L1	109	109	103	102	106	109	118	122
R2L2	-	-	-	-	-	-	-	-
R3	108	109	104	108	109	113	121	120

000026

Individual body weights 10 ppm **Males**

Week:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L																
Z	70	88	91	93	95	101	100	99	100	101	102	103	103	103	107	114
R1	76	96	106	108	113	117	123	119	125	127	125	120	119	115	114	118
R2	77	81	81	81	87	82	85	85	85	86	87	86	91	92	100	104
L1	80	91	96	91	96	101	104	107	109	114	110	110	107	101	96	92
L2	95	106	115	121	131	136	137	139	141	141	138	134	130	117	120	120
R1L1	89	90	92	93	98	98	101	105	108	111	114	116	119	116	120	126
R1L2	88	91	93	94	98	97	99	102	102	104	102	106	110	108	107	112
R2L1	89	39	93	92	89	88	91	89	91	91	89	92	93	90	90	94
R2L2	99	106	105	104	105	106	109	109	110	111	108	114	114	109	110	115
R3	119	112	113	113	109	111	113	116	113	117	114	117	119	117	116	122

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L																
Z	115	115	115	120	112	113	115	112	109	105	95	95	99	96	106	111
R1	118	121	117	121	120	122	123	125	127	124	121	126	126	121	130	121
R2	105	104	103	104	98	98	98	92	90	85	83	82	89	87	93	92
L1	86	91	87	88	89	93	93	94	95	93	91	95	95	92	102	98
L2	112	118	119	119	117	117	116	118	117	116	114	116	114	112	117	113
R1L1	129	128	127	128	125	127	125	127	118	115	106	104	109	110	113	112
R1L2	111	110	109	110	109	110	107	110	105	100	85	88	98	98	105	105
R2L1	93	88	88	92	87	89	91	90	90	88	76	70	79	82	83	83
R2L2	115	114	116	116	119	116	117	115	115	111	97	100	107	111	113	109
R3	123	122	125	123	119	120	119	120	121	121	109	108	110	114	116	116

Week:	52	56	60	64	68	72	76	78
L								
Z	108	110	106	102	96	101	99	101
R1	122	125	124	118	117	122	121	121
R2	94	93	88	88	88	89	95	99
L1	96	97	90	92	89	94	98	99
L2	116	119	113	98	99	91	* 23/2	.78
R1L1	114	116	110	110	109	109	106	110
R1L2	108	107	106	104	104	103	100	106
R2L1	88	91	86	76	75	70	73	76
R2L2	115	113	110	104	104	102	101	106
R3	118	119	118	110	110	111	110	115

* DEAD

000027

TABLE 3 - MEAN MATERNAL BODY WEIGHTS, WEIGHT-GAIN, FOOD INTAKE AND FOOD EFFICIENCY OF DAMS
PRELIMINARY STUDY

parameter	ppm β-chloroprene dosed by inhalation				
	0	10	25	75	175
number of dams examined	7	7	4	6	6
mean maternal body weight (g) at:					
day 0	186.4	186.4	180.5	185.1	187.8
day 6 (start of treatment)	206.6	204.1	199.0	203.0	206.0
day 16 (end of treatment)	238.7	233.7	222.5	222.3	217.6
day 21	286.1	285.4	275.8	271.8	267.5
weight gain during:					
treatment	32.1	29.6	23.5**	19.3**	11.7
gestation	99.7	99.0	95.3	86.7**	79.7
mean food intake (g/rat/day) from:					
day 0-6	16.4	16.0	15.9	16.4	16.0
day 6-16 (treatment period)	17.1	16.8	15.1*	14.2**	12.8
day 16-21	18.9	19.4	18.8	18.4	16.5
mean food efficiency $\frac{\text{weight gain(g)}}{\text{food consumption(g)}}$					
day 0-6	0.21	0.18	0.19	0.15	0.
day 6-16 (treatment period)	0.19	0.18	0.16	0.13*	0.(
day 16-21	0.50	0.53	0.57	0.54	0..

* $0.05 > P > 0.01$

** $0.01 > P > 0.001$

*** $P < 0.001$

Individual body weights 50 ppm Males

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	80	81	80	80	80	80	82	79	85	83	85	84	84	84	87	85
R1	87	87	85	86	81	82	79	88	95	85	85	80	82	78	83	83
R2	106	106	102	103	100	100	102	102	99	101	101	101	100	100	105	106
L1	111	110	110	110	110	108	103	103	101	91	95	101	104	101	105	105
L2	105	104	104	110	108	107	105	104	117	107	108	105	106	104	106	104
R1L1	98	98	98	96	98	97	100	99	111	100	103	98	99	101	96	95
R1L2	120	120	122	126	127	127	128	130	123	120	115	100	98	103	104	101
R2L1	110	111	107	109	102	110	110	116	113	113	112	107	108	96	112	107
R2L2	109	106	104	108	106	105	107	105	103	104	101	99	100	97	102	99
R3	107	108	100	101	100	98	101	101	101	103	103	102	102	99	97	97
B																
Z	101	101	98	92	96	95	95	94	91	90	89	85	86	83	84	80
R1	100	101	103	104	106	105	105	104	100	100	98	93	92	89	90	107
R2	114	113	107	112	106	106	107	108	115	103	101	98	98	91	95	96
L1	97	91	90	92	99	93	96	97	94	95	95	92	90	88	101	99
L2	104	105	106	110	109	107	108	106	93	91	88	87	92	96	96	92
R1L1	116	117	113	116	104	114	110	110	103	106	113	111	110	106	111	106
R1L2	96	95	90	94	88	79	80	73	70	70	73	74	78	75	78	77
R2L1	124	120	112	114	107	105	105	108	102	109	111	108	109	106	110	108
R2L2	86	85	84	86	85	80	82	82	80	78	78	80	83	83	86	83
R3	104	102	100	102	100	97	96	96	94	95	97	93	90	91	92	94
C																
Z	115	117	118	120	122	119	120	117	116	116	118	114	110	107	113	111
R1	90	90	88	88	88	89	88	90	87	89	91	91	92	91	92	90
R2	108	109	108	107	106	108	109	109	107	108	107	100	101	97	100	100
L1	112	113	112	115	116	112	112	108	102	100	87	90	91	96	100	98
L2	86	86	86	87	86	85	85	83	82	84	85	79	78	78	84	81
R1L1	* -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R1L2	87	87	84	84	82	78	77	76	70	76	80	82	84	87	89	88
R2L1	107	108	106	106	101	99	98	93	95	90	89	92	94	93	100	97
R2L2	117	116	110	110	106	104	101	101	90	97	97	101	102	101	106	100
R3	100	99	98	96	96	96	93	93	89	91	93	90	92	94	98	97

* DEAD

000029

Individual body weights 50 ppm Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	66	74	79	81	87	88	95	95	101	105	106	103	99	98	94	87
R1	74	84	85	88	87	91	90	91	92	93	97	97	99	99	105	104
R2	81	93	97	107	112	120	121	125	120	114	112	105	106	108	109	105
L1	77	89	102	110	116	119	115	112	104	96	90	86	89	85	88	87
L2	81	89	90	92	88	91	96	96	96	98	99	100	101	105	110	109
R1L1	88	92	101	105	109	113	117	125	115	112	107	95	91	97	93	99
R1L2	93	95	95	102	104	108	116	117	117	122	125	122	114	112	101	92
R2L1	85	86	88	89	91	93	94	95	96	97	97	98	94	98	100	92
R2L2	84	90	92	92	94	100	98	95	94	96	94	88	86	86	83	78
R3	91	93	93	91	89	89	90	89	91	94	92	91	87	90	91	87
E																
Z	63	70	80	78	83	86	86	89	89	90	93	91	92	94	92	93
R1	75	86	101	106	115	120	121	118	118	114	116	112	106	107	108	105
R2	75	85	97	101	109	113	116	115	115	110	115	109	104	104	100	95
L1	83	83	84	84	85	87	89	92	94	96	103	103	104	105	108	110
L2	84	93	102	110	112	115	126	120	120	118	117	113	108	107	106	100
R1L1	91	99	112	123	125	131	134	134	137	141	140	136	136	135	138	138
R1L2	102	107	119	127	129	134	141	137	140	143	146	143	142	140	141	138
R2L1	94	95	99	101	101	103	103	102	106	108	112	112	115	116	123	127
R2L2	103	109	116	118	126	122	125	124	125	125	127	126	129	128	126	125
R3	105	99	100	96	95	95	95	92	95	97	102	101	107	104	107	110
F																
Z	69	73	76	79	77	79	87	84	86	92	97	98	106	112	118	128
R1	82	92	93	103	103	106	112	109	112	118	116	111	116	121	116	120
R2	75	80	83	88	91	94	96	94	95	92	97	94	98	104	107	108
L1	90	96	95	108	104	105	107	106	110	111	113	109	116	119	126	130
L2	86	89	91	96	95	101	107	99	99	100	102	97	102	104	100	102
R1L1	79	84	84	91	89	88	91	89	92	97	102	102	105	108	111	114
R1L2	90	103	111	119	119	124	133	129	134	138	146	147	147	146	151	146
R2L1	102	117	121	134	143	150	157	154	154	156	159	155	156	160	161	168
R2L2	93	98	93	101	110	114	115	114	117	127	134	134	136	133	134	136
R3	95	103	107	116	118	122	126	122	120	117	119	119	123	123	123	125

Individual body weights 50 ppm **Males**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
D																
Z	84	80	80	81	80	81	80	79	77	78	78	77	81	77	85	79
R1	105	104	105	102	98	96	95	93	87	88	77	86	86	88	96	94
R2	105	107	102	105	106	104	106	105	101	101	102	103	102	99	101	100
L1	89	88	86	86	84	86	86	86	82	83	82	82	82	78	82	78
L2	112	108	107	110	108	105	105	105	94	91	80	78	82	84	89	88
R1L1	93	94	90	96	98	94	98	93	96	81	73	85	96	94	101	89
R1L2	90	88	89	94	93	95	98	100	87	99	101	96	97	95	95	92
R2L1	98	98	96	95	98	92	95	83	81	80	99	95	88	88	89	98
R2L2	76	75	73	80	78	80	83	112	82	80	85	83	84	84	91	86
R3	89	90	86	89	90	82	85	81	75	70	72	74	76	77	80	79
E																
Z	91	91	90	95	96	94	94	91	89	88	82	73	80	81	81	82
R1	103	103	102	103	106	105	106	106	106	106	106	103	110	106	110	93
R2	94	98	94	98	96	94	94	93	94	90	91	88	88	86	91	90
L1	110	112	106	111	112	108	109	108	101	105	98	96	98	98	92	93
L2	100	100	98	98	98	99	100	100	96	97	95	93	96	92	97	98
R1L1	136	135	136	128	134	133	83	131	124	125	127	135	130	126	132	130
R1L2	140	138	134	136	132	136	136	135	131	130	136	135	135	128	135	132
R2L1	126	124	124	133	124	126	124	120	110	112	111	112	118	113	117	111
R2L2	124	128	124	128	124	121	125	124	117	114	125	124	128	125	128	124
R3	108	110	109	110	105	106	104	106	98	96	92	89	94	94	99	100
F																
Z	120	120	119	119	122	119	124	124	126	125	129	126	120	115	114	102
R1	130	120	130	139	134	130	132	128	128	127	122	110	109	104	110	102
R2	108	108	105	106	106	106	108	107	110	113	106	103	100	93	97	91
L1	128	130	128	130	102	98	99	102	96	94	*10/4.77		-	-	-	-
L2	104	101	103	94	106	104	108	108	108	108	103	100	98	92	95	88
R1L1	110	108	108	106	106	104	107	100	99	97	100	103	104	101	108	104
R1L2	145	148	144	146	142	140	140	137	141	140	133	131	126	124	128	117
R2L1	160	161	161	160	160	156	157	156	157	158	155	150	154	146	151	142
R2L2	135	136	132	128	124	127	126	116	119	120	116	112	110	109	119	108
R3	124	122	122	120	120	118	120	118	117	118	115	110	106	109	112	103

* DEAD

000031

Individual body weights 50 ppm Males

Week	52	56	60	64	68	72	76	78
D								
Z	80	80	79	75	75	72	76	81
R1	96	95	92	93	96	96	97	98
R2	102	101	98	86	81	79	87	88
L1	80	75	72	71	68	72	75	75
L2	88	92	89	80	77	74	79	86
R1L1	99	100	97	84	85	80	91	99
R1L2	96	97	90	89	89	89	91	93
R2L1	90	91	84	80	75	80	82	84
R2L2	88	87	86	84	82	80	79	83
R3	81	82	79	73	67	62	68	71
E								
Z	84	82	81	75	76	75	73	77
R1	95	95	92	87	88	89	90	96
R2	92	93	89	83	84	85	88	88
L1	94	92	83	75	70	68	69	68
L2	97	96	93	88	89	94	99	100
R1L1	131	131	127	125	128	121	124	12[illegible]
R1L2	128	132	139	144	144	139	134	127
R2L1	114	116	124	119	120	113	108	104
R2L2	122	126	127	123	120	116	113	*1/4.78
R3	98	102	106	103	101	95	92	94
F								
Z	101	92	96	83	74	72	81	80
R1	100	98	85	87	94	91	94	86
R2	92	88	86	92	84	81	81	80
L1	-	-	-	-	-	-	-	-
L2	*26/9.77		-	-	-	-	-	-
R1L1	110	107	100	97	93	*8/2.78		-
R1L2	131	122	120	123	123	120	117	115
R2L1	147	144	145	146	145	152	152	151
R2L2	102	116	114	118	114	114	114	110
R3	110	110	108	107	106	107	108	105

*DEAD

000032

Individual body weights 50 ppm Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G																
Z	61	66	69	71	72	73	73	71	71	71	70	68	66	65	65	64
R1	73	78	81	83	85	87	91	91	90	94	94	94	96	95	96	95
R2	84	87	95	94	95	98	97	99	101	104	103	102	105	108	108	108
L1	81	84	89	90	93	103	109	105	108	111	112	112	111	111	107	99
L2	81	87	89	90	90	93	91	91	95	94	95	96	96	100	100	98
R1L1	82	102	112	118	124	129	127	126	121	122	123	128	117	110	111	117
R1L2	92	89	98	107	110	115	119	117	120	122	121	118	116	116	119	122
R2L1	93	93	97	99	99	100	99	97	99	102	106	107	98	112	116	116
R2L2	87	92	97	99	102	104	111	109	108	112	114	112	113	112	108	100
R3	98	95	97	98	97	96	97	95	97	97	102	103	105	107	111	111
H																
Z	69	75	83	79	83	35	85	87	89	90	91	97	88	89	96	97
R1	69	77	84	96	107	111	112	115	113	118	116	110	116	116	116	118
R2	71	91	96	101	113	123	127	128	130	130	130	123	125	120	121	120
L1	77	96	107	110	111	108	110	109	110	112	113	112	113	115	119	126
L2	77	81	84	85	86	86	87	86	88	88	94	92	92	92	95	98
R1L1	75	77	81	81	85	86	84	86	85	106	98	93	87	96	100	105
R1L2	83	89	99	86	105	110	115	115	112	90	91	87	81	75	73	72
R2L1	83	89	89	90	92	92	93	92	95	89	89	98	91	95	95	94
R2L2	85	84	88	100	88	90	90	87	87	96	98	86	75	88	90	88
R3	96	98	99	103	105	106	117	106	110	112	113	112	104	108	108	105
K																
Z	75	82	90	93	97	100	99	96	101	103	104	102	98	98	97	100
R1	67	71	78	82	85	88	91	90	94	95	98	94	96	98	95	93
R2	63	73	80	84	90	98	98	99	103	105	103	98	92	89	83	80
L1	83	89	89	90	95	98	99	99	100	104	105	105	106	109	110	108
L2	80	79	83	86	87	89	89	90	94	98	100	99	100	103	106	105
R1L1	75	86	93	105	114	115	122	125	129	130	129	120	113	108	98	90
R1L2	91	99	103	110	102	106	104	101	110	132	131	128	116	113	120	121
R2L1	88	97	105	110	114	114	121	115	106	106	108	104	108	107	118	117
R2L2	91	99	105	111	114	116	121	124	128	114	107	113	123	120	118	107
R3	96	99	103	113	112	114	120	124	123	124	123	122	119	116	112	108

0000033

Individual body weights 50 ppm **Males**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
G																
Z	62	65	64	66	67	73	75	82	83	86	91	95	98	85	89	91
R1	94	95	92	91	89	85	86	86	89	94	102	94	100	94	98	101
R2	109	112	106	106	105	104	107	107	106	110	119	125	130	126	126	126
L1	94	91	88	89	90	89	89	87	92	94	111	119	124	114	115	114
L2	97	95	98	95	94	94	96	96	97	107	111	112	110	108	108	108
R1L1	123	127	126	125	124	122	119	120	113	118	113	108	108	111	110	109
R1L2	122	118	116	117	115	112	113	110	107	105	106	93	106	104	105	104
R2L1	120	114	110	114	112	104	105	103	95	96	101	105	106	105	105	106
R2L2	96	94	90	92	94	94	92	94	91	92	95	103	93	94	96	99
R3	114	115	107	117	116	112	115	113	115	107	101	90	92	97	100	101
H																
Z	99	102	102	104	108	104	107	106	103	102	98	94	92	92	93	92
R1	118	119	120	124	130	129	130	129	129	134	134	132	132	127	124	121
R2	119	120	120	122	123	119	122	118	116	123	121	118	112	107	109	109
L1	129	133	134	132	134	128	131	123	103	118	116	126	124	121	122	120
L2	100	102	104	106	108	108	109	107	105	107	102	92	95	92	94	95
R1L1	107	108	112	112	110	113	85	112	106	107	99	97	96	92	94	93
R1L2	73	78	78	80	80	81	81	81	81	82	84	82	82	83	88	87
R2L1	93	93	92	86	82	79	86	75	71	73	77	81	86	86	89	90
R2L2	89	90	90	88	89	85	86	85	81	79	75	73	78	79	84	82
R3	101	97	90	86	82	74	74	75	73	78	81	82	86	84	87	82
K																
Z	97	98	96	98	94	88	90	85	76	75	73	82	84	86	93	93
R1	95	95	94	95	96	96	99	94	87	90	88	80	84	85	89	91
R2	83	86	86	86	88	86	90	89	85	91	90	86	87	86	92	90
L1	108	109	110	110	109	104	105	100	94	96	96	99	98	94	98	98
L2	107	110	105	101	94	94	87	84	75	80	87	92	92	91	95	94
R1L1	91	91	92	92	92	91	96	96	86	96	95	92	95	93	97	97
R1L2	120	124	124	124	123	122	125	120	107	116	108	100	100	98	108	98
R2L1	118	116	113	114	114	116	115	113	109	111	112	111	110	97	101	111
R2L2	105	105	105	106	106	116	115	116	97	105	103	100	101	104	103	102
R3	110	111	116	115	107	100	106	105	107	113	117	108	106	103	106	107

Individual body weights 50 ppm **Males**

Week	52	56	60	64	68	72	76	78
G								
Z	94	99	100	96	89	*13/2.78		-
R1	100	100	98	94	93	88	85	86
R2	126	122	127	127	121	113	108	105
L1	109	108	114	118	112	97	90	92
L2	108	110	110	106	104	98	94	101
R1L1	112	108	101	98	97	100	95	95
R1L2	107	102	95	92	93	95	93	93
R2L1	100	104	106	90	86	84	84	88
R2L2	88	96	93	91	90	96	90	92
R3	106	105	104	90	91	93	93	93
H								
Z	97	92	87	80	83	83	83	90
R1	120	122	127	120	116	114	106	107
R2	112	108	99	93	95	96	96	108
L1	126	129	124	121	124	126	121	122
L2	128	99	109	101	102	94	91	93
R1L1	94	97	86	83	74	77	78	78
R1L2	87	86	80	77	78	72	74	75
R2L1	91	84	79	79	83	86	86	89
R2L2	84	85	83	80	84	84	87	92
R3	78	76	69	73	73	64	75	76
K								
Z	96	97	88	85	88	89	95	93
R1	89	91	85	78	75	78	81	82
R2	91	68	79	72	69	72	75	76
L1	102	94	88	87	90	89	92	95
L2	92	84	78	71	77	76	79	85
R1L1	97	98	95	88	82	90	90	95
R1L2	102	96	89	83	89	90	92	101
R2L1	102	110	96	98	90	85	84	87
R2L2	112	96	105	87	97	99	102	102
R3	109	107	101	101	103	103	105	109

* DEAD

0000035

Individual body weights 50 ppm Males

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L																
Z	73	84	85	89	91	96	96	95	95	92	88	85	84	85	87	86
R1	81	77	92	99	102	105	111	114	119	116	116	114	85	114	113	106
R2	71	86	90	95	102	110	111	115	116	115	114	106	107	101	100	99
L1	85	96	96	91	102	107	109	108	110	110	104	102	102	100	101	98
L2	71	76	81	81	83	85	88	85	87	90	88	88	116	90	89	88
R1L1	87	83	89	93	91	91	117	94	97	126	118	117	134	132	131	122
R1L2	94	96	100	108	116	110	117	119	114	96	101	98	106	106	106	105
R2L1	90	100	108	114	108	117	95	116	120	116	120	114	116	117	116	112
R2L2	98	107	107	119	124	127	137	132	132	121	130	130	117	116	117	118
R3	114	118	121	131	128	134	144	143	149	157	153	148	147	138	130	127

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L																
Z	86	87	88	88	90	91	92	91	98	91	93	93	92	94	96	94
R1	106	105	103	100	102	101	102	100	99	102	103	101	104	100	104	100
R2	100	101	102	102	100	103	104	102	91	100	103	99	96	97	98	96
L1	96	94	95	96	95	94	97	96	94	96	97	94	96	94	96	97
L2	89	86	83	84	84	75	79	75	71	68	69	72	78	79	83	82
R1L1	121	119	118	118	117	119	120	117	112	118	117	102	104	82	85	83
R1L2	106	103	106	106	108	106	108	105	107	96	85	81	108	109	112	109
R2L1	109	109	106	108	109	106	110	109	106	108	105	102	84	99	106	99
R2L2	114	113	113	114	114	114	116	115	115	114	116	110	110	109	106	109
R3	110	102	101	104	104	104	104	103	101	104	109	108	108	108	113	114

Week	52	56	60	64	68	72	76	78
L								
Z	101	98	95	92	93	90	94	94
R1	103	100	96	92	94	92	89	93
R2	94	90	81	75	72	76	82	84
L1	97	99	95	92	90	92	92	92
L2	88	82	81	76	79	77	80	80
R1L1	88	100	92	73	79	89	73	74
R1L2	109	110	98	104	101	103	103	102
R2L1	103	84	81	84	85	81	92	93
R2L2	111	107	109	98	102	104	107	110
R3	116	114	106	100	98	103	105	106

Individual body weights Control group Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A																
Z	65	86	98	104	106	114	114	105	114	120	124	124	126	135	134	147
R1	70	87	89	94	99	97	95	93	98	104	107	115	110	112	113	114
R2	72	93	105	115	123	133	135	135	142	142	142	140	136	142	146	146
L1	74	87	91	109	97	101	101	95	104	110	116	124	123	122	128	130
L2	83	100	100	93	113	117	115	111	120	125	130	133	136	138	141	146
R1L1	90	93	92	98	100	101	101	102	103	104	107	110	98	106	108	109
R1L2	91	107	109	111	116	125	127	129	132	137	137	137	130	138	138	142
R2L1	85	97	100	109	116	122	121	121	125	125	124	124	111	118	120	123
R2L2	104	110	105	109	110	116	117	121	114	114	114	118	108	114	118	122
R3	104	117	117	119	121	126	129	125	126	128	132	134	121	130	136	138
B																
Z	65	76	88	97	98	113	104	102	111	110	111	110	102	108	110	110
R1	75	89	102	117	124	128	136	133	136	136	138	136	136	136	136	131
R2	78	78	82	91	93	94	93	91	92	92	93	93	94	96	98	101
L1	83	92	101	106	110	110	113	117	120	126	125	104	130	128	133	132
L2	85	87	91	99	108	111	110	104	110	110	107	127	105	106	106	104
R1L1	90	108	101	102	100	101	98	100	104	106	108	111	102	100	108	112
R1L2	91	92	91	97	101	106	107	109	113	116	119	101	124	121	120	126
R2L1	96	94	97	106	109	111	110	113	118	120	124	128	125	122	129	135
R2L2	88	101	96	100	107	107	111	115	115	118	126	131	116	113	128	128
R3	104	123	103	129	140	145	148	152	156	156	154	154	146	138	135	134
C																
Z	56	78	89	95	104	120	127	128	131	134	132	133	136	134	136	138
R1	73	89	94	97	102	103	103	106	107	113	120	121	126	120	124	127
R2	74	81	90	89	95	97	101	101	102	109	117	119	122	126	126	132
L1	81	86	90	101	103	105	107	109	112	118	125	128	136	138	140	138
L2	84	93	106	121	131	137	140	144	145	138	132	122	108	100	95	96
R1L1	82	91	92	101	89	96	99	98	96	98	107	105	106	104	104	102
R1L2	90	95	90	92	99	109	107	108	118	120	124	123	130	130	130	129
R2L1	93	103	100	102	106	108	113	109	108	109	110	110	112	108	108	104
R2L2	100	104	100	96	99	103	107	107	108	112	113	114	115	114	116	114
R3	107	120	131	141	150	144	137	127	124	122	117	112	109	104	107	104

000037

Individual body weights — Control group — Females

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	135	143	145	144	146	148	150	138	140	135	133	128	135	134	137	142
R1	121	120	118	123	122	126	130	122	126	127	118	114	119	113	114	106
R2	146	152	154	152	150	150	146	138	138	146	143	144	149	147	151	143
L1	130	135	132	140	136	144	147	136	141	137	144	133	131	128	126	129
L2	148	151	158	154	157	160	158	150	162	158	154	146	144	135	134	131
R1L1	108	111	111	112	110	114	115	114	110	107	101	95	101	95	101	100
R1L2	136	138	136	136	134	130	118	125	119	115	94	100	107	104	107	103
R2L1	124	123	129	128	126	127	127	124	121	118	105	93	98	103	109	105
R2L2	122	122	123	124	118	117	120	116	113	110	101	98	105	100	108	107
R3	136	139	142	144	140	143	143	142	136	136	128	122	123	118	117	114
B																
Z	108	110	112	112	108	110	110	102	101	96	82	84	101	106	117	110
R1	133	130	126	120	118	118	119	112	113	112	107	105	113	99	108	100
R2	100	102	104	102	98	99	97	94	96	94	83	71	81	86	92	91
L1	134	135	136	134	132	136	136	134	134	130	130	110	116	114	119	119
L2	106	102	103	101	100	100	98	100	103	95	93	88	85	94	105	107
R1L1	110	113	113	114	114	114	113	113	120	119	110	102	108	117	132	128
R1L2	128	135	135	134	134	134	131	126	124	124	120	122	121	98	100	96
R2L1	136	140	141	141	146	149	145	147	146	144	144	138	136	127	128	125
R2L2	130	130	133	134	128	126	126	128	131	135	133	132	126	108	116	113
R3	134	127	125	123	124	120	122	120	123	123	121	126	129	122	131	131
C																
Z	138	139	143	143	142	142	144	135	133	140	139	132	136	129	137	136
R1	132	136	135	134	136	144	145	136	143	142	130	120	101	89	89	90
R2	137	140	134	133	128	132	126	114	113	112	110	100	113	111	118	118
L1	134	133	133	128	132	138	140	132	132	133	135	120	115	106	113	115
L2	105	107	108	108	114	120	128	118	123	127	126	128	118	113	128	123
R1L1	102	103	100	103	100	100	97	95	96	95	78	70	80	93	102	98
R1L2	131	132	126	126	124	120	114	113	111	106	93	98	103	103	105	110
R2L1	104	103	99	100	96	94	88	85	85	85	82	70	73	76	80	82
R2L2	114	119	112	112	114	114	108	108	110	109	104	90	102	96	104	103
R3	104	101	102	100	98	102	104	106	103	103	104	100	104	101	104	102

Individual body weights Control group Females

Week	52	56	60	64	68	72	76	78
A								
Z	149	148	140	127	114	106	106	125
R1	110	110	114	96	85	77	84	76
R2	147	138	134	115	105	92	99	110
L1	140	142	142	133	140	149	*3/3.78	
L2	133	150	150	*3/2.78		-	-	-
R1L1	113	106	103	87	80	79	87	87
R1L2	108	110	104	90	85	*15/2.78		-
R2L1	126	110	106	97	99	95	102	98
R2L2	111	118	114	113	117	117	118	119
R3	126	118	106	96	95	88	91	94
B								
Z	115	113	103	93	91	93	101	104
R1	103	*21/10.77		-	-	-	-	-
R2	93	94	86	80	83	83	89	90
L1	127	124	122	114	104	106	116	116
L2	104	108	104	102	101	108	118	102
R1L1	129	122	107	96	*27/12.77		-	-
R1L2	101	102	91	85	87	80	90	89
R2L1	130	126	130	123	119	*27/1.78		-
R2L2	119	112	102	97	88	83	96	89
R3	131	133	118	*22/12.77		-	-	-
C								
Z	*28/9.77		-	-	-	-	-	-
R1	87	84	82	82	81	*2/2.78		-
R2	120	122	105	88	88	83	85	87
L1	112	110	108	99	101	*1/2.78		-
L2	119	118	*16/11.77		-	-	-	-
R1L1	100	101	98	89	*4/1.78		-	-
R1L2	113	114	118	100	95	94	96	96
R2L1	80	80	79	73	78	71	78	73
R2L2	100	100	99	91	94	81	85	81
R3	100	103	99	95	98	89	101	95

* DEAD

000039

Individual body weights 0 ppm Control group Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	63	86	88	90	97	103	103	103	111	116	118	118	119	116	120	127
R1	71	87	94	94	103	109	109	115	120	126	133	134	132	131	138	145
R2	73	87	84	83	92	91	91	93	92	96	98	99	98	96	100	105
L1	82	91	89	94	101	109	113	116	117	118	123	128	124	124	128	131
L2	87	103	114	124	130	140	144	142	145	140	137	136	132	118	118	116
R1L1	86	103	110	118	126	136	139	144	149	151	153	154	152	146	146	138
R1L2	91	101	95	101	104	107	104	115	108	114	112	115	121	128	133	133
R2L1	94	111	102	114	122	124	126	130	128	124	124	134	138	144	146	151
R2L2	97	115	125	136	147	151	153	149	148	144	139	138	142	144	144	145
R3	106	107	103	108	113	109	100	109	105	108	108	114	122	122	128	136
E																
Z	41	65	68	72	84	88	89	93	97	100	108	112	114	115	118	122
R1	56	78	80	88	95	103	111	111	116	122	135	140	146	146	153	158
R2	64	91	91	104	106	108	109	109	112	116	126	130	123	124	132	132
L1	68	96	95	104	116	122	129	126	127	130	130	126	120	116	120	115
L2	72	105	116	126	135	142	143	145	143	146	146	143	137	134	136	132
R1L1	80	91	92	93	93	97	97	94	103	108	117	125	120	124	130	133
R1L2	90	98	100	100	98	93	99	96	103	103	112	115	108	114	111	120
R2L1	94	117	112	116	125	124	132	139	140	142	149	153	150	154	150	154
R2L2	96	144	127	141	148	153	161	161	161	162	152	140	132	137	133	132
R3	103	122	129	132	135	136	136	133	130	132	140	148	142	144	146	153
F																
Z	69	90	94	101	104	104	107	108	107	110	115	115	114	116	122	120
R1	74	89	86	87	92	97	97	100	100	107	108	106	110	114	112	111
R2	80	95	99	102	105	111	110	109	107	114	112	112	114	*29/12.76		-
L1	80	98	100	106	112	115	113	118	114	121	124	128	129	132	128	128
L2	83	96	96	97	104	109	105	107	102	105	105	108	109	108	106	105
R1L1	89	102	103	110	116	121	122	126	126	132	130	135	132	136	137	134
R1L2	92	108	106	109	117	125	130	125	127	130	132	134	133	136	135	138
R2L1	85	105	100	104	113	112	111	110	113	114	111	110	114	112	111	111
R2L2	99	92	90	92	97	99	103	101	103	106	107	105	105	107	1081	108
R3	117	130	132	136	136	131	127	123	124	124	123	122	124	124	125	126

* DEAD

000040

Individual body weights **Control group** **Females**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
D																
Z	134	139	145	146	150	146	149	149	153	157	161	158	148	138	147	147
R1	147	154	157	159	160	138	163	164	167	170	164	148	155	143	136	132
R2	110	112	120	125	124	124	125	130	132	130	128	110	119	119	120	106
L1	137	137	143	140	143	144	146	139	136	136	133	132	128	136	140	138
L2	120	127	126	125	121	122	122	127	128	131	128	127	133	133	139	137
R1L1	124	115	113	107	108	107	116	116	115	121	121	130	141	126	131	119
R1L2	136	140	144	145	140	144	140	132	132	127	121	122	116	121	120	118
R2L1	155	152	169	162	160	162	163	159	15o	*24/3.77		-	-	-	-	-
R2L2	142	144	143	140	138	136	132	123	119	126	120	124	134	126	123	125
R3	143	144	148	146	147	149	152	149	141	145	141	121	123	127	132	127
E																
Z	129	126	129	132	137	140	148	148	144	140	135	130	131	127	131	127
R1	164	164	166	166	164	162	167	166	165	159	151	140	148	133	144	141
R2	135	134	137	140	144	141	147	151	147	143	148	146	143	138	145	140
L1	118	122	125	125	128	126	126	127	130	127	133	136	124	116	125	123
L2	134	138	143	145	150	151	146	149	151	148	155	147	135	126	133	135
R1L1	138	142	146	152	149	151	155	156	158	156	154	160	156	143	132	128
R1L2	122	125	121	127	125	124	122	119	123	119	115	115	120	117	127	125
R2L1	160	158	164	162	166	160	167	162	165	168	164	138	141	127	152	143
R2L2	138	138	135	139	143	134	142	136	139	137	137	131	130	129	139	134
R3	157	162	165	172	170	170	175	178	174	178	171	155	149	127	139	130
F																
Z	116	118	111	118	115	112	112	105	104	101	85	72	81	93	101	101
R1	112	112	111	109	108	115	113	109	106	106	88	76	87	97	96	92
R2	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L1	122	127	128	126	128	128	125	120	116	113	100	98	108	109	114	110
L2	95	97	93	94	96	96	98	92	88	88	79	78	87	89	93	96
R1L1	132	130	127	130	128	126	128	120	119	114	100	90	101	103	104	110
R1L2	140	140	138	140	139	136	139	133	137	131	122	113	119	119	119	122
R2L1	114	115	113	114	114	114	116	112	107	104	86	69	74	85	87	93
R2L2	108	106	107	108	106	104	102	101	97	93	84	80	96	96	92	96
R3	128	130	128	126	128	125	130	125	121	121	113	98	102	104	104	106

* DEAD

Individual body weights Control group Females

Week	52	56	60	64	68	72	76	78
D								
Z	148	148	143	135	127	111	102	96
R1	134	130	114	109	104	*28/1.78		-
R2	115	120	122	112	102	100	*6/3.78	
L1	138	138	150	133	121	111	106	98
L2	135	134	122	129	132	141	142	145
R1L1	109	*7/10.77		-	-	-	-	-
R1L2	119	110	103	101	99	98	104	110
R2L1	*-	-	-	-	-	-	-	-
R2L2	132	130	127	110	101	100	110	114
R3	138	130	136	*20/12.77		-	-	-
E								
Z	129	134	133	121	98	91	97	96
R1	142	142	*6/11.77		-	-	-	-
R2	144	136	128	110	*28/12.77		-	-
L1	127	124	119	109	94	87	87	87
L2	145	140	138	112	107	110	122	128
R1L1	126	136	126	113	108	102	100	102
R1L2	130	130	126	121	112	108	110	116
R2L1	139	136	122	112	108	99	101	*11/4.78
R2L2	141	142	132	123	114	108	115	119
R3	129	133	130	114	111	96	99	99
F								
Z	103	102	90	76	76	107	*20/2.78	
R1	93	90	86	86	86	85	82	85
R2	*-	-	-	-	-	-	-	-
L1	109	108	110	116	128	146	79	79
L2	97	86	82	77	78	70	74	85
R1L1	109	104	94	91	91	91	* 8/3.78	
R1L2	123	124	119	116	107	102	111	114
R2L1	95	102	104	98	97	96	95	99
R2L2	97	93	92	93	88	94	86	90
R3	109	115	116	114	108	95	100	103

* DEAD

000042

Individual body weights — Control group — Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G																
Z	66	91	90	95	103	110	115	121	129	127	131	129	132	134	132	128
R1	72	88	96	107	112	121	120	119	123	128	132	134	140	142	142	140
R2	81	104	106	119	128	137	129	128	120	119	115	111	104	100	100	101
L1	80	94	95	99	105	109	111	109	112	116	114	116	122	119	124	128
L2	87	95	88	99	100	106	105	108	107	102	103	104	102	98	92	90
R1L1	85	110	113	116	120	118	119	121	129	126	124	129	133	136	136	142
R1L2	91	91	91	95	103	111	115	120	130	132	130	120	116	116	115	112
R2L1	96	99	94	100	106	106	110	115	120	122	122	126	127	127	128	130
R2L2	97	111	114	117	118	121	121	115	117	119	125	130	132	136	142	145
R3	106	116	116	120	117	118	121	122	123	123	129	129	125	127	128	128
H																
Z	62	69	75	81	86	88	90	91	95	96	102	102	100	106	108	110
R1	70	89	96	108	117	121	129	133	136	142	144	145	144	146	148	149
R2	80	97	98	102	119	115	117	117	125	126	131	135	135	136	133	145
L1	84	101	94	95	103	109	110	112	120	124	126	130	128	133	134	138
L2	84	90	86	93	100	104	104	106	112	110	113	112	110	112	114	115
R1L1	92	113	109	118	123	124	124	127	128	126	127	130	126	134	138	144
R1L2	92	102	101	111	119	123	124	131	141	142	148	149	149	151	156	155
R2L1	93	107	104	109	118	117	120	123	126	127	131	131	132	139	140	140
R2L2	99	103	100	109	115	118	117	117	120	122	124	135	128	134	138	145
R3	104	118	110	118	124	126	126	130	137	134	138	146	136	146	152	152
K																
Z	56	68	84	95	101	108	112	118	120	118	117	116	121	117	116	111
R1	72	78	87	93	96	102	103	101	102	100	103	108	108	108	114	110
R2	71	74	91	106	117	121	127	131	132	134	130	126	120	118	116	114
L1	75	84	97	106	112	112	115	115	119	111	12	8	124	124	126	125
L2	85	95	112	120	131	143	151	152	151	150	149		146	144	144	141
R1L1	83	106	111	118	127	136	144	147	149	148	154	160	164	163	170	177
R1L2	89	102	110	104	115	120	125	129	131	132	132	135	140	138	146	155
R2L1	93	109	104	107	111	109	110	113	111	112	112	113	118	114	117	120
R2L2	103	122	116	120	127	129	131	131	136	136	134	131	132	127	132	134
R3	107	115	117	127	134	140	143	148	148	148	152	153	148	146	144	138

Individual body weights Control group Females

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
G																
Z	125	123	114	107	99	96	94	88	89	82	97	108	109	103	113	112
R1	138	136	133	130	126	128	125	119	116	110	107	114	113	91	114	110
R2	101	102	102	106	104	103	105	104	101	103	103	105	104	104	108	106
L1	130	133	130	132	131	130	133	126	124	120	120	94	91	89	100	99
L2	84	81	82	79	76	78	77	75	76	74	86	82	95	91	98	96
R1L1	144	144	150	151	145	148	147	142	136	134	135	148	149	143	139	131
R1L2	112	112	115	116	112	118	125	120	114	123	125	124	123	113	109	112
R2L1	130	134	133	134	134	138	136	140	139	138	127	129	129	118	112	119
R2L2	146	151	149	148	158	166	163	164	163	166	163	126	133	116	111	114
R3	133	131	138	139	144	146	153	150	150	154	158	142	147	131	120	113
H																
Z	113	114	110	102	116	116	117	108	108	110	99	100	98	99	102	101
R1	147	142	136	136	134	132	130	123	124	125	130	121	126	124	125	126
R2	150	155	154	145	140	130	140	134	128	125	118	112	107	111	118	108
L1	142	143	141	144	148	150	149	140	138	140	136	128	113	121	127	133
L2	116	117	114	113	120	120	127	121	121	126	133	126	117	119	119	*11/8.77
R1L1	145	149	145	132	130	136	127	127	113	123	121	122	132	109	118	114
R1L2	158	154	156	152	150	154	150	148	146	147	143	142	150	133	141	133
R2L1	146	147	148	148	148	151	147	148	144	149	146	116	138	105	111	110
R2L2	148	155	157	156	154	150	150	148	144	146	139	128	136	129	138	132
R3	154	160	166	167	172	174	158	159	164	162	163	136	118	125	133	137
K																
Z	102	*2/1.77	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R1	110	109	110	108	108	106	110	106	113	112	95	78	73	74	86	91
R2	116	113	112	112	110	110	116	108	104	108	105	93	104	103	109	109
L1	126	126	124	120	114	116	114	112	108	112	103	103	106	110	115	112
L2	137	133	130	129	133	134	132	133	132	134	133	128	128	126	129	127
R1L1	184	178	180	184	184	186	190	186	177	165	164	146	143	136	140	135
R1L2	166	159	163	167	170	172	175	172	179	169	160	158	151	145	153	152
R2L1	124	122	126	125	128	130	131	126	124	124	120	122	121	112	112	107
R2L2	136	132	136	133	134	135	119	114	119	126	123	130	128	106	104	85
R3	138	129	128	128	130	131	134	129	128	135	135	134	135	129	122	121

* DEAD

0000044

Individual body weights — Control group — Females

Week	52	56	60	64	68	72	76	78
G								
Z	117	118	111	97	96	87	92	90
R1	117	116	107	88	86	86	97	102
R2	109	108	102	95	*12/1.78		-	-
L1	100	96	94	84	82	82	89	86
L2	97	96	92	83	76	82	89	90
R1L1	124	104	98	96	96	99	104	*29/3.78
R1L2	115	106	104	94	93	77	89	93
R2L1	121	116	110	104	94	88	77	90
R2L2	116	112	102	91	84	91	92	81
R3	103	101	102	99	92	89	86	95
H								
Z	109	112	104	96	86	84	93	105
R1	122	118	104	89	79	*23/1.78		-
R2	109	112	104	90	90	85	84	90
L1	137	136	138	115	111	106	96	100
L2	* -	-	-	-	-	-	-	-
R1L1	113	112	106	107	99	94	95	109
R1L2	141	136	134	113	111	100	99	98
R2L1	115	120	108	100	88	87	88	92
R2L2	129	124	115	95	102	91	*3/3.78	
R3	129	132	126	107	107	96	107	112
K								
Z	*-	-	-	-	-	-	-	-
R1	94	99	92	85	80	81	81	87
R2	107	104	92	83	66	*22/1.78		-
L1	111	112	109	96	86	86	87	96
L2	124	128	126	124	125	118	115	115
R1L1	128	128	130	136	138	136	141	142
R1L2	143	150	150	142	126	123	112	126
R2L1	107	104	98	97	93	84	85	83
R2L2	*16/9.77		-	-	-	-	-	-
R3	115	116	118	117	106	100	103	99

*DEAD

000045

Individual body weights Control group Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L																
Z	47	74	86	99	110	121	123	126	130	134	138	139	140	138	137	147
R1	60	88	100	112	122	127	134	129	133	134	127	121	118	113	105	99
R2	66	92	97	103	110	108	105	105	103	108	117	122	125	124	133	136
L1	74	94	96	109	113	116	117	116	113	117	123	122	134	140	142	151
L2	76	89	90	97	98	103	103	104	105	105	114	129	126	130	133	142
R1L1	85	100	102	108	115	116	117	116	118	114	115	112	114	114	118	116
R1L2	89	91	101	94	98	96	97	97	98	99	98	106	107	106	106	112
R2L1	95	95	100	111	122	128	130	132	136	132	129	127	120	116	116	117
R2L2	97	101	91	105	114	115	113	121	131	130	133	136	136	136	138	136
R3	104	115	107	113	121	127	130	135	143	142	148	148	147	149	153	148

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L																
Z	147	143	143	142	138	136	130	127	129	132	137	140	141	136	134	130
R1	100	97	96	94	92	90	85	85	92	96	108	106	103	99	114	104
R2	136	125	121	116	126	122	120	119	118	113	116	128	138	136	116	128
L1	151	145	146	148	154	152	145	142	148	152	149	140	139	135	145	133
L2	143	138	137	140	120	144	138	137	131	129	125	130	127	128	139	129
R1L1	112	108	107	107	104	108	111	105	103	102	101	102	101	97	105	106
R1L2	108	108	107	114	118	118	117	119	121	120	114	116	119	116	124	120
R2L1	115	110	108	111	109	110	113	109	112	114	104	104	108	104	112	115
R2L2	133	130	122	122	118	111	113	109	109	109	112	119	122	122	132	130
R3	149	140	133	131	129	130	134	135	136	134	133	134	135	128	134	127

Week	52	56	60	64	68	72	76	78
L								
Z	127	119	110	94	96	*7/2.78		-
R1	105	106	94	92	90	85	*8/3.78	
R2	*9/9.77		-	-	-	-	-	-
L1	136	135	*10/11.77		-	-	-	-
L2	125	104	92	*19/12.77		-	-	-
R1L1	110	110	101	97	95	*13/2.78		-
R1L2	118	108	106	98	86	86	*2/3.78	
R2L1	118	113	105	97	88	83	92	89
R2L2	128	125	110	98	95	88	95	100
R3	132	*26/10.77		-	-	-	-	-

* DEAD

000046

Individual body weights 10 ppm

Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A																
Z	67	75	75	80	82	95	93	99	97	99	104	102	105	107	108	110
R1	77	95	102	99	103	105	105	107	106	105	108	107	107	110	110	109
R2	83	98	100	110	113	117	114	113	114	109	109	104	102	98	98	102
L1	78	94	95	97	93	96	98	102	101	107	104	109	106	107	104	108
L2	81	82	91	95	92	95	100	101	101	105	107	105	106	107	108	109
R1L1	93	104	120	128	137	139	146	141	140	134	132	126	123	126	126	124
R1L2	90	102	111	109	119	127	131	133	134	136	138	136	132	131	130	139
R2L1	97	102	101	103	111	110	115	117	119	123	132	135	138	140	140	145
R2L2	102	122	130	135	144	142	144	148	148	145	142	134	131	130	126	135
R3	113	115	125	117	122	124	128	130	135	133	140	139	142	136	138	143
B																
Z	66	91	105	122	138	141	151	149	130	131	138	136	140	141	138	137
R1	75	98	107	110	123	122	125	129	132	133	136	135	134	138	138	139
R2	79	92	98	106	113	115	117	121	121	121	124	128	131	130	130	138
L1	83	93	103	107	115	120	122	122	128	134	138	140	146	148	150	153
L2	84	90	96	97	101	104	107	109	111	116	119	120	124	122	124	128
R1L1	87	101	104	95	94	94	95	99	102	107	108	116	120	121	120	128
R1L2	90	105	115	120	135	137	142	147	145	151	147	149	149	144	146	135
R2L1	98	110	121	120	125	122	120	116	108	116	115	121	118	115	120	124
R2L2	99	109	110	112	130	128	133	133	134	143	137	139	148	147	140	150
R3	105	115	117	120	133	133	135	137	136	143	140	154	154	148	154	155
C																
Z	57	68	81	88	100	106	109	114	113	109	107	105	107	110	110	110
R1	74	92	107	119	128	133	129	130	124	127	132	138	148	153	162	167
R2	78	92	97	102	111	110	117	121	118	120	120	120	123	121	123	130
L1	83	100	115	125	134	142	147	149	150	150	150	147	141	138	136	135
L2	77	77	80	75	81	85	89	94	98	103	105	104	113	111	111	114
R1L1	82	93	99	100	109	105	107	108	105	101	102	102	103	98	100	99
R1L2	90	96	100	100	115	118	118	118	116	107	102	110	83	83	80	90
R2L1	93	105	108	105	118	123	122	125	121	124	120	119	120	120	120	122
R2L2	100	106	116	114	130	136	143	151	154	155	163	163	168	164	166	167
R3	106	124	121	117	133	140	141	143	144	147	145	148	149	146	140	150

0000047

Individual body weights 10 ppm **Females**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	108	108	108	113	107	109	106	103	106	95	88	81	85	82	93	108
R1	111	113	110	112	111	115	110	110	101	99	86	75	81	86	94	94
R2	98	100	98	94	97	96	97	97	95	94	96	93	98	102	101	108
L1	108	105	108	110	105	116	102	100	96	94	83	82	83	94	102	103
L2	108	112	107	92	80	78	83	90	90	93	81	70	72	82	95	93
R1L1	138	140	140	134	133	127	130	130	132	128	129	128	133	122	132	135
R1L2	125	127	127	122	125	124	119	122	125	119	116	112	113	108	111	108
R2L1	148	151	158	156	156	154	154	160	160	153	143	120	138	127	133	137
R2L2	130	129	129	127	121	120	120	120	125	119	119	114	121	111	124	123
R3	140	138	145	142	139	142	143	146	144	137	136	130	129	124	138	132
B																
Z	132	136	132	130	129	124	126	130	127	130	127	124	123	112	121	120
R1	135	137	141	138	138	130	135	139	130	139	139	140	135	128	135	132
R2	137	141	144	150	151	148	149	146	140	151	148	120	111	104	113	104
L1	152	155	154	158	153	150	140	130	129	122	118	124	130	118	124	117
L2	123	126	132	132	130	125	122	124	129	133	127	119	106	101	105	104
R1L1	127	133	135	136	140	141	138	144	141	136	143	139	139	121	126	114
R1L2	130	136	138	141	143	142	139	142	150	143	142	147	151	139	148	136
R2L1	123	131	130	133	135	135	136	137	135	138	136	130	112	124	123	113
R2L2	152	149	145	150	149	148	150	149	141	153	150	149	141	121	127	118
R3	154	162	154	148	143	139	132	136	132	132	133	137	141	134	137	124
C																
Z	110	108	107	114	108	110	105	108	109	106	108	101	113	93	95	91
R1	165	175	175	174	164	160	155	143	155	145	139	127	105	109	112	110
R2	130	130	132	133	131	132	128	134	132	130	123	108	85	75	83	92
L1	135	135	135	140	134	135	132	127	132	125	131	136	135	119	124	112
L2	114	115	109	114	115	120	116	115	117	126	117	93	104	102	*17/7.77-	
R1L1	99	99	98	98	98	98	99	98	97	89	81	68	81	80	92	89
R1L2	94	90	92	94	89	94	95	94	93	86	90	93	97	89	97	92
R2L1	120	121	117	94	121	124	125	124	125	110	114	102	101	102	112	109
R2L2	166	160	155	152	143	138	132	120	118	111	109	106	105	102	108	104
R3	145	148	148	146	142	146	147	148	145	130	125	128	135	131	137	131

* DEAD

Individual body weights 10 ppm Females

Week	52	56	60	64	68	72	76	78
A								
Z	107	99	87	74	*27/12.77		-	-
R1	96	99	94	84	91	89	100	97
R2	98	97	92	74	68	67	72	78
L1	104	104	92	80	84	85	86	88
L2	94	93	86	75	72	68	71	72
R1L1	140	137	130	116	112	95	100	106
R1L2	110	112	110	108	96	92	95	89
R2L1	134	134	138	144	137	125	122	112
R2L2	116	123	120	121	114	106	95	92
R3	136	129	130	130	125	122	124	127
B								
Z	122	117	110	100	95	93	94	92
R1	134	128	124	120	116	106	110	107
R2	108	107	104	100	82	81	82	78
L1	120	113	110	99	97	96	100	107
L2	96	*19/10.77		-	-	-	-	-
R1L1	110	107	108	102	100	96	103	94
R1L2	138	144	134	140	128	122	124	111
R2L1	118	114	114	116	109	110	104	108
R2L2	126	130	116	103	94	90	98	99
R3	122	115	118	114	101	98	99	104
C								
Z	82	76	75	75	*19/1.78		-	-
R1	100	98	86	85	88	84	95	92
R2	90	97	93	87	87	88	87	84
L1	99	99	94	92	91	98	102	97
L2	* -	-	-	-	-	-	-	-
R1L1	94	95	94	86	87	96	92	93
R1L2	94	94	87	83	86	92	88	88
R2L1	116	112	112	106	104	103	106	100
R2L2	112	113	114	100	103	107	105	105
R3	134	136	134	130	129	125	128	134

* DEAD

000049

Individual body weights 10 ppm **Females**

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	67	65	97	105	114	122	131	133	145	148	149	153	152	151	149	146
R1	68	75	89	98	105	112	115	119	126	132	132	131	127	118	110	107
R2	74	79	87	89	93	95	95	97	100	100	96	102	103	104	106	111
L1	77	79	85	83	90	88	89	89	91	92	96	93	98	98	100	101
L2	85	84	86	87	90	92	97	99	107	113	116	121	120	122	125	132
R1L1	80	95	107	109	120	127	128	131	132	133	134	135	132	128	125	127
R1L2	89	86	87	89	91	91	90	91	93	95	100	103	103	101	110	105
R2L1	94	100	103	99	101	102	113	105	103	106	106	106	107	107	110	110
R2L2	99	108	115	115	115	117	118	121	123	126	126	127	129	126	126	137
R3	105	108	109	105	107	111	111	113	112	117	116	118	118	121	123	129
E																
Z	51	68	69	69	71	75	77	79	81	83	84	87	88	91	98	105
R1	71	78	81	87	101	111	115	126	130	134	139	141	142	132	133	137
R2	72	80	86	92	98	104	109	116	119	123	128	133	147	130	140	150
L1	76	82	90	90	91	94	94	99	98	99	102	104	108	104	103	122
L2	83	87	106	110	121	137	145	150	159	159	165	164	161	157	154	141
R1L1	80	106	106	118	131	136	139	144	150	147	144	142	143	138	131	121
R1L2	91	98	104	98	115	107	115	125	130	131	133	137	137	140	142	146
R2L1	90	96	108	105	109	112	117	123	121	116	122	125	123	123	129	129
R2L2	87	104	115	119	115	121	121	128	125	127	126	134	139	141	144	150
R3	101	106	109	110	104	112	116	117	117	124	128	131	132	132	142	146
F																
Z	68	68	70	83	93	100	107	110	118	123	131	134	141	140	140	144
R1	69	84	87	86	95	94	93	102	101	105	107	108	109	113	114	113
R2	78	82	90	92	89	92	95	99	103	103	106	107	110	109	110	113
L1	79	85	93	97	104	105	113	119	124	128	134	137	134	132	130	125
L2	82	83	92	99	105	107	113	119	125	129	138	138	143	142	143	152
R1L1	89	104	103	105	118	121	131	133	133	135	138	146	145	141	132	124
R1L2	92	102	110	112	125	130	133	137	143	141	145	142	145	153	162	160
R2L1	97	99	114	114	123	129	122	137	147	147	150	156	161	158	160	165
R2L2	104	99	113	114	119	130	136	141	147	153	156	168	162	168	174	175
R3	116	123	120	121	129	137	138	143	145	148	150	148	152	155	164	153

050050

Individual body weights 10 ppm

Females

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
DZ	144	143	145	152	151	154	155	155	157	145	155	144	144	127	125	116
R1	103	100	103	118	108	110	112	117	119	109	112	106	111	104	110	102
R2	111	117	122	127	136	140	141	145	147	140	143	132	106	111	110	107
L1	102	104	107	116	116	116	117	120	126	109	104	88	95	99	108	109
L2	136	139	134	132	120	122	119	123	127	113	115	112	114	114	114	112
R1L1	126	125	125	129	134	134	134	137	131	132	138	131	132	128	123	115
R1L2	106	110	110	112	110	115	117	115	116	112	115	88	111	106	108	92
R2L1	111	116	116	116	122	126	125	130	128	125	116	104	103	104	105	90
R2L2	127	127	121	124	126	126	124	118	118	113	97	111	93	97	108	106
R3	127	125	125	124	122	122	117	114	112	108	115	116	113	109	110	103
EZ	110	114	115	122	116	120	116	114	116	108	95	82	91	91	104	98
R1	133	132	134	131	130	128	129	122	123	118	115	114	115	106	112	106
R2	152	156	156	157	162	166	168	167	163	160	158	148	132	123	127	118
L1	127	130	132	139	142	147	149	150	145	135	128	118	119	114	134	123
L2	135	131	124	124	127	126	125	125	127	127	126	126	126	114	127	118
R1L1	115	115	126	116	113	119	120	111	120	118	118	118	*18/5.77		-	-
R1L2	149	155	156	159	158	160	161	155	155	149	132	136	141	127	129	122
R2L1	132	140	138	142	132	139	136	134	142	138	131	118	117	111	127	111
R2L2	156	161	154	154	148	152	152	147	147	143	143	144	148	151	158	147
R3	149	150	148	152	146	154	150	143	149	142	138	131	125	119	133	128
FZ	146	146	142	147	138	134	130	136	137	135	124	126	134	119	124	124
R1	117	124	128	131	135	132	138	139	139	129	129	130	129	124	125	*9/8.77
R2	117	124	122	132	126	122	122	129	126	120	102	98	114	104	*13/7.77	
L1	122	118	108	101	92	88	82	78	76	73	84	95	109	109	118	117
L2	154	154	149	149	146	148	147	144	143	135	137	134	145	141	127	130
R1L1	117	116	122	121	126	132	134	129	131	123	126	140	*31/5.77		-	-
R1L2	164	166	165	164	158	158	161	170	173	160	155	140	141	135	131	121
R2L1	166	170	169	178	176	172	165	173	175	162	168	161	150	126	127	121
R2L2	174	176	170	174	177	178	178	184	183	174	170	160	141	116	119	110
R3	152	158	154	156	154	153	158	164	160	150	146	132	126	116	112	107

* DEAD

000051

Individual body weights 10 ppm **Females**

Week	52	56	60	64	68	72	76	78
D								
Z	112	105	101	88	*4/1.78		-	-
R1	100	97	88	80	77	75	81	80
R2	104	107	104	92	*9/1.78		-	-
L1	108	117	117	110	98	81	84	81
L2	108	107	109	96	91	78	83	83
R1L1	114	119	108	102	92	87	90	86
R1L2	104	108	104	88	78	77	82	79
R2L1	92	94	84	79	76	80	88	*29/3.78
R2L2	108	109	102	90	78	85	89	82
R3	108	104	107	100	86	85	98	92
E								
Z	100	102	94	92	86	73	73	68
R1	110	110	104	102	96	86	*18/2.78	
R2	131	133	131	118	129	125	129	119
L1	122	121	123	120	122	109	106	109
L2	121	129	122	100	93	80	94	99
R1L1	* -	-	-	-	-	-	-	-
R1L2	123	120	126	117	108	86	90	89
R2L1	113	110	110	104	105	*9/2.78		-
R2L2	135	135	132	114	105	94	104	110
R3	130	134	134	125	118	102	110	105
F								
Z	124	119	110	97	101	92	*23/2.78	
R1	* -	-	-	-	-	-	-	-
R2	* -	-	-	-	-	-	-	-
L1	118	111	106	90	93	80	82	87
L2	134	130	126	116	121	115	110	99
R1L1	* -	-	-	-	-	-	-	-
R1L2	123	114	105	92	*9/1.78		-	-
R2L1	126	139	128	132	129	128	111	*21/3.78
R2L2	121	135	137	138	132	133	132	124
R3	109	*28/10.77		-	-	-	-	-

* DEAD

000052

Individual body weights 10 ppm **Females**

book	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G																
Z	69	90	104	112	122	131	131	139	138	134	130	136	136	127	128	126
R1	76	89	92	95	101	99	99	99	104	109	112	109	108	101	110	125
R2	78	88	94	95	101	104	107	113	113	117	120	128	128	118	122	134
L1	85	96	110	114	116	116	119	121	121	127	138	145	149	147	154	166
L2	83	95	97	94	95	100	101	107	109	112	115	125	127	119	130	138
R1L1	88	102	116	122	132	138	146	146	147	146	152	152	154	142	142	146
R1L2	91	96	92	113	99	104	109	113	114	115	120	124	128	127	124	133
R2L1	92	100	115	106	107	109	110	111	113	116	116	117	120	122	121	128
R2L2	101	115	119	113	117	113	117	117	116	114	118	116	115	111	110	110
R3	107	110	123	129	130	130	131	136	132	134	135	140	144	141	139	143
H																
Z	58	80	86	88	89	92	97	96	92	93	92	100	107	115	114	127
R1	77	88	104	111	118	126	125	125	127	123	116	113	111	106	102	100
R2	69	84	82	82	85	89	99	100	103	106	106	109	116	123	122	121
L1	79	88	100	98	98	103	106	109	102	104	107	110	117	119	125	128
L2	82	82	86	88	87	89	89	91	89	91	94	103	110	114	120	124
R1L1	83	88	97	103	105	107	109	108	107	107	109	107	110	108	110	110
R1L2	87	86	94	96	99	103	100	101	97	99	99	98	99	100	102	99
R2L1	95	93	109	113	114	123	123	127	127	131	134	134	134	137	136	134
R2L2	96	98	106	108	111	114	113	115	113	113	112	115	115	113	114	114
R3	97	92	95	94	101	105	103	105	101	103	106	107	107	105	107	106
K																
Z	59	80	93	95	101	105	107	115	116	122	126	127	131	132	134	138
R1	74	94	93	81	85	86	90	97	101	105	111	116	121	120	121	127
R2	75	82	87	91	95	93	95	97	101	103	106	108	108	110	110	113
L1	79	82	94	100	96	95	93	94	92	93	94	91	95	88	93	97
L2	85	96	106	106	107	107	109	113	117	122	126	125	127	125	124	128
R1L1	83	90	85	91	93	97	97	99	95	101	104	106	108	106	108	110
R1L2	89	94	98	96	101	107	111	111	115	120	122	124	130	130	130	132
R2L1	95	102	110	108	111	114	113	116	116	121	124	125	120	136	131	133
R2L2	98	114	120	120	130	139	139	145	145	155	155	161	167	164	166	170
R3	100	98	107	107	110	111	113	115	113	117	127	125	130	132	132	134

000053

Individual body weights 10 ppm **Females**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
G																
Z	125	125	133	131	121	126	131	132	125	125	129	130	120	114	123	122
R1	128	136	133	132	129	124	135	138	136	128	123	108	115	109	119	116
R2	136	140	139	142	140	138	137	145	140	137	138	120	129	127	130	121
L1	166	171	173	174	176	174	170	163	160	151	151	161	154	139	144	143
L2	144	153	151	154	156	156	158	161	155	155	158	151	144	131	125	118
R1L1	142	145	142	142	134	131	129	133	133	119	133	126	99	*19/6	.77	-
R1L2	132	138	137	138	134	130	127	127	128	110	113	114	121	113	120	119
R2L1	122	123	127	128	131	132	133	135	138	128	127	139	137	129	142	135
R2L2	109	112	115	115	116	116	116	120	120	115	120	126	120	114	125	123
R3	143	142	143	146	147	144	140	148	150	136	145	144	151	145	154	140
H																
Z	120	128	121	120	115	112	114	112	110	101	101	94	79	76	86	91
R1	106	104	104	104	110	105	105	103	106	102	104	103	99	96	104	98
R2	114	112	98	98	98	94	93	94	95	89	88	93	94	95	100	100
L1	131	133	127	134	131	130	127	125	123	115	109	78	91	99	111	111
L2	126	127	121	125	123	120	115	113	106	97	99	76	74	80	91	90
R1L1	109	113	112	116	108	108	107	99	92	95	86	82	93	87	94	97
R1L2	98	102	98	100	91	96	94	92	88	83	85	70	73	76	91	93
R2L1	133	132	128	125	118	116	116	107	110	107	104	106	107	104	111	114
R2L2	112	115	114	111	105	107	102	103	92	84	77	72	76	75	87	92
R3	107	112	110	106	99	102	94	87	84	79	75	92	99	97	106	102
K																
Z	140	146	141	144	144	144	145	144	137	134	135	98	102	93	98	100
R1	130	131	134	132	132	130	125	123	116	110	108	96	109	105	109	102
R2	114	113	116	114	116	118	117	117	117	111	115	120	121	111	111	103
L1	96	99	97	102	103	104	102	104	100	98	96	90	95	99	105	107
L2	127	131	133	135	133	134	131	133	135	135	135	129	131	126	133	129
R1L1	108	110	112	115	108	110	107	111	120	107	105	101	94	86	93	92
R1L2	130	127	122	121	117	113	108	105	101	94	100	104	105	100	108	106
R2L1	135	140	136	140	141	143	148	150	145	144	139	143	128	97	95	106
R2L2	168	171	175	178	178	182	184	185	184	180	170	140	141	129	132	113
R3	137	135	135	134	137	136	130	130	110	119	108	120	123	117	122	116

* DEAD

000054

Individual body weights 10 ppm **Females**

Week	52	56	60	64	68	72	76	78
G								
Z	124	133	132	120	105	107	114	124
R1	116	115	111	106	91	87	97	107
R2	122	121	122	122	121	119	121	122
L1	142	139	136	128	139	132	132	134
L2	122	119	114	110	111	96	*22/2.78	
R1L1	*-	-	-	-	-	-	-	-
R1L2	123	125	121	116	115	120	103	107
R2L1	124	135	131	131	109	107	101	103
R2L2	116	113	120	116	118	105	103	108
R3	126	119	112	110	105	*14/2	-	-
H								
Z	95	97	92	96	101	95	96	103
R1	100	103	92	86	78	80	85	87
R2	98	100	98	98	101	104	107	105
L1	112	112	110	110	109	111	111	107
L2	92	97	95	95	93	90	85	83
R1L1	96	93	82	72	77	83	88	90
R1L2	96	93	98	75	71	69	73	79
R2L1	116	116	115	112	114	115	110	116
R2L2	96	103	98	98	95	93	102	105
R3	104	107	106	102	105	104	105	99
K								
Z	102	108	98	84	76	78	81	92
R1	96	90	86	*8/12.77		-	-	-
R2	101	101	99	88	77	78	83	84
L1	106	103	93	78	72	70	73	*1/4.78
L2	130	130	128	110	96	96	97	108
R1L1	94	94	92	88	77	72	77	75
R1L2	104	106	96	92	86	80	90	86
R2L1	110	113	115	104	90	82	94	87
R2L2	116	105	103	100	86	84	86	87
R3	118	116	116	107	94	90	90	93

* DEAD

000055

Individual body weights 10 ppm

Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L																
Z	65	84	92	94	103	106	115	117	128	133	138	137	148	154	156	163
R1	70	93	101	112	133	145	149	153	157	157	158	153	156	154	151	154
R2	72	87	89	95	95	101	103	109	104	106	106	104	108	114	113	115
L1	81	104	104	110	120	126	132	137	139	137	138	132	136	135	130	128
L2	88	98	103	106	109	114	114	122	122	129	132	124	128	125	124	129
R1L1	85	96	96	100	102	108	111	115	119	124	126	131	137	136	132	136
R1L2	90	102	115	117	118	119	121	117	112	113	112	113	118	120	116	117
R2L1	89	102	107	109	116	115	116	117	117	118	115	113	118	118	116	113
R2L2	95	106	103	107	119	119	118	120	124	124	132	129	132	129	124	130
R3	102	108	118	120	120	121	125	127	127	127	134	135	136	130	128	127

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L																
Z	164	168	168	164	156	156	148	144	137	135	126	138	133	138	130	116
R1	151	148	144	146	143	149	133	142	143	136	142	135	131	136	141	124
R2	123	124	131	132	136	139	144	135	130	126	119	126	116	*2/7.77		-
L1	125	123	124	126	133	134	131	133	138	133	132	124	124	118	123	119
L2	131	134	129	128	125	140	135	135	126	118	101	112	120	*7/7.77		-
R1L1	136	141	140	144	142	130	121	116	110	107	96	106	106	102	89	72
R1L2	117	118	114	124	126	126	117	122	120	119	113	96	98	97	106	103
R2L1	112	112	111	116	114	114	114	115	112	112	115	88	77	86	99	99
R2L2	131	129	127	133	133	132	129	128	123	124	113	104	107	106	110	106
R3	123	122	118	114	112	118	100	100	95	90	89	90	91	91	97	95

Week	52	56	60	64	68	72	76	78
L								
Z	121	113	121	104	111	104	109	114
R1	*4/9.77		-	-	-	-	-	-
R2	*-	-	-	-	-	-	-	-
L1	112	112	114	97	*30/12.77		-	-
L2	*-	-	-	-	-	-	-	-
R1L1	94	99	100	93	89	89	93	94
R1L2	107	110	114	103	100	97	100	104
R2L1	102	105	106	98	97	98	101	105
R2L2	108	108	106	100	94	91	95	87
R3	98	96	96	88	87	91	94	102

* DEAD

000056

Individual body weights 50 ppm Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A																
Z	71	80	83	91	93	103	104	104	110	114	120	121	120	126	129	128
R1	76	88	97	107	109	118	121	126	136	142	146	145	145	151	156	154
R2	78	90	101	101	103	113	104	125	134	139	144	149	139	148	152	148
L1	81	88	95	101	102	104	105	105	108	110	110	110	111	111	117	115
L2	86	86	89	94	99	105	111	111	114	121	120	126	122	129	134	138
R1L1	89	92	96	98	101	99	100	107	112	114	114	125	118	118	125	125
R1L2	88	92	91	95	96	100	99	105	109	104	105	113	114	116	120	132
R2L1	98	97	108	114	117	120	110	114	117	124	130	133	141	142	145	148
R2L2	95	98	104	105	103	105	105	109	105	104	106	102	100	99	103	103
R3	104	124	125	139	144	150	151	155	160	159	156	155	152	151	153	149
B																
Z	63	77	82	94	103	109	115	119	124	120	120	117	110	109	107	105
R1	75	81	81	86	87	85	85	86	85	88	84	86	85	87	87	90
R2	75	73	73	82	81	82	84	82	87	86	86	86	88	86	90	93
L1	79	82	92	100	101	105	106	103	107	105	106	114	105	100	111	114
L2	84	80	83	88	87	86	87	87	90	88	86	86	86	87	91	92
R1L1	83	84	92	104	104	105	113	109	113	109	106	100	98	95	95	90
R1L2	84	92	99	102	101	93	105	101	107	105	107	101	96	99	100	100
R2L1	88	96	96	94	95	92	101	99	103	105	109	107	106	107	110	113
R2L2	93	101	95	94	96	89	103	98	99	98	100	105	100	104	105	104
R3	96	108	108	112	112	102	109	110	108	106	103	104	101	98	100	98
C																
Z	64	80	91	101	108	114	118	117	115	106	112	107	100	95	92	97
R1	78	94	105	115	114	116	121	121	125	128	125	124	119	119	112	115
R2	81	85	94	100	103	105	107	109	110	111	114	121	110	109	108	110
L1	80	86	92	97	95	100	102	100	103	104	106	106	102	105	107	106
L2	84	85	97	106	107	108	112	113	113	116	110	112	112	115	117	119
R1L1	87	91	98	101	100	105	105	103	106	107	109	114	113	117	117	117
R1L2	87	91	100	119	111	117	129	126	130	128	133	129	122	121	110	110
R2L1	101	91	94	96	98	97	101	101	98	98	102	100	100	100	102	100
R2L2	97	109	112	106	121	125	125	127	128	129	132	130	135	137	141	145
R3	107	108	113	114	114	110	115	107	109	104	106	111	102	101	102	100

0000057

Individual body weights 50 ppm Females

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
A																
Z	131	133	139	136	136	136	137	130	128	126	122	116	118	106	121	117
R1	158	160	162	168	166	165	163	160	161	155	157	130	142	134	136	132
R2	152	157	158	156	158	162	161	165	168	161	171	174	173	154	152	137
L1	119	117	122	120	126	125	124	124	127	123	136	122	114	116	114	114
L2	130	132	136	136	138	138	139	136	136	132	140	133	134	132	132	127
R1L1	130	140	140	142	144	141	140	146	138	142	138	125	124	122	120	119
R1L2	128	129	138	136	136	138	148	141	131	133	126	98	90	104	116	114
R2L1	148	144	141	148	152	161	157	163	151	151	157	140	134	124	131	134
R2L2	107	110	114	108	104	104	107	110	109	112	107	102	102	104	107	105
R3	145	143	144	146	142	140	139	145	131	140	142	128	130	123	*27/1.77	
B																
Z	95	96	100	96	94	94	100	100	95	101	103	104	104	109	115	111
R1	92	91	94	94	92	89	94	95	93	92	92	94	94	94	104	103
R2	93	95	98	96	92	90	91	95	88	89	78	71	82	83	92	94
L1	114	118	122	122	120	120	125	129	119	118	117	115	114	115	121	124
L2	90	90	93	88	85	85	88	86	81	81	76	85	88	87	88	86
R1L1	89	89	94	94	92	94	91	92	87	89	89	91	92	88	92	92
R1L2	95	94	93	92	88	88	96	88	80	76	71	67	70	62	88	76
R2L1	110	111	116	114	113	112	115	114	110	107	103	99	106	101	103	100
R2L2	102	103	106	106	98	96	98	94	95	91	87	78	76	78	92	91
R3	98	98	100	96	96	90	91	92	86	81	76	69	70	79	92	95
C																
Z	93	93	91	92	93	89	102	90	95	87	86	81	84	84	92	81
R1	114	115	116	112	108	101	103	107	98	96	91	79	90	94	99	96
R2	112	114	113	114	112	113	113	111	107	104	92	80	76	86	99	99
L1	104	103	104	102	100	99	91	96	87	96	82	68	74	79	91	89
L2	123	126	130	132	128	134	136	130	123	114	96	92	96	99	103	103
R1L1	115	116	122	120	122	122	123	119	120	115	113	112	112	110	118	112
R1L2	90	80	91	70	72	72	72	74	74	70	78	80	84	84	84	86
R2L1	146	146	151	150	144	151	152	145	138	137	127	122	132	133	137	131
R2L2	107	108	109	118	105	115	104	103	98	98	81	78	89	94	98	94
R3	98	99	102	96	96	94	98	90	83	83	72	78	82	87	89	91

* DEAD

Individual body weights 50 ppm

Females

Week	52	56	60	64	68	72	76	78
A								
Z	109	114	112	99	84	72	77	83
R1	133	134	130	120	105	94	92	97
R2	124	120	113	108	96	99	103	104
L1	105	110	111	109	101	98	99	110
L2	119	126	123	109	92	78	85	90
R1L1	119	*13/10.77		-	-	-	-	-
R1L2	112	112	111	100	88	85	81	82
R2L1	131	128	123	111	86	84	87	88
R2L2	105	110	104	94	101	80	84	84
R3	*-	-	-	-	-	-	-	-
B								
Z	112	112	111	104	104	111	123	121
R1	103	102	101	98	91	93	95	99
R2	97	92	84	72	69	*6/2.78		-
L1	127	123	125	114	108	112	116	117
L2	81	72	68	69	66	*31/1.78		-
R1L1	96	90	89	79	76	76	83	82
R1L2	78	72	71	70	64	61	*25/2.78	
R2L1	98	90	91	89	86	*19/1.78		-
R2L2	93	94	93	87	84	80	84	81
R3	97	101	104	96	92	85	84	82
C								
Z	74	71	68	67	63	63	63	73
R1	93	86	85	76	82	79	80	78
R2	101	98	94	86	94	97	90	*3/4.78
L1	91	88	85	75	74	71	75	73
L2	104	106	105	93	90	81	80	83
R1L1	113	118	110	99	94	94	100	99
R1L2	87	86	75	65	64	64	71	*11/4.78
R2L1	135	130	125	120	116	117	127	*28/3.78
R2L2	92	94	83	78	75	76	82	*31/3.78
R3	94	90	81	89	83	97	96	*31/3.78

* DEAD

000059

Individual body weights 50 ppm Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D																
Z	57	66	85	95	104	108	107	107	105	102	96	94	88	84	78	91
R1	71	90	107	115	121	121	125	126	127	137	143	141	138	149	151	146
R2	76	91	96	107	103	104	105	110	110	122	134	135	132	140	144	148
L1	80	79	81	85	87	84	87	93	95	103	107	107	104	107	102	102
L2	87	92	90	88	93	93	95	106	106	117	126	125	119	130	128	122
R1L1	87	95	101	109	111	109	109	106	103	102	100	104	104	98	100	98
R1L2	87	98	103	107	112	107	108	105	109	106	105	106	110	103	117	114
R2L1	89	100	105	112	114	114	110	109	109	111	106	106	105	108	110	109
R2L2	97	102	102	105	118	105	103	103	106	104	105	110	104	106	107	108
R3	100	100	104	103	103	101	99	97	98	101	100	104	102	105	106	102
E																
Z	65	75	79	81	82	84	91	92	97	102	98	97	94	103	95	97
R1	78	87	96	105	111	117	106	118	122	127	131	137	138	145	154	156
R2	73	86	93	101	100	97	91	94	95	96	93	92	91	102	114	115
L1	79	89	95	104	105	106	106	111	112	112	117	119	119	112	110	115
L2	79	89	99	103	99	102	103	101	104	105	110	110	106	115	127	134
R1L1	82	89	91	89	91	91	95	94	97	95	98	96	96	95	98	92
R1L2	85	93	96	96	99	101	105	103	103	106	109	106	103	105	108	107
R2L1	96	95	90	95	98	100	106	110	108	107	108	107	101	104	104	103
R2L2	99	110	115	114	112	109	109	109	110	110	110	105	103	105	107	104
R3	113	106	116	117	119	120	121	119	125	123	124	121	120	121	122	118
F																
Z	66	81	91	107	114	124	129	134	142	158	152	154	150	150	147	143
R1	76	90	102	112	119	127	131	133	137	141	139	139	130	129	125	119
R2	73	85	95	101	101	107	105	107	111	113	117	117	116	117	123	124
L1	86	86	93	101	104	100	103	108	115	114	116	118	111	113	116	109
L2	86	95	101	107	102	105	110	110	114	120	123	124	120	123	128	130
R1L1	90	87	100	109	110	107	111	109	113	115	118	117	106	118	125	125
R1L2	87	93	112	115	117	117	117	111	110	113	105	96	86	98	100	98
R2L1	98	101	111	117	125	130	133	139	142	151	152	153	147	157	150	153
R2L2	100	91	95	99	100	99	103	101	105	107	108	109	108	121	120	115
R3	106	104	111	126	119	117	118	114	116	120	124	120	115	127	130	136

090000

Individual body weights 50 ppm Females

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
D																
Z	101	101	100	92	88	89	96	90	101	110	120	101	112	111	110	86
R1	148	147	151	148	150	148	150	136	139	137	146	145	136	136	130	93
R2	149	153	155	158	160	159	156	139	134	128	128	131	128	128	129	113
L1	95	94	97	96	98	90	90	82	83	86	85	83	86	85	82	78
L2	120	118	125	132	134	132	137	120	126	126	139	100	110	103	92	124
R1L1	104	104	110	102	104	113	106	99	100	90	89	87	88	94	106	100
R1L2	123	124	134	124	127	123	124	118	111	106	91	92	90	99	82	96
R2L1	109	115	116	112	116	116	115	112	107	105	102	100	88	89	96	98
R2L2	108	107	110	108	108	108	110	106	101	103	94	105	97	106	102	104
R3	105	109	110	1..	106	104	105	104	88	82	72	75	74	74	85	89
E																
Z	98	95	95	88	83	85	88	88	91	93	97	99	88	87	81	74
R1	159	163	161	164	162	162	162	156	153	154	146	138	146	154	159	161
R2	115	110	116	116	120	116	119	114	106	105	113	103	99	109	112	104
L1	119	120	124	120	112	114	114	117	116	117	118	118	114	108	105	102
L2	136	141	146	144	146	150	150	147	146	145	139	118	102	122	118	111
R1L1	94	92	94	92	90	86	93	82	81	90	95	100	104	98	113	102
R1L2	106	107	106	106	108	107	115	113	114	119	113	109	104	111	113	105
R2L1	100	96	96	92	86	88	93	88	88	88	91	93	98	108	111	103
R2L2	104	104	104	102	99	101	99	89	88	91	91	87	100	106	108	103
R3	121	115	120	118	106	117	120	115	117	116	122	122	114	114	119	111
F																
Z	140	137	136	126	123	122	125	125	128	130	134	122	112	111	118	114
R1	120	119	118	112	110	108	113	108	109	110	112	114	109	106	93	94
R2	124	125	127	124	122	121	127	121	121	114	111	117	110	97	97	91
L1	110	110	110	112	118	120	115	103	97	92	95	110	100	98	114	104
L2	129	132	136	138	140	142	144	139	141	141	140	140	136	127	132	132
R1L1	126	127	136	136	139	146	144	145	143	147	140	115	82	89	95	96
R1L2	97	94	102	98	96	96	98	95	99	102	95	88	88	86	90	93
R2L1	153	151	152	144	140	144	142	139	137	138	121	84	114	68	86	86
R2L2	115	123	120	120	120	126	125	118	115	115	118	73	66	117	113	114
R3	130	130	132	132	128	132	135	136	136	134	126	93	79	77	88	96

190061

Individual body weights 50 ppm **Females**

Week	52	56	60	64	68	72	76	78
D								
Z	104	98	87	79	82	74	*12/3.78	
R1	109	88	87	85	*13/1.78		-	-
R2	123	124	108	111	100	97	104	111
L1	79	74	69	69	66	61	66	63
L2	89	82	89	74	67	*19/1.78		-
R1L1	94	96	97	79	87	83	78	84
R1L2	94	100	99	89	83	85	90	82
R2L1	95	95	89	78	81	72	75	83
R2L2	99	104	96	95	92	82	82	89
R3	85	92	93	80	77	76	81	74
E								
Z	75	66	61	*27/11.77		-	-	-
R1	159	154	131	97	93	88	89	87
R2	110	102	99	81	81	77	79	78
L1	102	97	96	90	82	75	79	79
L2	114	114	111	109	107	104	112	103
R1L1	93	84	82	77	78	83	87	82
R1L2	105	96	*7/11.77		-	-	-	-
R2L1	104	90	88	83	78	87	92	94
R2L2	98	89	84	85	78	83	88	87
R3	98	92	95	79	76	87	87	93
F								
Z	117	114	113	113	109	107	117	123
R1	105	109	99	76	84	78	81	89
R2	96	90	84	86	72	62	*5/3.78	
L1	106	106	98	89	84	81	*12/3.78	
L2	132	140	132	111	96	81	83	88
R1L1	99	98	102	102	101	97	103	98
R1L2	95	94	95	93	91	91	89	85
R2L1	115	110	107	110	108	106	103	98
R2L2	89	88	87	86	82	76	69	74
R3	103	104	104	100	95	85	84	85

* DEAD

000062

Individual body weights 5 0 ppm **Females**

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G																
Z	67	82	84	88	92	87	95	93	97	97	96	99	98	102	104	100
R1	76	86	93	105	118	120	129	128	132	129	124	122	116	112	112	115
R2	75	81	82	84	86	87	90	93	98	97	104	102	102	109	108	106
L1	80	91	100	104	103	99	105	103	105	108	109	111	112	104	112	113
L2	87	84	95	96	101	96	106	106	108	108	108	110	106	113	111	122
R1L1	83	98	101	107	108	109	115	113	116	116	119	120	112	123	124	126
R1L2	89	98	107	112	113	111	113	111	115	115	116	119	98	115	119	116
R2L1	95	95	100	104	108	103	99	100	99	100	95	100	104	101	101	102
R2L2	101	103	103	109	102	100	101	103	100	105	103	106	115	111	112	107
R3	105	103	107	101	101	98	101	103	100	101	101	96	98	101	101	100
H																
Z	64	80	89	91	103	101	111	109	116	115	109	106	100	100	98	100
R1	71	74	78	77	86	89	89	89	91	96	96	96	98	100	103	93
R2	80	90	91	89	92	95	97	95	96	99	98	100	102	105	112	112
L1	81	92	97	105	105	103	106	104	104	108	110	116	112	114	122	122
L2	89	90	91	96	100	100	106	102	108	107	104	105	107	108	116	118
R1L1	87	90	97	104	107	110	115	117	120	132	126	127	125	119	130	123
R1L2	85	92	99	102	107	114	119	126	132	130	138	140	142	137	129	150
R2L1	91	93	96	96	99	100	104	105	110	108	109	106	104	98	106	101
R2L2	100	106	102	106	111	115	121	125	132	122	134	133	109	128	132	127
R3	103	116	106	120	120	113	101	111	110	110	108	110	109	106	120	122
K																
Z	62	70	79	85	89	95	96	97	94	91	88	80	75	71	68	65
R1	71	85	90	88	91	93	92	93	92	91	92	91	92	96	94	93
R2	82	81	94	105	114	120	126	126	130	134	137	134	130	131	133	130
L1	80	84	89	90	91	94	95	96	96	95	97	97	98	99	102	102
L2	87	93	97	101	102	100	101	103	101	101	102	100	99	101	104	104
R1L1	81	96	107	118	117	116	118	125	122	126	126	126	126	127	130	132
R1L2	85	92	102	109	107	107	109	114	112	114	115	113	120	116	118	111
R2L1	92	112	119	139	143	153	157	159	162	163	162	156	155	152	155	155
R2L2	90	98	107	111	102	113	117	121	125	127	130	124	123	123	120	115
R3	101	101	105	105	105	107	110	109	114	114	119	118	122	126	128	130

0000063

Individual body weights 50 ppm **Females**

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
G																
Z	104	102	100	100	98	99	102	98	99	97	97	80	82	94	91	93
R1	116	113	110	108	106	104	113	103	104	106	105	97	100	102	94	98
R2	108	108	108	104	106	104	109	105	100	95	101	96	94	97	92	94
L1	112	113	112	112	104	116	107	112	112	112	110	85	99	101	105	106
L2	111	115	109	106	104	102	105	106	103	103	101	93	94	95	94	95
R1L1	125	125	114	122	118	114	114	110	103	98	98	70	73	76	90	86
R1L2	110	108	114	108	110	106	102	100	98	94	83	87	80	85	93	95
R2L1	106	108	108	106	106	104	101	103	99	95	94	89	90	92	92	93
R2L2	114	111	113	106	106	103	105	93	92	90	87	72	84	86	98	96
R3	100	100	100	96	96	96	96	94	91	88	81	78	88	94	99	99
H																
Z	98	98	98	94	94	90	104	86	89	90	91	89	90	93	94	96
R1	101	102	100	96	102	102	102	96	96	95	86	68	63	71	83	93
R2	117	120	118	116	114	110	110	100	95	90	77	68	68	83	91	91
L1	126	130	130	128	128	125	124	118	110	108	98	83	98	101	105	100
L2	116	121	124	120	116	120	124	116	114	114	98	93	97	96	105	99
R1L1	126	127	130	134	134	135	136	136	139	134	126	123	112	104	106	111
R1L2	150	158	156	154	152	150	152	150	134	138	124	115	116	114	118	121
R2L1	101	114	104	104	105	110	100	102	101	91	92	78	67	71	72	82
R2L2	112	117	116	112	115	112	110	108	113	114	111	95	96	94	100	98
R3	125	127	128	127	133	128	130	130	121	120	111	100	84	77	78	74
K																
Z	63	63	66	66	72	75	80	78	78	81	82	85	86	85	81	88
R1	96	98	98	95	92	92	98	95	94	93	87	74	74	86	92	92
R2	127	121	124	127	126	124	122	119	119	116	115	111	112	114	115	109
L1	103	104	106	104	108	106	106	105	102	100	95	87	77	80	88	89
L2	104	104	106	104	108	105	105	105	103	100	95	97	96	97	96	93
R1L1	136	133	132	134	134	136	124	134	129	129	121	100	106	118	118	116
R1L2	101	120	122	124	125	126	123	110	109	106	106	96	94	89	97	96
R2L1	159	157	158	154	158	154	137	147	142	136	142	140	144	137	140	128
R2L2	120	92	92	90	90	93	93	88	89	88	89	100	96	98	96	100
R3	131	127	128	128	132	130	130	125	109	114	116	107	106	101	96	89

000064

Individual body weights 50 ppm **Females**

Week	52	56	60	64	68	72	76	78
G								
Z	96	98	98	89	88	93	90	91
R1	98	91	97	83	92	88	92	94
R2	90	81	89	94	85	95	100	108
L1	99	92	89	81	77	*10/2.78		-
L2	95	92	88	74	74	69	72	68
R1L1	92	90	89	87	89	93	89	88
R1L2	94	94	100	99	103	104	110	113
R2L1	94	92	91	81	79	80	73	75
R2L2	100	102	90	89	90	81	93	92
R3	102	108	110	117	106	108	116	122
H								
Z	98	101	105	98	101	98	97	100
R1	95	98	96	83	83	86	76	74
R2	93	98	83	78	82	77	75	76
L1	99	96	89	83	91	96	88	*4/4.78
L2	101	100	94	87	92	90	92	99
R1L1	107	104	99	90	95	94	94	102
R1L2	115	114	105	87	81	90	99	113
R2L1	86	84	87	87	81	80	85	85
R2L2	97	92	90	85	91	78	92	87
R3	69	*19/10.77		-	-	-	-	-
K								
Z	85	102	69	59	54	58	59	60
R1	88	106	91	92	94	102	105	132
R2	111	112	113	101	95	94	85	90
L1	86	80	87	81	74	73	73	72
L2	89	86	81	70	71	63	63	62
R1L1	116	98	102	91	92	126	102	*18/3.78
R1L2	100	76	102	95	96	100	108	*22/3.78
R2L1	128	122	125	113	122	100	*21/2.78	
R2L2	98	88	97	93	90	92	93	*21/3.78
R3	91	94	93	79	70	76	83	85

* DEAD

000065

Individual body weights 50 ppm

Females

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L Z	61	68	78	81	89	99	102	106	107	110	114	113	115	105	118	116
R1	73	81	87	89	89	93	94	95	98	99	104	103	108	111	110	109
R2	66	70	81	83	87	91	93	93	94	94	96	98	102	102	106	106
L1	72	89	95	99	95	95	103	101	106	105	104	100	105	106	108	106
L2	85	87	94	93	99	99	100	99	100	98	99	94	97	99	103	102
R1L1	86	91	92	96	97	99	99	97	99	98	104	101	99	101	105	105
R1L2	88	85	87	89	90	91	92	93	92	92	96	93	92	94	96	95
R2L1	94	92	94	102	104	110	105	107	110	112	113	110	109	118	114	113
R2L2	95	100	102	101	103	107	105	109	112	112	112	113	107	112	112	113
R3	115	110	118	120	116	116	111	113	120	119	124	117	119	121	124	128

Week	16	17	18	19	20	21	22	23	24	25	28	32	36	40	44	48
L Z	117	116	112	112	96	103	106	105	106	103	103	103	100	99	108	100
R1	114	115	110	112	112	110	110	110	111	105	105	109	116	114	119	114
R2	110	112	111	112	114	112	114	114	110	111	106	88	106	103	110	106
L1	110	110	108	114	110	106	109	107	108	105	106	83	90	97	96	92
L2	104	109	104	108	110	110	107	104	107	103	104	93	102	104	95	95
R1L1	105	107	104	106	106	101	105	100	96	98	99	86	80	89	96	96
R1L2	94	96	94	96	95	94	95	92	88	88	92	80	78	83	88	86
R2L1	117	124	118	118	118	116	116	112	108	96	92	88	96	94	94	94
R2L2	112	115	114	114	114	113	114	110	106	105	101	97	90	97	104	98
R3	132	131	129	131	144	132	135	135	128	123	118	114	118	116	120	118

Week	52	56	60	64	68	72	76	78
L Z	101	74	102	103	105	93	89	85
R1	113	90	103	93	93	83	82	83
R2	110	110	105	94	84	72	73	72
L1	93	86	88	83	85	77	87	*13/3.78
L2	94	80	95	92	87	78	80	84
R1L1	96	102	97	85	89	77	70	*22/3.78
R1L2	84	102	70	71	68	65	68	*20/3.78
R2L1	96	92	91	88	90	87	89	90
R2L2	94	91	89	87	85	78	84	75
R3	122	124	121	110	108	103	106	115

* DEAD

000096

Appendix 3

Individual data on time and cause of death of animals that died spontaneously or were killed in extremis, and individual data on time of death and on tumours of tumour-bearing animals

– Pathology code:

chloroprene concentration (ppm)	males	females
0	1 - 100	301 - 400
10	101 - 200	401 - 500
50	201 - 300	501 - 600

Animal house code : see page 6

Animal house code/pathology code :

Subgroup A:	1-10	101-110	201-210	301-310	401-410	501-510
Subgroup B:	11-20	111-120	211-220	311-320	411-420	511-520
	etc.	etc.				

The animals in each subgroup A: 1-10, 201-210, etc. and in each subgroup B, C, etc. are individually coded as follows:

Animal house code		Pathology code
Z	=	1, 11, etc.
R1	=	2, 12, etc.
R2	=	3, 13, etc.
L1	=	4, 14, etc.
L2	=	5, 15, etc.
R1L1	=	6, 16, etc.
R1L2	=	7, 17, etc.
R2L1	=	8, 18, etc.
R2L2	=	9, 19, etc.
R3	=	10, 20, etc.

- periods in which the experimental period was divided:

period 1 = week 1 - 38
period 2 = week 39 - 48
period 3 = week 49 - 58
period 4 = week 59 - 68
period 5 = week 69 - 78
period 6 = terminal sacrifice period (week 79 - 81)

- The week of the experiment in which the animal died and the period (see before) in which this week fell, are listed for each animal as well as the number of primary tumours.
- A few animals were completely lost for examination because of too advanced autolysis and/or cannibalism or loss of material during processing.
- The diagnose of the cause of death were based on a combination of clinical, macroscopic and microscopic pathological data.
- Tumours observed macroscopically but not examined microscopically because of loss during processing are indicated as unidentified tumours lost for microscopy.
- Abbreviation:
  poss. = possibly

MALES 0 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
11	60	4	D	0	unknown (partially lost by cannibalism)	
13	78	6	K	1		pancreas : small islet cell adenom
20	77	5	D	0	unknown (partially lost by cannibalism and autolysis)	
29	37	1	K	0	pneumonia	
41	78	6	D	0	heart thrombus	
42	78	6	K	1		thyroid: small parafollicular cell adenoma
47	74	5	K	0	unknown	
56	78	6	K	1		thyroid: small cystadenoma
61	78	6	K	1		thyroid: small follicular adenoma
62	78	6	K	1		testis: Leydig cell tumour
64	78	6	K	1		thyroid: small follicular adenoma
66	9	1	D	0	poss. heart thrombus (partially lost by cannibalism)	
67	78	6	K	1		gall bladder: multiple polyps
69	9	1	D	0	poss. heart thrombus	
70	8	1	K	0	poss. heart thrombus (partially lost by cannibalism)	
71	78	6	D	1	reticulum cell sarcoma	abdominal cavity: reticulum cell sarcom
73	78	6	K	1		stomach: unidentified papilloma-li lesion, lost for microscopy

000070

MALES 0 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
77	9	1	D	0	unknown	
78	14	1	D	0	unknown	
79	13	1	D	0	poss. heart thrombus	
80	78	6	K	2		kidney: cortical adenocarcinoma adrenals: medium-sized cortical aden
81	67	4	D	0	heart thrombus	
84	78	6	K	1		adrenals: small corticol adenoma
93	78	6	K	1		kidney: cortical adenocarcinoma
94	78	6	K	1		adrenals: medium-sized cortical aden
96	78	6	K	1		adrenals: large cortical adenoma
100	68	4	D	0	heart thrombus	

000071

MALES 10 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
106	78	6	K	1		adrenal: small phaeochromocytoma
108	66	4	D	?	unknown (completely lost by cannibalism	
109	78	6	K	1		gall bladder: polyp
110	78	6	K	1		adrenal: medium-sized phaeochromocytoma
112	78	6	K	1		adrenal: small phaeochromocytoma
117	78	6	K	1		gallbladder: polyp
120	78	6	K	1		liver: neoplastic nodule
128	78	6	K	1		thyroid: small papillary adenoma
133	3	1	D	?	unknown (completely lost by cannibalism)	
146	78	6	K	1		stomach: unidentified papilloma-like lesion, lost for microscopy
147	45	2	D	0	unknown	
149	78	6	K	1		gallbladder: polyp
150	8	1	D	?	unknown (completely lost by cannibalism)	
152	73	5	K	1		skin: unidentified tumour-like lesion lost for microscopy by cannibalism
156	76	6	D	0	unknown	
162	78	6	K	2		adrenal: cortical adenocarcinoma, small phaeochromocytoma
164	78	6	K	1		gallbladder: polyp
176	78	6	K	1		thyroid: small follicular adenoma

000072

MALES 10 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
180	78	6	K	1		adrenal: small cortical adenoma
187	78	6	K	1		liver: unidentified tumour-like lesion, lost for microscopy
188	78	6	K	1		gallbladder: multiple polyps
189	12	1	K	0	unknwon	
195	73	5	D	0	poss. heart thrombus	
199	78	6	K	1		gallbladder: polyp

000073

MALES 50 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
202	73	5	D	0	unknown	
205	78	6	K	1		pancreas: islet cell adenoma
207	78	6	D	1	unknown	pancreas: islet cell adenoma
208	78	6	K	1		adrenal: small cortical adenoma
212	78	6	K	1		adrenal: large cortical adenoma
215	70	5	D	?	unknown (completely lost by cannibalism)	
226	15	1	D	0	unknown	
231	78	6	K	1		pituitary: small adenoma
233	78	6	K	1		adrenal: small phaeochromocyto-
237	78	6	K	1		adrenal: medium-sized cortical adenoma
238	78	6	K	1		gallbladder: polyp
243	78	6	K	1		Zymbal gland: sebaecous adenoma
244	78	6	D	0	unknown (partially lost by autolysis)	
249	78	6	D	?	unknown (completely lost)	
250	78	6	K	1		adrenal: small cortical adenoma
252	78	6	K	2		adrenal: medium-sized phaeochromocytoma stomach: unidentified papilloma-like lesion, lost for microscopy
254	28	1	D	?	unknown (completely lost by cannibalism)	

0000074

MALES 50 ppm

rat	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
255	52	3	D	0	poss. heart thrombus	
256	71	5	K	0	abscess abdominal cavity	
257	78	6	K	1		adrenal: small cortical adenoma
258	78	6	K	1		adrenal: small cortical adenoma
260	78	6	K	1		adrenal: small cortical adenoma
261	72	5	D	0	poss. heart thrombus and abscesses in liver and salivary gland	
262	78	6	K	2		thyroid: small papillary adenoma stomach: papilloma
263	78	6	K	1		adrenal: medium-sized cortical adenoma
266	78	6	K	1		adrenal: small cortical adenoma
279	78	6	K	1		adrenal: malignant phaeochromocytoma
280	78	6	K	1		stomach: papilloma
290	78	6	K	2		adrenals: small cortical adenoma, medium-sized phaeochromocytoma

000075

FEMALES 0 ppm

rat no.	time of death (week)	period	killed(K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
304	74	5	K	0	unknown	
305	62	4	D	?	unknown (completely lost by cannibalism)	
307	72	5	D	0	unknown (partially lost by cannibalism)	
312	56	3	D	0	heart thrombus	
313	78	6	K	1		stomach: unidentified papilloma-like lesion, lost for microscopy
314	78	6	K	1		colon: adenomatous polyp
316	66	4	D	?	unknown (completely lost by cannibalism)	
318	69	5	D	1	heart thrombus	gallbladder: polyp
320	65	4	D	0	unknown	
321	52	3	D	0	unknown	
322	70	5	D	0	heart thrombus	
324	70	5	D	0	heart thrombus	
325	60	4	D	0	unknown	
326	67	4	D	0	unknown	
332	69	5	D	0	heart thrombus	
333	75	5	K	0	heart thrombus	
334	78	6	K	1		thyroid: medium-sized papillary adenoma
336	54	3	D	0	heart thrombus	
338	25	1	D	0	heart thrombus	

000076

FEMALES 0 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
339	78	6	K	1		pituitary: medium-sized adenoma
340	65	4	D	0	heart thrombus	
342	61	4	D	0	heart thrombus	
343	66	4	D	0	heart thrombus	
347	78,	6	K	2		pituitary: large adenoma; pancreas: islet cell adenocarcinoma
348	78	6	D	0	poss. renal disease	
350	78	6	K	1		thyroid: medium-sized follicular adenoma
351	73	5	K	0	liver cysts	
353	13	1	D	0	poss. heart thrombus	
356	75	5	K	0	heart thrombus	
363	?.	?	?	?	completely lost	
366	78	6	K	1		rib (bone): osteosarcoma (metastazizing)
372	69	5	D	0	heart thrombus	
375	45	5	D	?	unknown (completely lost by cannibalism)	
378	78	6	K	1		nose: adenoma of Bowman's glands
379	74	5	D	0	heart thrombus	
381	17	1	D	0	unknown	

000077

FEMALES 0 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
383	69	5	D	?	unknown (completely lost by cannibalism)	
385	78	6	K	1		adrenal: cortical carcinoma
389	50	3	D	0	poss. heart thrombus	
391	71	5	D	0	heart thrombus	
392	75	5	D	0	heart thrombus	
393	49	3	D	0	unknown (partially lost by cannibalism)	
394	59	4	D	0	unknown	
395	65	4	D	0	heart thrombus	
396	72	5	K	0	pleuro-pneumonia + heart thrombus	
397	74	5	D	0	heart thrombus	
400	57	3	D	0	unknown (partially lost by cannibalism)	

0000078

FEMALES 10 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
401	66	4	D	0	unknown	
406	78	6	K	1		ovary: granulosa-theca cell tumour
411	78	6	K	1		stomach: unidentified papilloma-like lesion lost for micros-copy
415	56	3	K	0	poss. stomach ulcer	
421	69	5	D	?	unknown (completely lost by cannibalism)	
425	42	2	D	?	unknown (completely lost by cannibalism)	
431	67	4	D	0	unknown (partially lost by cannibalism)	
433	68	4	D	0	unknown (partially lost by cannibalism)	
438	78	5	D	0	unknown	
442	72	5	D	0	unknown	
446	33	1	D	?	unknown (completely lost by cannibalism)	
448	71	5	D	0	unknown (partially lost by cannibalism)	
450	78	6	K	1		gallbladder: polyp parathroid: adenoma
451	73	5	D	1	unknown	
452	45	2	K	0	unknown	
453	41	2	D	?	unknown (completely lost cannibalism)	

0000079

FEMALES 10 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
456	35	1	D	?	heart thrombus	
457	68	4	K	0	unknown	
458	77	5	D	1	heart thrombus	thyroid: medium-sized follicular adenoma
460	57	3	K	0	unknown	
464	78	6	K	1		thyroid: small follicular adenoma
465	73	5	D	0	unknown	
466	38	1	D	0	unknown (partially lost by cannibalism)	
468	78	6	K	1		ovary: granulosa-theca cell tumour
470	72	5	D	0	unknown (partially lost by cannibalism)	
471	78	6	K	1		stomach: unidentified papilloma-like lesion, lost for microscopy
474	78	6	K	1		gallbladder: polyp
476	78	6	K	1		thyroid: small parafollicular cell adenoma
481	63	4	D	0	unknown	
484	78	6	D	0	unknown	
492	49	3	D	?	unknown (completely lost by cannibalism)	
493	39	2	D	0	unknown (partially lost by cannibalism)	

000080

FEMALES 10 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
494	66	4	K	1	unknown	thyroid: small parafollicular cell adenoma
495	40	2	D	0	unknown	

180000

FEMALES 50 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
502	78	6	K	1		thyroid: medium-sized parafollicular cell adenoma
503	55	3	K	1	cannibalism	addipose tissue: lipoma
510	43	2	D	0	heart thrombus (partially lost by cannibalism)	
513	71	5	D	0	unknown poss. cannibalism	
515	70	5	K	0	poss. cannibalism	
517	73	5	K	0	heart thrombus and renal disease	
518	69	5	D	0	poss. heart thrombus	
523	78	6	D	0	poss. heart thrombus	
525	78	6	K	1		thyroid: small papillary adenoma
526	78	6	D	0	heart thrombus	
528	78	6	D	0	unknown	
530	78	6	D	0	poss. heart thrombus	
531	76	5	D	0	unknown (completely lost by autolysis)	
532	68	4	K	2	poss. heart thrombus and pneumonia	gallbladder: polyp; nose: adenomacarcinoma of Bowman's glands
533	78	6	K	1		gallbladder: polyp
535	68	4	K	0	poss. heart thrombus	

0000082

FEMALES 50 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
540	78	6	K	1		thyroid: small papillary adenoma
541	62	4	D	?	unknown (completely lost by cannibalism)	
547	60	4	D	0	heart thrombus	small cortical adenoma adrenal
549	78	6	K	1		
553	75	5	D	?	unknown (completely lost by cannibalism)	
554	76	5	D	?	unknown (completely lost by cannibalism)	
555	78	6	K	1		liver: unidentified tumour-like lesion, lost for microscopy
563	78	6	K	1		thyroid: medium-sized follicular adenoma
564	71	5	D	0	poss. abscess haed-neck region	
569	78	6	K	2		ovary: granulosa-theca cell tumour parotid salivary gland: adenoma
574	78	6	D	0	heart thrombus, renal disease and hepatic disease	
578	78	6	K	2		gallbladder: polyp adrenal: cortical carcinoma
579	78	6	K	1		adrenal: medium-sized cortical adenoma
580	56	3	K	0	unknown	

0000083

FEMALES 50 ppm

rat no.	time of death (week)	period	killed (K) or found dead (D)	no. of primary tumours	CAUSE OF DEATH	TUMOURS
586	76	5	D	0	heart thrombus	
587	77	5	D	0	unknown (partially lost by cannibalism)	
588	73	5	D	0	unknown (partially lost by cannibalism)	
589	77	5	D	0	unknown (partially lost by cannibalism)	
590	78	6	K	1		adrenal: small cortical adenoma
592	78	6	K	1		pancreas: islet cell adeno-carcinoma
594	76	5	D	0	heart thrombus	
596	77	5	D	?	unknown (completely lost by cannibalism)	
597	77	5	D	0	unknown (partially lost by cannibalism)	
600	78	6	K	1		adrenals: medium-sized phaeochromocytoma

8782 1503

000084

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Organ. Name	E I DUPONT DENEMOURS & CO INC 11
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D & B NO. (11)	20
Contractor	21
Doc Type	• RI • UP • HEASD 8D. SU HS FN 22
Doc Title	SUBCHRONIC (26-WEEK) INHALATION TOXICITY STUDY WITH BETA — CHLOROPRENE IN RATS WITH COVER LETTER 23

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

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3/11/85 RS.

878215014

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56 Wuppertal-Elberfeld
Germany
Attn. Dr. E. Löser

date 22- 1-1979
our ref. 7463/VJF/MV
your ref.
your date
encl. 10
on. B77/2650

Dear Dr. Löser,

Please find enclosed 10 copies of a concise report on the gross and microscopic pathology concerning the 26-week inhalation study with β-chloroprene in rats.
A preliminary summary of this study (attached) not including the histopathology, has been sent to you in December 1978.

Kind regards,

Victor J. Feron

Chloropren
Tox Com.

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CENTRAAL INSTITUUT VOOR VOEDINGSONDERZOEK TNO

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Zeist

CENTRAL INSTITUTE FOR NUTRITION AND FOOD RESEARCH
INSTITUT CENTRAL DE LA NUTRITION ET DE L'ALIMENTATION
ZENTRALINSTITUT FÜR ERNÄHRUNGSFORSCHUNG

Subchronic (26-week) inhalation toxicity study with ß-chloroprene in rats

(preliminary summary)

1. Groups

A subchronic (26-week) inhalation toxicity study including interim kills after 4 and 13 weeks was carried out with chloroprene in Wistar rats. Four groups of 40 male and 40 female rats each were exposed to atmospheres containing 0, 10, 33 and 100 ppm chloroprene respectively during 6 hours a day, 5 days a week. Interim kills comprised 10 rats/sex/group.

2. Concentrations of ß-chloroprene in the atmospheres

group no.	concentration in ppm		
	nominal	actual	standard error
8197 (control)	0	0	0
8198	10	9.8	0.8
8199	33	32.4	1.6
8200	100	100.2	5.6

3. Mortality, condition and behaviour

No mortality occurred. Condition and behaviour were not visibly affected by ß-chloroprene exposure.

Signs of treatment-related alopecia were not observed.

4. Body weights

In males slight growth retardation occurred at the 100 ppm level, while at the 33 ppm level relatively low body weights were seen. In females body weights of test animals were occasionally statistically significantly lower than those of the controls, but there was no evidence of a consistent or dose-related effect.

5. Haematology

No relevant changes.

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Total or partial publication of this report without written assent is not allowed.

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6. Biochemistry

No relevant changes.

7. Urinalysis

Females of the high dose group produced more urine with a decreased creatinine content. This effect was not seen in males.

8. Organ-to-body weight ratios (tables 1 and 2)

A statistically significant increase of the relative liver weight was seen in males of the high dose group after 26 weeks, in females of the high dose group after 13 weeks, and in females of each of the test groups after 26 weeks. The last-mentioned increases were positively dose-related.

The relative weight of the kidneys of males of the 100 ppm group and of females of the 33 and 100 ppm group was increased after 26 weeks. In addition, the relative kidney weight of males killed after 13 weeks was high, though not statistically significantly higher than that of the controls.

A slightly increased adrenal-to-body weight ratio was seen in females of the top-dose group after 26 weeks only.

9. Gross pathology

Macroscopic examination of the animals killed after 13 weeks did not reveal any relevant abnormalities. The observed changes were only minor, unrelated to treatment and occurred in test as well as in control animals.

Macroscopic examination of the animals killed after 26 weeks did not reveal any tumours or lesions suspected of being tumours. Focal alopecia occurred in several animals (table 3) mainly of the 33 ppm group. The other changes observed macroscopically consisted of slight hydrometra of the uterus, discoloured liver or lungs and small superficial lesions in liver or kidneys; these changes were observed in test and control animals as well.

Table 1 .Mean body weights (g), mean relative organ weights (g/100 g body weight) and the standard error of the mean of groups of ten male and ten female rats after an experimental period of 13 weeks

group no.	β-chloroprene ppm	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	thymus	pituitary	thyroid	adrenals	lungs
						MALES							
8197B	0	334.	.319	.71	3.54	.159	.57	.91	.113	.0041	.0069	.0134	.40
		(8.)	(.012)	(.06)	(.08)	(.004)	(.02)	(.03)	(.008)	(.0004)	(.0014)	(.0008)	(.02)
8198B	10	312.*	.331	.67	3.38	.161	.59	.94	.102	.0046	.0069	.0130	.41
		(5.)	(.012)	(.02)	(.12)	(.009)	(.01)	(.03)	(.007)	(.0004)	(.0007)	(.0008)	(.01)
8199B	33	331.	.332	.70	3.68	.156	.58	.95	.110	.0041	.0059	.0129	.42
		(12.)	(.015)	(.03)	(.10)	(.006)	(.02)	(.03)	(.003)	(.0005)	(.0004)	(.0009)	(.01)
8200B	100	309.	.333	.78	3.83	.144	.59	.99	.104	.0048	.0068	.0152	.43
		(10.)	(.011)	(.03)	(.12)	(.007)	(.02)	(.04)	(.004)	(.0006)	(.0007)	(.0007)	(.01)
						FEMALES							
8197B	0	193.	.384	.75	3.51	.186	.90	.036	.180	.0104	.0095	.0287	.59
		(2.)	(.006)	(.05)	(.08)	(.004)	(.01)	(.002)	(.013)	(.0012)	(.0006)	(.0017)	(.04)
8198B	10	196.	.371	.70	3.64	.196	.88	.036	.162	.0088	.0107	.0295	.54
		(5.)	(.010)	(.02)	(.09)	(.008)	(.03)	(.001)	(.010)	(.0011)	(.0020)	(.0008)	(.02)
8199B	33	190.	.381	.74	3.72	.176	.89	.033	.147*	.0093	.0086	.0274	.57
		(4.)	(.011)	(.02)	(.10)	(.006)	(.02)	(.002)	(.006)	(.0016)	(.0014)	(.0018)	(.05)
8200B	100	196.	.376	.76	4.56*	.189	.87	.037	.144*	.0106	.0089	.0272	.57
		(3.)	(.006)	(.02)	(.45)	(.005)	(.02)	(.002)	(.010)	(.0013)	(.0007)	(.0021)	(.03)

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

UNDER EACH MEAN THE STANDARD ERROR OF THE MEAN (IN BRACKETS) IS GIVEN.

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Table 2 .Mean body weights (g), mean relative organ weights (g/100 g body weight) and the standard error of the mean of groups of 20 male and 20 female rats after an experimental period of 26 weeks

group no.	S-chloroprene ppm	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	thymus	pituitary	thyroid	adrenals	lungs
						MALES							
8197C/D	0	389.	.298	.64	3.20	.133	.49	.86	.060	.0020	.0044	.0095	.39
		(9.)	(.006)	(.01)	(.06)	(.004)	(.01)	(.02)	(.004)	(.0002)	(.0002)	(.0004)	(.01)
8198C/D	10	399.	.297	.60*	3.15	.138	.48	.83	.074*	.0025	.0045	.0099	.37
		(10.)	(.006)	(.01)	(.07)	(.005)	(.01)	(.02)	(.005)	(.0002)	(.0003)	(.0005)	(.01)
8199C/D	33	375.	.297	.66	3.24	.128	.51	.81	.064	.0024	.0050	.0097	.38
		(9.)	(.005)	(.01)	(.05)	(.003)	(.01)	(.04)	(.003)	(.0002)	(.0002)	(.0004)	(.01)
8200C/D	100	374.	.296	.68*	3.42*	.126	.51	.76	.059	.0024	.0044	.0094	.38
		(8.)	(.004)	(.01)	(.07)	(.003)	(.01)	(.05)	(.004)	(.0002)	(.0004)	(.0005)	(.01)
						FEMALES							
8197C/D	0	228.	.357	.67	3.03	.172	.76	.030	.091	.0053	.0055	.0212	.49
		(3.)	(.008)	(.01)	(.06)	(.004)	(.01)	(.001)	(.004)	(.0002)	(.0002)	(.0004)	(.01)
8198C/D	10	223.	.344	.69	3.24*	.163	.76	.029	.092	.0050	.0059	.0225	.50
		(4.)	(.006)	(.01)	(.08)	(.003)	(.01)	(.001)	(.003)	(.0003)	(.0004)	(.0007)	(.01)
8199C/D	33	224.	.347	.71*	3.37***	.170	.78	.030	.089	.0056	.0063*	.0216	.50
		(5.)	(.006)	(.01)	(.07)	(.006)	(.02)	(.001)	(.003)	(.0003)	(.0004)	(.0007)	(.01)
8200C/D	100	220.	.351	.72*	3.52***	.170	.78	.031	.092	.0050	.0059	.0247***	.50
		(3.)	(.005)	(.02)	(.07)	(.004)	(.01)	(.001)	(.005)	(.0002)	(.0003)	(.0006)	(.01)

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

UNDER EACH MEAN THE STANDARD ERROR OF THE MEAN (IN BRACKETS) IS GIVEN.

Table 3. Incidence of focal alopecia in animals killed at week 26

	♂♂				♀♀			
	ppm β-chloroprene							
	0	10	33	100	0	10	33	100
Number of animals examined	20	20	20	20	20	20	20	20
alopecia: slight	1	0	4*)	0	0	2	0	0
moderate to severe	0	0	0	1	0	0	4**)	0

*) Three out of the four affected rats were present in one cage containing a total of four rats.

**) All four affected rats were present in one and the same cage containing a total of four rats; thus each rat in the cage showed focal alopecia. Three of these rats showed slight alopecia already after a test period of three months.

SUB-CHRONIC (26-WEEK) INHALATION TOXICITY STUDY WITH β-CHLOROPRENE IN RATS

PATHOLOGY

Macroscopic examination

Macroscopic examination of the animals killed after 13 weeks did not reveal any relevant abnormalities. The observed changes were only minor, unrelated to treatment and occurred in test as well as in control animals.
Gross examination of the animals killed after 26 weeks did not reveal any tumours or lesions suspected of being tumours. Focal alopecia occurred in several animals mainly of the 33 ppm group. The other changes observed macroscopically consisted of slight hydrometra of the uterus, discoloured liver or lungs and small superficial lesions in liver or kidneys; these changes were observed in test and control animals as well. Testicular atrophy was noticed in one rat of the 33 ppm group and in one rat of the 100 ppm group.

Microscopic examination

Degree and incidence of the histopathological changes observed (see table) were similar in the control and high-dose group both after 13 and 26 weeks. None of these abnormalities, which are common findings in the strain of rats used, could, therefore, be related to the test substance.
Bilateral testicular atrophy, which occurred in two of the 20 high-dose males killed after 26 weeks, is not unusual in the strain of Wistar rats used; even in 13-week studies an incidence of bilateral testicular atrophy of 10% is not uncommon, and recently even a "spontaneous" 20% incidence has been found in a 13-week study. Since, moreover, the microscopic examination of the testes of the other high-dose males did not produce any indication of degenerative changes of seminiferous tubules, the bilateral testicular atrophy found in 10% of the top-dose animals is considered a fortuitous finding without any toxicological relevance.

In conclusion, gross and microscopic pathological examinations did not reveal any alterations that could be attributed to β-chloroprene.

TABLE HISTOPATHOLOGICAL CHANGES AND THE NUMBER OF ANIMALS SHOWING THE OBSERVED LESIONS IN THE VARIOUS GROUPS*

site and type of lesions	incidence of lesions							
	males				females			
	13 wk		26 wk		13 wk		26 wk	
	ppm β chloroprene							
	0	100	0	100	0	100	0	100
Kidneys	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20
1. Tubular nephrosis a) slight	6	4	13	11	2	0	5	3
b) moderate	0	2	1	0	0	0	0	0
2. A few proteinaceous droplets in the cytoplasm of tubular epithelial cells	2	5	1	0	0	0	0	0
3. Calcareous deposits in the intercortico/medullary layer and/or papilla	1	0	0	0	0	0	0	0
4. A few aggregates of mononuclear inflammatory cells	0	1	3	2	0	0	1	3
5. A few dilated tubules in the cortex	0	1	2	3	0	0	1	0
Liver	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20
1. Increased number of RES cell aggregates accompanied by a few necrotic hepatocytes	2	2	5	6	1	3	7	7
2. Periportal aggregates of a few mononuclear inflammatory cells	2	3	3	2	5	1	10	7
3. Slight bile duct proliferation	0	0	0	1	0	0	0	2
4. Focal vacuolation of hepatocytes	0	0	0	1	0	0	0	1
Pancreas	(9)	(10)	(20)	(20)	(9)	(10)	(20)	(20)
1. Aggregates of mononuclear inflammatory cells	2	0	1	0	0	3	1	0
2. Granuloma	1	0	0	2	0	0	0	0
Lungs	(10)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. Peribronchial, peribronchiolar and/or perivascular lymphoid aggregates a) slight	8	9	19	19	10	7	17	15
b) moderate	1	1	0	1	0	1	1	0
2. Focal pneumonitis	0	0	0	0	0	1	0	0
3. A few calcareous deposits in arterial walls	0	0	1	0	0	0	1	0
Trachea	(10)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. A few subepithelial aggregates of mononuclear inflammatory cells	5	2	8	7	6	2	6	2

* the number of animals examined is given in brackets

TABLE (CONTINUED 1)

site and type of lesions	incidence of lesions							
	males				females			
	13 wk		26 wk		13 wk		26 wk	
	ppm β chloroprene							
	0	00	0	100	0	100	0	100
Larynx	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. A few subepithelial aggregates of mononuclear inflammatory cells	0	0	1	0	0	0	0	0
Heart	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. A few focal aggregates of mononuclear inflammatory cells	1	1	2	1	0	0	0	0
Stomach	(9)	(10)	(20)	(20)	(10)	(10)	(18)	(20)
1. A few dilated fundic glands	0	0	3	4	0	0	2	3
Testicles	(8)	(9)	(20)	(20)				
1. A few atrophic tubules	0	0	1	0				
2. Bilateral atrophy	0	0	0	2				
Epididymides	(9)	(10)	(20)	(20)				
1. A few aggregates of mononuclear inflammatory cells	1	3	3	2				
Prostate	(9)	(10)	(20)	(20)				
1. A few aggregates of mononuclear inflammatory cells	0	1	2	1				
Uterus					(10)	(10)	(20)	(19)
1. Slightly to moderately dilated uterine horn					0	1	3	1
2. Slight endometritis					1	1	0	1
Small intestines	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. Parasites	0	0	1	0	0	0	0	0
Urinary bladder	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(18)
1. A few subepithelial infiltrates of mononuclear inflammatory cells	0	0	1	1	0	0	0	2
2. Proteinaceous plug	1	0	0	1	0	0	0	0
3. Chronic cystitis	0	0	0	1	0	0	0	0
Pituitary	(7)	(9)	(14)	(18)	(9)	(10)	(19)	(18
1. Colloid-filled cyst.	0	0	2	1	0	0	0	0
Thyroid	(9)	(10)	(20)	(19)	(10)	(10)	(20)	(20
1. Slightly activated appearance	3	2	3	6	1	1	1	2
2. Ultimobranchial remnant	0	0	0	1	0	2	0	0

TABLE (CONTINUED 2)

site and type of lesions	incidence of lesions							
	males				females			
	13 wk		26 wk		13 wk		26 wk	
	ppm β chloroprene							
	0	100	0	100	0	100	0	100
Adrenals	(9)	(10)	(20)	(19)	(10)	(10)	(20)	(20)
1. Slight vacuolation of cells in the zona fasciculata	0	0	2	2	0	0	0	0
2. Haemocyst	0	0	0	0	0	0	0	2
Submaxillary salivary glands	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. A few aggregates of mononuclear inflammatory cells	0	0	1	1	0	0	0	0
Sublingual salivary glands	(9)	(10)	(19)	(16)	(10)	(6)	(20)	(15)
1. Focal proliferation of demi-lune cells	1	0	0	0	0	0	1	0
2. Slight hyperplasia of ductular epithelium	0	0	0	0	0	0	1	0
Exorbital lachrymal glands	(9)	(10)	(19)	(20)	(10)	(10)	(20)	(20)
1. Dacryoadenitis	0	0	1	1	0	0	0	2
2. A few foci of mononuclear inflammatory cells	0	0	2	4	0	0	2	1
Nose	(10)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. Rhinitis	0	0	0	0	0	0	2	0
Mesenteric lymph nodes	(9)	(10)	(20)	(20)	(9)	(8)	(19)	(20)
1. Slightly increased number of histiocytes in the medulla	1	0	0	0	1	0	1	0
Skin/subcutis	(9)	(9)	(20)	(20)	(10)	(9)	(19)	(18)
1. Acanthosis	1	0	0	0	0	0	0	0
Thymus	(9)	(10)	(20)	(20)	(10)	(9)	(20)	(19)
1. Focal "starry sky" appearance	0	0	0	0	1	0	0	0
2. Slightly increased number of histiocytes	0	0	0	0	0	0	0	1
Coecum	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
1. Parasite	0	0	0	0	0	0	2	0
Spleen	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Skeletal muscle	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Coagulating glands	(9)	(10)	(20)	(20)				

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TABLE (CONTINUED 3)

site and type of lesions	incidence of lesions							
	males				females			
	13 wk		26 wk		13 wk		26 wk	
	ppm β chloroprene							
	0	100	0	100	0	100	0	100
Seminal vercicles	(9)	(10)	(20)	(20)				
Mammary glands					(9)	(8)	(13)	(17)
Aorta	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Oesophagus	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Spinal cord	(7)	(10)	(18)	(17)	(8)	(8)	(18)	(16)
Colon	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Sciatic nerve	(9)	(10)	(16)	(15)	(10)	(10)	(14)	(13)
Brain	(9)	(9)	(20)	(20)	(10)	(10)	(20)	(20)
Ovaries					(10)	(10)	(20)	(20)
Parotid salivary glands	(9)	(10)	(20)	(20)	(10)	(10)	(20)	(20)
Eyes	(8)	(10)	(20)	(20)	(10)	(10)	(20)	(19)
Axillary lymph nodes	(8)	(10)	(20)	(20)	(9)	(10)	(19)	(18)
Abdominal wall	(8)	(10)	(20)	(20)	(10)	(10)	(18)	(20)
Sternum with bone marrow	(10)	(10)	(20)	(20)	(10)	(10)	(20)	(20)

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Contractor —	21
Doc Type • RI • U.P • H.E.A.S.D 8D. . S.U H.S F.N	22
Doc Title INHALATION EMBRYOTOXICITY/ TERATOGENICITY STUDIES WITH BETA—CHLOROPRENE IN RATS (SUMMARY AND TABLES)	23

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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INHALATION EMBRYOTOXICITY/TERATOGENICITY STUDIES WITH B-CHLOROPRENE IN RATS (SUMMARY AND TABLES)

H.B.W.M. Koëter

December 1979

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INHALATION EMBRYOTOXICITY/TERATOGENICITY STUDIES WITH β-CHLOROPRENE IN RATS

SUMMARY

1. Pregnant rats were exposed to atmospheres containing 0 (control), 10, 25, 75 or 175 ppm β-Chloroprene for 6 hours/day from day 6-16 of gestation in a preliminary study, and from day 4-16 of gestation in a final study.

2. During the first day of treatment in both the preliminary and the final study salivation was observed in several rats of the 75 ppm group and in nearly all rats of the 175 ppm group.
In the final study, treatment-related focal alopecia was observed at the highest level.

3. Diminished food consumption, weight gain and food efficiency occurred in the 25, 75 and 175 ppm dose groups in both studies during treatment. These effects were still present during the post-treatment period, in the 75 and 175 ppm dose groups.

4. In the preliminary study, no signs of embryotoxicity or teratogenicity were observed.
In the final study, several significant differences occurred in numbers of corpora lutea, implantation sites, embryonic resorptions, post-implantation loss and live foetuses per litter between controls and lowest and highest levels. However, these differences were not considered to be related to the treatment.
Empty uterus weight and foetal weight were lower in the two highest dose groups than in the control group.

5. Skeletal and visceral examination of the foetuses of the control and high-dose group of the final study did not reveal any abnormalities attributable to the treatment. Ossification of the foetal skeletons was slightly returned in top-dose rats as compared to the controls.

6. On the basis of maternal growth depression in the 25, 75 and 175 ppm dose groups, it is concluded that β-Chloroprene when dosed by inhalation from day 4-16 of pregnancy resulted in a no-toxic effect level for pregnant rats of 10 ppm.
Decreased foetal weight and slight retardation in bone development justify to consider 175 ppm as slightly foeto-toxic.
The absence of relevant changes in foetuses justifies the conclusion that the exposure of rats to β-Chloroprene at levels up to 175 ppm during day 4-16 of pregnancy did not exert any teratogenic effect on foetuses.

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TABLE 1 - NOMINAL AND MEAN ACTUAL CONCENTRATIONS OF β-CHLOROPRENE IN THE TEST ATMOSPHERE
PRELIMINARY STUDY

concentration	group numbers			
	1375	1376	1377	1378
nominal concentration	10	25	75	175
mean actual concentration during an exposure period of 12 days	10.18	24.38	75.05	175.2
standard deviation	0.35	1.53	2.09	6.9
standard error of the mean	0.10	0.44	0.61	2.0
mean concentration/rat/day during 10 days of treatment	10.14	24.44	75.22	174.7
standard deviation	0.02	0.20	0.18	0.2
standard error of the mean	0.01	0.08	0.07	0.0

TABLE 2 - MATERNAL PERFORMANCE
PRELIMINARY STUDY

observations	ppm β-chloroprene dosed by inhalation				
	0	10	25	75	175
number of females placed with males	7	7	7	7	7
number of females mated	7	7	7	6	7
number of females pregnant	7	7	4	6	6
pregnancy rate (percentage)	100	100	57.14	100	85.
number of females bearing live foetuses	7	7	4	6	6
number of animals showing focal alopecia	0	0	0	1	1

TABLE 3 - MEAN MATERNAL BODY WEIGHTS, WEIGHT-GAIN, FOOD INTAKE AND FOOD EFFICIENCY OF DAMS
PRELIMINARY STUDY

parameter	ppm β-chloroprene dosed by inhalation				
	0	10	25	75	175
number of dams examined	7	7	4	6	6
mean maternal body weight (g) at:					
day 0	186.4	186.4	180.5	185.1	187.8
day 6 (start of treatment)	206.6	204.1	199.0	203.0	206.0
day 16 (end of treatment)	238.7	233.7	222.5	222.3	217.6
day 21	286.1	285.4	275.8	271.8	267.5
weight gain during:					
treatment	32.1	29.6	23.5**	19.3**	11.7
gestation	99.7	99.0	95.3	86.7**	79.7
mean food intake (g/rat/day) from:					
day 0-6	16.4	16.0	15.9	16.4	16.0
day 6-16 (treatment period)	17.1	16.8	15.1*	14.2**	12.8
day 16-21	18.9	19.4	18.8	18.4	16.5
mean food efficiency $\frac{\text{weight gain(g)}}{\text{food consumption(g)}}$					
day 0-6	0.21	0.18	0.19	0.15	0.
day 6-16 (treatment period)	0.19	0.18	0.16	0.13*	0.(
day 16-21	0.50	0.53	0.57	0.54	0..

* $0.05 > P > 0.01$

** $0.01 > P > 0.001$

*** $P < 0.001$

TABLE 4 - AUTOPSY FINDINGS, ORGAN WEIGHTS AND LITTER DATA
PRELIMINARY STUDY

parameter	ppm 8-chloroprene dosed by inhalation														
	0			10			25			75			175		
	total	mean	group range	total	mean	group range	total	mean	group range	total	mean	group range	total	mean	gr ra
number of litters	7			7			4			6			6		
number of live foetuses	79	11.3	8-14	78	11.1	10-13	50	12.5	12-13	69	11.5	9-13	68	11.3	8
number of foetuses with externally visible malformations	0			0			0			0			0		
number of corpora lutea		15.0	10-20		15.7	12-20		14.8	14-17		15.0	12-19		15.8	13
number of implantation sites		11.7	8-14		11.6	10-13		12.8	12-14		12.5	11-14		11.8	9
pre-implantation loss (%)		20.11	0-31.6		25.09	83-40.0		12.71	0-29.4		14.83	0-33.3		24.44	15
number of embryonic resorptions	3	0.43	0-1	3	0.43	0-2	0			5	0.83	0-3	3	0.50	(
number of foetal resorptions	0			0			0			1	0.17	0-1	0		
number of dead foetuses	0			0			1	0.25	0-1	0			0		
post-implantation loss (%)		3.59	0-9.1		3.39	0-15.4		1.79	0-7.1		8.39	0-25.0		4.35	(

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TABLE 5 - NOMINAL AND MEAN ACTUAL CONCENTRATIONS OF β-CHLOROPRENE IN THE TEST ATMOSPHERE
FINAL STUDY

concentration	group numbers			
	1386	1387	1388	1389
nominal concentration	10	25	75	175
mean actual concentration during an exposure period of 25 days	10.29	24.69	73.47	171.02
standard deviation	1.07	0.82	3.00	7.38
standard error of the mean	0.21	0.17	0.60	1.48
mean concentration/rat/day during 12 days of treatment	10.33	24.81	73.32	170.36
standard deviation	0.177	0.176	0.936	1.289
standard error of the mean	0.03	0.03	0.17	0.22

TABLE 6 - MATERNAL PERFORMANCE
FINAL STUDY

observations	ppm β-chloroprene dosed by inhalation				
	0	10	25	75	175
number of females placed with males	36	36	36	36	36
number of females mated	33	32	32	32	32
number of females pregnant	30	31	30	27	28
pregnancy rate (percentage)	90.9	96.9	93.8	84.4	87
number of females bearing live foetuses	30	31	30	27	28
number of animals showing focal alopecia	3	1	5	5	13

TABLE 7 - MEAN MATERNAL BODY WEIGHTS, WEIGHT GAIN, FOOD INTAKE AND FOOD EFFICIENCY
FINAL STUDY

paramater	ppm β-chloroprene dosed by inhalation				
	0	10	25	75	175
number of dams examined	30	31[1)]	30	27	28
mean maternal body weight (g) at:					
day 0	172.6	172.6	172.1	170.0	171.9
day 4 (start of treatment)	188.0	186.5	184.5	183.0	184.3
day 8	191.9	191.6	185.3	177.0***	171.5
day 12	205.5	205.2	197.1*	187.6***	181.5
day 16 (end of treatment)	226.0	221.5	214.4*	202.5***	195.1
day 18	246.2	241.7	236.6	223.3***	217.1
day 21	274.3	270.3	264.4	248.9***	243.5
weight gain during:					
treatment	40.5	37.6	32.45**	23.3***	13.6
gestation	101.7	97.6	95.7	78.6***	75.2
mean food intake (g/rat/day) from:					
day 0-4	14.6	14.7	15.0	15.1	14.7
day 4-16 (treatment period)	16.3	16.0	14.3***	12.3***	11.5
day 16-21	18.0	18.2	18.1	18.2	19.0
mean food efficiency $\frac{\text{weight gain(g)}}{\text{food consumption(g)}}$					
day 0-4	0.22	0.21	0.17	0.20	0.1
day 4-16 (treatment period)	0.20	0.19	0.18*	0.15***	0.0
day 16-21	0.54	0.53	0.55	0.52	0.5

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

*** $P < 0.001$

1) : during one day, one animal was exposed to β-chloroprene for 4,5 instead of 6 hours

TABLE 8 - AUTOPSY FINDINGS, ORGAN WEIGHTS AND LITTER DATA
FINAL STUDY

parameter	ppm 8-chloroprene dosed by inhalation																	
	0			10			25			75			175		
	total	mean	group range	total	mean	group range	total	mean	group range	total	mean	group range	total	mean	group range
number of litters	30			31[1]			30			27			28		
number of live foetuses	327	10.9	8 - 14	307	9.9	1 - 15	332	11.1	5 - 16	252	9.3**	3 - 12	258	9.2**	1 - 14
number of foetuses with externally visible malformations[2]	3			2			3			2			2		
number of corpora lutea		13.6	10 - 20		13.6	11 - 17		13.9	10 - 25		13.4	11 - 18		11.8*	3 - 17
number of implantation sites		11.1	8 - 15		11.0	1 - 15		11.3	5 - 16		10.2	4 - 13		10.1*	2 - 15
pre-implantation loss (%)		16.70	0 - 45.0		18.23	0 - 81.8		16.48	0 - 72.2		21.43	0 - 72.2		13.96	0 - 47.1
number of embryonic resorptions	6	0.20	0 - 1	32	1.03*	0 - 9	8	0.27	0 - 2	23	0.85***	0 - 3	23	0.86*	0 - 7
number of foetal resorptions	0			0			0			0			0		
number of dead foetuses	0			2	0.06	0 - 1	0			0			0		
post-implantation loss (%)		1.75	0 - 10.0		8.95*	0 - 69.2		2.29	0 - 20.0		9.44**	0 - 50.0		8.96*	0 - 87.5
ovary weight (mg)		85.3	57 -106		85.4	54 -148		83.0	52 -137		83.6	56 -120		81.7	52 -109
empty uterus weight (g)		4.09	2.88- 5.63		3.94	1.18- 5.73		3.99	2.12- 6.94		3.58*	1.54- 4.90		3.37***	1.33- 4.89
foetal weight (g)		4.58	4.07- 5.03		4.68	3.81- 6.08		4.68	3.88- 5.17		4.36*	3.75- 5.51		4.23**	2.74- 4.81
placental weight (g)		0.52	0.44- 0.67		0.55	0.42- 0.87		0.53	0.44- 0.86		0.56	0.45- 0.89		0.52	0.35- 0.70
male - female ratio:															
males per litter (%)		44.1			47.8			52.6*			46.7			51.7	
females per litter (%)		55.9			52.2			47.4*			53.3			48.3	

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$;

1) during one day, one animal was exposed to 8-chloroprene for 4,5 instead of 6 hours

2) the observed malformations consisted of: acrania and cleft lip in one foetus of the 25 ppm dose group and meningocele in one foetus of the 75 ppm dose group. A dysmature foetus was observed in the high-dose group. The other malformations consisted of subcutaneous haemorrhages.

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TABLE 9 - TYPE AND INCIDENCE OF VISCERAL ANOMALIES (FOETUSES/LITTERS)
FINAL STUDY

parameter		ppm β-chloroprene dosed by inhalati	
		0	175
number of litters examined		30	28
number of foetuses examined		161	135
number of litters with one or more foetuses showing minor visceral anomalies		16	15
number of foetuses showing minor visceral anomalies		26	19
number of litters with one or more foetuses showing visceral variants		26	25
number of foetuses showing visceral variants		48	57
1. minor visceral anomalies			
meningial haemorrhage in the olfactory region of the brain		4/4	2/2
slight dilatation of the lateral brain ventricles		0/0	1/1
focal disposition of the esophagus		0/0	1/1
subcutaneous haemorrhages		4/3	0/0
abdominal cavity containing haemorrhagic fluid		2/2	4/4
unilateral testis ectopy		0/0	1/1
slight torsio or flexure of one limb		17/12	12/8
hydronephrosis, unilateral		0/0	1/1
2. visceral variants			
increased renal pelvic cavitation,	unilateral	18/13	30/17 *
	bilateral	3/3	4/4
slight hydroureter,	unilateral	2/2	5/5
	bilateral	1/1	0/0
dilatation of the esophagus		2/2	0/0
crooked curly or ringtail		23/15	23/18
one or more cysts in the placenta		10/10	4/4

* $0.05 > P > 0.01$

TABLE 10 - TYPE AND INCIDENCE OF SKELETAL ANOMALIES (FOETUSES/LITTERS)
FINAL STUDY

parameter	ppm B-chloroprene dosed by inhalatic	
	0	175
number of litters examined	30	26
number of foetuses examined	153	118
number of litters with one or more foetuses showing skeletal malformations	0	1
number of foetuses with skeletal malformations	0	1
number of litters with one or more foetuses showing minor skeletal anomalies	2	3
number of foetuses with minor skeletal anomalies	2	4
number of litters with one or more foetuses showing skeletal variants	21	14
number of foetuses with skeletal variants	38	21
1. skeletal malformations		
misshapen first sternebra	0/0	1/1
2. minor skeletal anomalies		
separated sternebrae	1/1	2/2
fused sternebrae	0/0	2/2
slight torsio of one hindlimb	2/1	1/1
3. skeletal variants		
dislocated sternebrae	13/10	11/9
minor dislocation of one or more digits	2/2	1/1
reduced sternebrae	0/0	1/1
supernumerary sternebrae	1/1	3/3
supernumerary skull bones	4/4	0/0
supernumerary rib no. 14, unilateral	18/13	7/5
bilateral	4/4	3/3

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TABLE 11 - VARIATION IN OSSIFICATION OF FOETAL SKELETONS EXPRESSED AS TRANSFORMED PERCENTAGES

parameter		ppm β-chloroprene dosed by inhalatio	
		0	175
number of litters examined		30	26
number of foetuses examined		153	118
1. ossification absent			
forelimb:	metacarpals	26.56	26.56**
	phalanges	44.23	46.47
hindlimb:	metatarsals	4.03	9.27*
	phalanges	52.00	53.66
cervical vertebral bodies		38.15	44.70*
2. ossification incomplete			
forelimb:	metacarpals	5.13	9.65**
	phalanges	18.39	20.49
hindlimb:	metatarsals	9.25	10.85
	phalanges	19.60	21.01
cervical vertebral bodies		24.76	28.58*
thoracal vertebral bodies		2.33	1.44
skull bones		2.46	1.93
sternebrae		9.23	12.25

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

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CHLOROPRENE: OBSERVATIONS OF CARCINOGENESIS AND MUTAGENESIS

Chemical Name (300 per name) 25	CAS No. (10) 24
2-CHLORO-1-3-BUTADIENE	126-99-8

1A

3/11/85 RS

(28)

December 16, 1976

S78215016

CHLOROPRENE: OBSERVATIONS OF CARCINOGENESIS AND MUTAGENESIS

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Running Head: Mutagenic Effects of Chloroprene

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Paper presented at meeting on Origins of Human Cancer, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, September 7-14, 1976. In Press, Proceedings from Conference.

PRE-PUBLICATION

In 1931, 2-chlorobutadiene, more commonly called chloroprene, was introduced into American commerce. This colorless liquid is used for the manufacture of a synthetic rubber, polychloroprene. World-wide, an estimated one billion pounds of chloroprene are currently produced annually (Lloyd, Selevan, Woolf, 1976). In the U.S. alone, an estimated four hundred million pounds of polychloroprene, marketed under the trade name Neoprene are produced annually (Lloyd, Decoufle, Moore, 1975). It has been estimated that approximately 2,500-3,000 workers in the U.S. are currently exposed to chloroprene during its manufacture and polymerization. It is not known how many thousands of individuals may be exposed to chloroprene as a result of working daily with Neoprene or with the liquid polymer(latex). Because of the close structural similarity between chloroprene and vinyl chloride (VC), Figure 1, the latter a known carcinogen (Waxweiler, et al., 1976) and mutagen (Bartsch, Malaveille, Montesano, 1975; Rannug, et al., 1974; Garro, Guttenplan, Milvey, 1976; Greim, et al., 1975; Andrews, Zawistowski, Valentine, 1976; McCann, et al., 1975; Loprieno, et al., 1976), it was only natural that scientific attention should be focused on the assessment of potential adverse effects associated with exposure to chloroprene. With this in mind, the National Institute for Occupationa Safety and Health (NIOSH) in 1976 initiate' an intensive search and synthesis of chloroprene-related research from sources both within and outside the U.S.

OBSERVATIONS OF CARCINOGENESIS

Laboratory Observations

One animal study has addressed itself to the carcinogenicity of chloroprene (Zilfian, Fichigian, Pogosova, 1975). In three series of experiments, the authors reported that skin application to mice, and subcutaneous and stomach tube administration to rats did not induce tumors. In several of the test series, the authors do not state the specific period of time for tumor observation, making it difficult to determine the number of surviving animals for specific periods of time on which observations were based. The investigators indicated that pure chloroprene rather than technical grade chloroprene was administered to the animals. Since workers are exposed to technical grade chloroprene, which may contain other contaminants, it is difficult to determine the public health significance of these study results.

Human Observations

The earliest assessment of the possible carcinogenicity of chloroprene was undertaken by Khachatryan in the Yerevan district of the Soviet Union (Khachatryan, 1972a; Khachatryan, 1972b). A gradient in the exposure to chloroprene was established. As shown in Table 1, a zero exposure level was assumed for group 1 and the relatively highest exposure level was assumed for group 5 (Khachatryan, 1972a). The prevalence-rate of skin cancer during the period 1956-70 was directly related to the degree of chloroprene exposure. The rate increased from 0.13% in exposure

group 1, composed of cultural and civic workers, to 0.40% in non-chemical industrial workers, composed of electricians, carpenters, tinsmiths, etc., to 0.67% in non-chloroprene chemical workers, composed of truck drivers and service station attendants, to 1.69% in chloroprene derivative workers, to 3.07% in chloroprene production workers having extensive exposure. An inverse relation was demonstrated between mean age at diagnosis of skin cancer and degree of chloroprene exposure, i.e., the groups with relatively greater exposure to chloroprene had an earlier age at diagnosis of skin cancer. A similar inverse relation also was demonstrated for duration of employment i.e., higher exposure groups had a shorter duration of employment by the time of skin cancer diagnosis.

As shown in Table 2, during this same time period, (1956-70), lung cancer morbidity also was directly associated with the degree of chloroprene exposure (Khachatryan, 1972b). This increased from 0.06% in group 1 to 0.71% and 2.63% in groups 4 and 5, respectively. As demonstrated in the study of skin cancer mentioned above, similar inverse relations between degree of exposure to chloroprene and mean age of diagnosis of lung cancer and mean duration of employment were observed.

Although these findings were reported to have been instrumental in the lowering of the Maximum Allowable Concentration in the USSR from 20 mg to 2 mg/M^3, 5 ppm to 0.5 ppm, (Prosser, 1975) recent correspondence from the Soviet Union now claims that a re-evaluation of this study has detected some methodological errors (Savelev, 1975). To date, however,

a reanalysis of the data has not been released, and discussions of the findings with the author, other involved medical personnel or toxicologists have been unsuccessful (Zapp, 1975).

Since no mention is made of adjustments for age and sex differences in comparisons among the groups, these variables may account for some of the methodological aspects needing reconsideration. Although reference is made to length of employment for cancer cases (both lung and skin) in these reports, no consideration was given to the importance of latency. Total employment histories also appear to be lacking as suggested by a difference of 40-50 years between mean age of cases and mean length of employment for cases. If one assumes that most workers begin employment in their mid-twenties, the differences between mean age of cases and mean length of employment should be less than 25 years.

More recently, Pell (1976) reported preliminary analyses from a study of mortality among workers exposed to chloroprene in the U.S. In this study (Table 3), the cohort consisted of 1,661 men who were on the wage roll as chloroprene workers at a single facility on June 30, 1957. Although no significant excesses are apparent, the data do show an excessive risk of respiratory cancer at each calendar time period for the total study cohort.

Four deaths from lung cancer have been observed among the maintenance mechanics (Table 3). Additionally, four currently active maintenance mechanics have been diagnosed with lung cancer and these data are not shown in the Table. The eight lung cancers in this subcohort account for 40% (8/20) of the lung cancer cases in the total

study cohort. Since only 17% of the total study cohort is composed of maintenance mechanics, (Pell, 1976) this observation may be highly significant. (Two respiratory cancer deaths in the total cohort apparently were categorized as other than lung cancer.) Since the task of the maintenance mechanics is to replace leaking pipe-fittings, to install equipment and to do general maintenance in the reactor areas, this group of workers would be expected to have relatively high chloroprene exposures.

In terms of methodology for this study, data for chloroprene monomer production workers and polymerization workers were combined, making it impossible to determine the relative contribution of each of these groups to the risk of lung cancer in the total study cohort. (The greatest cancer risk in the VC industry was identified in polymerization workers and not in monomer production workers. Thus, it would seem essential that data for these groups be analyzed separately in the chloroprene rubber industry.) Since the study cohort included any active worker, who was on the chloroprene wage roll on June 30, 1957, it is not clear whether any person classified as a member of the control group may have had extensive exposure to chloroprene at a much earlier date. Adequate consideration also was not given to length of exposure and latency, two variables of prime importance in the epidemiologic study of occupationally-related cancer.

In the absence of adequate consideration of these variables, a final evaluation of the carcinogenicity of chloroprene cannot be made.

The importance of exposure duration and latency has been demonstrated clearly by Nelson (1977) in his report of lung cancer among workers occupationally exposed to BCME and by Selikoff (1977) in his report of lung cancer and mesothelioma among populations exposed to asbestos, from both occupational and environmental sources.

Case Report of Angiosarcoma of the The Liver

More recently, a confirmed case of angiosarcoma of the liver in a worker who had extensive exposure to finished polychloroprene (Neoprene) has been identified (Herbert, 1976). The worker had been employed as a roll builder from the period 1952-1967. During this period, he applied Neoprene to metal cylinders, which were then vulcanized. After this procedure, the material often would be cut to the desired size with a metal saw. Dust control was attempted by water sprays and the worker did not wear a mask. A history of exposure indicated that this worker never had occupational exposure to vinyl chloride, nor had he ever received Thorotrast, an agent also associated with angiosarcoma of the liver (da Silva Horta, et al., 1965). Because of the structural similarity of vinyl chloride and chloroprene and the rare occurrence of angiosarcoma, this observation is considered of great public health concern. It also indicates the need to determine the amount of residual chloroprene in polychloroprene as well as the need to identify other industrial populations which may have potential exposure to chloroprene from working with finished polychloroprene or the liquid polymer(latex). The latter material is estimated to contain

5,000 ppm chloroprene. (J.J. Clary, personal communication.) After 45 years of occupational exposure to chloroprene, not one investigation addressed to its carcinogenic potential, either through epidemiologic or animal study, either by Government or by Industry has been completed in the United States.

OBSERVATIONS OF MUTAGENESIS

Laboratory Observations

Because of the lack of data on the carcinogenic potential of chloroprene, efforts have been undertaken to assess existing data regarding the potential for mutagenic effects of chloroprene. This approach seemed scientifically appropriate given the high correlation between carcinogenesis and mutagenesis (McCann, et al., 1975), and the close similarity in chemical structure between chloroprene and VC, an agent with strong evidence for both carcinogenicity and mutagenicity.

As far back as 1936, Von Oettingen, et al. (Table 4) of DuPont's Haskell Laboratories reported infertility associated with chloroprene exposure to male mice (Von Oettingen, et al., 1936). As shown, the fertility rate in mice exposed to levels ranging from 12 to 150 ppm was 43% versus 100% for controls. (Most exposures were at relatively lower levels.) In rats, the fertility rate was 32% at higher levels versus 100% for controls as shown in the Table.

This report served as the basis upon which the U.S.P.H.S., in 1945, recommended at 25 ppm Maximum Allowable Concentration (MAC) for the

workplace (Cook, 1945). With the passage of the Occupational Safety and Health Act in 1971, this 25 ppm level was established as the legal occupational standard for chloroprene in the U.S.

As shown in Table 5, in 1973, the first in-depth industrial hygiene air monitoring program conducted at a chloroprene polymerization facility revealed mean concentrations to be as high as 1,000 ppm, with individual samples ranging as high as 6,760 ppm (NIOSH, unpublished data). Data for cancer mortality among personnel employed at this plant were presented above (Pell, 1976).

During this same period of time, 1972, Davtyan reported the mutagenic effects of chloroprene on male rats at an exposure level of 0.06 ppm. He observed a dominant lethal effect, i.e., a significant excess of embryonic mortality following male rat exposure only, testicular atrophy and a reduction in the numbers and motility of sperm in animals with non-atrophied testicles.

A year later, as shown in Table 6, Davtyan, Fomenko and Andreyva (1973) reported the results of chloroprene exposure to male rats at concentrations of 1.0 ppm. Again, a dominant lethal effect was observed. Chromosomal aberrations in bone marrow cells of these same animals also were increased. The authors concluded that both germinal and somatic cells were sensitive to the mutagenic action of chloroprene at these low levels. A year later, observations of chromosomal aberrations in bone marrow cells of chloroprene exposed rats were also reported by Bagramyan and Babayan (1974).

In 1975, Bartsch, et al., reported chloroprene vapor to be mutagenic in S. Typhimurium, TA 100 both with and without metabolic activation

when plates containing the bacteria and the mouse liver microsomal fraction were incubated to gaseous mixtures of 2-chlorobutadiene in air. As reported in 1976 (Vogel) and presented at this conference, Vogel has demonstrated chloroprene to induce sex-linked recessive lethal mutations in Drosophila. Thus, reports indicate that chloroprene is mutagenic in bacteria, is recessive lethal in Drosophila and causes both dominant lethality as well as chromosomal aberrations in bone marrow cells of the rat. Since 1936, chloroprene has been associated with sterility (Von Oettingen et al., 1936) and later with deceased numbers and motility of sperm as well as testicular atrophy in mice and rats at very low concentrations (Davtyan, 1972; Davtyan et al., 1973).

The question facing society is whether such data is scientifically adequate to institute prudent public health policy by controlling worker exposure to toxic substances. If such data for laboratory study of mutagenicity is not sufficient to allow for a decision to control exposure to chloroprene as a mutagen at this time, genetic toxicologists will have to come forward to indicate the data set they would accept as adequate for prediction of potential mutagenic response in humans.

Human Observations

Recently, as shown in Table 6, data has surfaced regarding the cytogenetic and mutagenic effects of chloroprene in humans. Katosova (1973) has reported a significant excess of chromosomal aberrations in chloroprene workers. As reported at an international conference held by the Environmental Mutagen Society in 1975,

Bochkov found a significant excess of chromosomal aberrations in workers exposed to chloroprene as compared to controls. In March, 1976, Sanotsky also reported an excess of chromosomal aberrations in chloroprene workers. More importantly, the latter paper contained two additional observations: 1) a 3-fold excess of miscarriages among the wives of chloroprene workers and 2) a reduction in the number of motility of sperm following occupational exposure to chloroprene.

Chloroprene Data Versus Vinyl Chloride Data for Mutagenesis

Given the mutagenic capacity of chloroprene, first demonstrated by laboratory assay and subsequently complimented by observations in humans, and given the close chemical similarity between chloroprene and VC, it seems to follow that one would contrast the mutagenicity data for chloroprene to data available for VC as shown in Table 7. VC has demonstrated mutagenicity in several microbial test systems (Bartsch, Malaveille, Montesano, 1975; Rannug, et al., 1974; Garro, Guttenplan, Milvy, 1976; Greim, et al., 1975; Andrews, Zawistowski, Valentine, 1976; McCann, et al., 1975; Loprieno, et al., 1976). Recessive lethal mutations associated with VC exposure also have been observed in Drosophila (Sobels ar Vogel 1976; Magnusson and Ramel, 1976) as well as in plants (Sparrow, 1976). VC metabolites have induced mutations in Chinese hamster cells (Huberman, Bartsch, Sachs, 1975). Several studies have demonstrated excessive chromosomal aberrations in circulating lymphocytes among workers exposed to VC as compared to controls (Ducatman, Hirschhorn, Selikoff, 1975; Funes-Cravioto, et al., 1975; Purchase, Richardson, Anderson, 1975;

Loken, Thiis-Evensen, 1976). In addition, excessive miscarriages have been reported among the wives of male workers exposed to VC as compared to industrial controls (Infante, et al., 1976a; Infante, et al., 1976b). Although these observations overwhelmingly document the mutagenicity of VC, even more data are available (Table 7) to demonstrate that chloroprene is both mutagenic and reaches the germinal tissue in mammals, including man.

SUMMARY

Even though observations of excessive lung and skin cancer associated with chloroprene production were reported in the Russian literature in 1972, not until 1974 with the identification of the epidemic of liver, brain and lung cancer among VC polymerization workers (Waxweiler, et al., 1976) did Government, Labor and Industry in the U.S. begin to focus attention upon the carcinogenic potential of chloroprene. Unfortunately, data sources and reports currently available do not permit the development of valid inferences regarding the carcinogenicity of chloroprene. Given the consistency of positive response for the mutagenicity of chloroprene in several test systems, plus the observations of adverse effects on the reproduction process among workers occupationally exposed to chloroprene, from a public health point of view whether or not chloroprene is carcinogenic may be only of academic interest.

Even more striking is the fact that 46 years after the introduction of this toxic agent into the workplace and 40 years after the report by

Von Oettingen et al., (1936) indicating adverse effects on reproduction, not one epidemiologic study of the effects of chloroprene on reproduction among workers populations in the U.S. has been reported. These observations clearly indicate the need for control of toxic substances prior to their introduction into the workplace. Perhaps, the data for VC and chloroprene may now serve as the basis for a more agressive role by genetic toxicologists in the assay of industrial chemicals and in the utilization of these assay results for making prudent public health policy decisions to control industry/environmental mutagens that are impacting on the present and future generations.

REFERENCES

Andrews, A.W., E.S. Zawistowski and C.R. Valentine. 1976. A comparison of the mutagenic properties of vinyl chloride and methyl chloride. Mutat. Res. 40:273.

Bagramyan, S.B. and E.A. Babayan. 1974. Cytogenetic study of the mutagenic activity of chemical substances isolated from nairet latexes MKh and LNT-1. Biol. Zh. Arm. 27:102.

Bartsch, H., C. Malaveille and R. Montesano. 1975. Human, rat and mouse liver-mediated mutagenicity of vinyl chloride in S. typhimurium strains. Int. J. Cancer. 15:429.

Bartsch, H., C. Malaveille, R. Montesano and L. Tomatis. 1975. Tissue mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in S. typhimurium. Nature 255:641.

Bochkov, N.P. 1976. Population studies in the chemical industry. International Symposium on New Developments in Mutagenicity Testing of Environmental Chemicals, Zinkovy Castle, Czechoslovakia, Oct. 1975. Mutat. Res. (In Press).

Cook, W.A. 1945. Maximum allowable concentrations of industrial atmospheric contaminants. Industr. Med. 14:936.

da Silva Horta, J., J.D. Abbatt, L.C. da Motta and M.L. Roriz. 1965. Malignancy and other late effects following administration of thorotrast. Lancet. ii:201.

Davtyan, R.M. 1972. Toxicological characteristics of the action of chloroprene on the reproductive function of male rats. Toksikol. Gig. Prod. Neftekhim. Neftekhim. Proizvod Vses. Konf. (Dokl.) 2nd 1971, p. 95.

Davtyan, R.M., V.N. Fomenko and G.P. Andreyva. 1973. Question of the effect of chloroprene on the generative function of mammals (males). USSR Academy of Medical Sciences. The toxicology of new industrial chemical substances, Issue No. 13:53.

Ducatman, A., K. Hirschhorn and I.J. Selikoff. 1975. Vinyl chloride exposure and human chromosome aberrations. Mutat. Res. 31:163.

Funes-Cravioto, F., B. Lambert, J. Lindsten, L. Ehrenberg, A.T. Natarajan and S. Osterman-Golkar. 1975. Chromosome aberrations in workers exposed to vinyl chloride. Lancet. i:459.

Garro, A.J., J.B. Guttenpaln and P. Milvy. 1976. Vinyl chloride dependent mutagenesis:effects of liver extracts and free radicals. Mutat. Res. 38(2):81.

Greim, H., G. Bonse, Z. Radwan, D. Reichert and D. Henschler. 1975. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes and a function of metabolic oxirane formation. Biochem. Pharmacol. 24:2013.

Herbert, J.T., April 12, 1976. Angiosarcoma of the liver in a rubber worker. CDC Memorandum.

Huberman, E., H. Bartsch and L. Sachs. 1975. Mutation induction in Chinese Hamster V79 cells by two vinyl chloride metabolites chloroethylene oxide and 2-chloroacetaldehyde. Int. J. Cancer. 16:639.

Infante, P.F., J.K. Wagoner, A.J. McMichael, R.J. Waxweiler and H. Falk. 1976a. Genetic risks of vinyl chloride. Lancet. i:734.

Infante, P.F., J.K. Wagoner, A.J. McMichael, R.J. Waxweiler and H. Falk. 1976b Genetic risks of vinyl chloride. Lancet. i:1239.

Katosova, L.D. 1973. Cytogenetic analysis of peripheral blood of workers engaged in the production of chloroprene. Gig. Tr. Prof. Zabol. 10:30.

Khachatryan, E.A. 1972b. The occurrence of lung cancer among people working with chloroprene. Problems in Oncology 18:85.

Khachatryan, E.A. 1972a. The role of chloropr[illegible] the process of skin neoplasm formation. Gig. Tr. Prof. Zabol. 18:54.

Lloyd, J.W., P. Decoufle and R.M. Moore. 1975. Background information on chloroprene. J. Occup. Med. 17:263.

Lloyd, J.W., S.G. Selevan and B. Woolf. February 10, 1976. Trip to E.I. duPont de Nemours and Company, Wilmington, Delaware, February 10, 1976, NIOSH Memorandum.

Loken, E. and E. Thiis-Evensen. Preliminary report on the medical examination of 288 employees at the PVC plant, Norsk Hydro a.s., Porsgrunn Fabrikker, Porsgrunn, Norway. (Unpublished manuscript).

Loprieno, N., R. Barale, S. Baroncelli, C. Bauer, G. Bronzetti, A. Cammellini, G. Cercignani, C. Corsi, G. Gervasi, C. Leporini, R. Nieri, A.M. Rossi, G. Stretti and G. Turchi. 1976. Evaluation of the genetic effects induced by vinyl chloride monomer (VCM) under mammalian metabolic activation:Studies in vitro and in vivo. Mutat. Res. 40:85.

Magnusson, J. and C. Ramel. 1976. Mutagenic effects of vinyl chloride in Drosophila melanogaster. Mutat. Res. 38:114.

McCann, J., E. Choi, E. Yamasaki and B.N. Ames. 1975. Detection of carcionogens as mutagens in the Salmonella/microsome test:Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72(12):5135.

Nelson, N. 1977. The carcinogenicity of chloro ethers and related compounds. Cold Springs Harbor Conferences on Cell Proliferation, Origins of Human Cancer. 4:In Press.

Pell, S. 1976. Mortality of workers exposed to chloroprene at the Louisville Works, 1957-74:A preliminary study. E.I. duPont de Nemours and Company, (Unpublished manuscript).

Prosser, R.M. April 14, 1975. DuPont visit to USSR, chloroprene toxicity, letter to E.J. Fairchild, NIOSH.

Purchase, I.F.H., C.R. Richardson and D. Anderson. 1975. Chromosomal and dominant lethal effects of vinyl chloride. Lancet. ii:410.

Rannug, U., A. Johansson, C. Ramel and C.A. Wachtmeister. 1974. The mutagenicity of vinyl chloride after metabolic activation. Ambio. 3:194.

Sanotsky, I.V. 1976. Problems of chloroprene toxicology (immediate and long-range effects). Symposium on Potential Environmental Hazards from Technological Developments in Rubber and Plastics Industry, National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina, March, 1976 (In Press).

Savelev, M.N. December 15, 1975. Letter from Ministry of Health, USSR, to John Zapp, E.I. duPont de Nemours and Company.

Selikoff, I.J. 1977. Cancer risk of asbestos exposure. Cold Spring Harbor Conferences on Cell Proliferation, Origins of Human Cancer. 4:In Press.

Sobels, F.H. and Vogel, E. 1976. The capacity of Drosophila for detecting relevant genetic damage. Mutat. Res. 41:95.

Sparrow, A.H. 1976. (Correspondence).

Vogel, E. 1976. Mutagenicity of carcinogens in Drosophila as function of genotype-controlled metabolism. Conference in vitro Metabolic Activation, National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina, February, 1976.

Von Oettingen, W. F., W.C. Hueper, W. Deichmann-Gruebler and F.H. Wiley. 1936. 2-chloro-butadiene (chloroprene):Its toxicity and pathology and the mechanism of its action. J. Ind. Hyg. Tox.18: 240.

Waxweiler, R.J., W. Stringer, J.K. Wagoner, J. Jones, H. Falk and C. Carter, 1976. Neoplastic risk among workers exposed to vinyl chloride. Ann. N.Y. Acad. Sci. 271:40.

Zapp, J.A. December 31, 1975. Letter to E.J. Baier, NIOSH.

Zilfian, V.N., B.S. Fichigian and D.M. Pogosova. 1975. Results of studies on the carcinogenicity of chloroprene. J. Exp. Clin. Med. 15:54.

Table 1

PREVALENCE OF SKIN CANCER AMONG WORKERS > 25 YEARS OF AGE, YEREVAN, SOVIET UNION, 1956-70

	Exposure Groups				
	1 No Industry	2 Non-Chem. Industry	3 Unrelated Chem. Industry	4 Chloroprene Derivatives	5 Chloroprene Production
Number Examined	8520	8755	4780	2250	684
Number with Skin Cancer	11	35	32	38	21
Percent	(0.13)	(0.40)	(0.67)	(1.69)	(3.07)
Mean Age of Cases	72.1	68.9	64.4	59.1	59.6
Mean Length of Employment for Cases (yrs.)	16.3	15.4	13.8	8.7	9.5

Source: Khachatryan, Gig Tr Prof Zabol 18:54, 1972.

020020

Table 2

PREVALENCE OF LUNG CANCER AMONG WORKERS > 25 YEARS OF AGE, YEREVAN, SOVIET UNION, 1956-70

	Exposure Groups				
	1 No Industry	2 Non-Chem. Industry	3 Unrelated Chem. Industry	4 Chloroprene Derivatives	5 Chloroprene
Number Examined	6220	6045	4780	2250	684
Number with Lung Cancer	4	11	22	16	18
Percent	(0.06)	(0.18)	(0.46)	(0.71)	(2.63)
Mean Age of Cases	60.2	59.3	54.9	44.5	
Mean Length of Employment for Cases (yrs.)	18.5	14.9	10.3	8.7	

Source: Khachatryan, Problems in Oncology 18:85, 1972.

000021

Table 3

PRELIMINARY FINDINGS FOR RESPIRATORY CANCER DEATHS FOR WORKERS EXPOSED TO CHLOROPRENE FOR TOTAL COHORT AND SPECIFIC EXPOSURE AREAS, BY PERIOD OF DEATH

	PERIOD OF DEATH (YEARS)											
	All Years 1957-1974			1957-1962			1963-1968			1969-1974		
	OBS	EXP	SMR	OBS	EXP	SMR	OBS	EXP	SMR	OBS	EXP	SMR
Total Cohort	18	13.2	136	3	2.2	136	4	3.1	129	11	7.9	139
Maintenance Mechanics	4	2.8	143	1	0.4	250	1	0.7	143	2	1.7	118
Chemical Operators	3	2.8	107	1	0.4	250	0	0.6	---	2	1.8	111
Other High Exposures	2	1.4	143	0	0.3	---	0	0.3	---	2	0.8	250

OBS = Observed Deaths

EXP = Expected Deaths (Based on Company Records)

Source: Pell, 1976

000022

TABLE 4

EFFECT OF INHALATION OF CHLOROPRENE ON THE REPRODUCTION OF MALE MICE AND RATS, 1936

SPECIES	NUMBER OF ANIMALS	PREGNANCIES	FERTILITY RATE (%)
Mice			
0.042 - 0.548 mg/L (11.6 - 151.4 ppm)	14	6	43
Controls	5	5	100
Rats			
0.434 - 22.419 mg/L (120 - 6194 ppm)	19	6	32
Controls	5	5	100

Source: Von Oettingen et al., 1936.

000023

Table 5

ATMOSPHERIC CHLOROPRENE CONCENTRATIONS AT A POLYMERIZATION PLANT, 1973

Area	No. Samples	Range (ppm)	Mean (ppm)
Make-up	10	14 - 1420	554
Reactor	21	130 - 6760	1015
Monomer Recovery	2	6 - 440	223
Latex	2	113 - 252	205

Grab samples collected with glass sampling flasks; analysis by gas chromatography.

Table 6

LABORATORY STUDIES INDICATING CYTOGENETIC, MUTAGENIC OR

REPRODUCTIVE EFFECTS OF CHLOROPRENE

LABORATORY TEST SYSTEM	OBSERVATION	INVESTIGATORS
Mice & Rats	sterility	Von Oettingen, Hueper, Deichmann-Gruebler, Wiley (1936)
Rat	1. dominant lethal 2. effects on sperm 3. testicular atrophy	Davtyan (1972)
Rat	1. chromosomal aberrations 2. dominant lethal	Davtyan, Fomenko, Andreyeva (1973)
Rat	chromosomal aberrations	Bagramyan and Babayan (1974)
S. Typhimurium	mutagenic	Bartsch, Malaveille, Montesano, Tomatis (1975)
Drosophila	recessive lethal	Vogel (1975)
HUMAN STUDIES	**OBSERVATION**	**INVESTIGATORS**
Workers	chromosomal aberrations	Katosova (1973)
Workers	chromosomal aberrations	Bochkov (1976)
Workers	1. chromosomal aberrations 2. decrease in motility and number of sperm 3. three-fold excess of miscarriages in wives of male workers	Sanotsky (1976)

000025

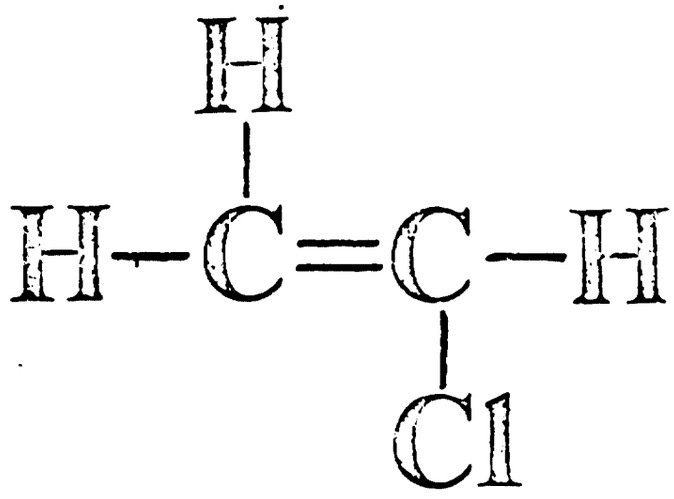
TABLE 7

EVIDENCE FOR CYTOGENETIC, MUTAGENIC OR REPRODUCTIVE EFFECTS OF VINYL CHLORIDE AND CHLOROPRENE

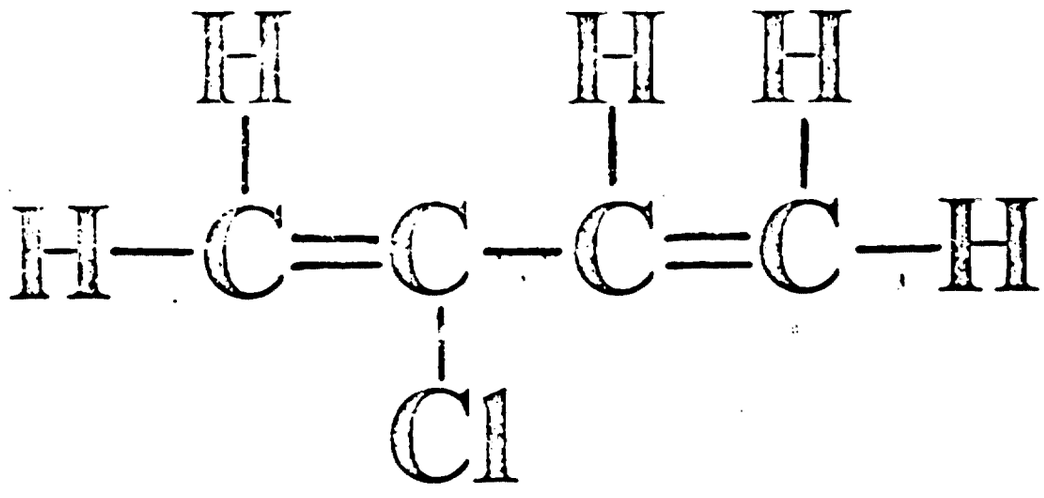
TEST SYSTEM	AGENT	
	VINYL CHLORIDE	CHLOROPRENE
LABORATORY OBSERVATIONS		
MICROBIAL (E. COLI; S. POMBE,* S. TYPHIMURIUM)	+	+
INSECT (RECESSIVE LETHAL IN DROSOPHILA)	+	+
PLANTS (TRADESCANTIA)	+	?
MAMMALS METABOLITES IN HAMSTER CELLS	+	?
CHROMOSOMAL ABERRATIONS IN MALE RATS	?	+
REPRODUCTION INTERFERENCE FOLLOWING EXPOSURE TO MALE MICE AND RATS	—	+
DECREASE IN MOTILITY AND NUMBER OF SPERM IN RATS	?	+
HUMAN OBSERVATIONS		
CHROMOSOMAL ABERRATIONS IN MALE WORKERS	+	+
EXCESS MISCARRIAGE IN WIVES OF MALE WORKERS	+	+
DECREASE IN MOTILITY AND NUMBER OF SPERM IN WORKERS	?	+

* MUTAGENIC ACTIVITY OF CHLOROPRENE HAS NOT BEEN TESTED IN THESE ORGANISMS.

Figure Legend: Chemical structures of vinyl chloride and chloroprene.



VINYL CHLORIDE



CHLOROPRENE

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MARCH 15, 1950

PRELIMINARY STUDY OF TOXIC EFFECTS WITH CHLOROPRENE

++ Just able to work
+ Moderate
Mild

MEN WORKING IN CHLOROPRENE

CLOCK NUMBER	NAME	AGE	TIME IN COMPANY	SHORTNESS OF BREA ON EXERTION	SUB-STERNAL OR PRECORDIAL PAIN OR TIGHTNESS ON EXERTION	DIZZY ON EXPOSURE TO FUMES	DIZZY FREQUENTLY	HEADACHE	NERVOUSNESS	WEAKNESS-TIREDNESS	PALPITATION ON EXERTION	INDIGESTION	NAUSEA & VOMITING	MUSCLE PAINS	
								ROUTINE EXAMINATIONS							
1	W.B.Smith	49	3 mo.	N	N	N	N	N	N	N	N	N	N	N	
7	C.A.Ingeman	38	5 yrs.	+	+	N	N	N	+	N	+	N	N	N	
0	H.A.Peterson	32	5 yrs.	+++	+++	N	+++	N	N	+++	++	N	N	N	
					Taken off job for time										
.	R.D.St.George	50	8 yrs. All oper.	++	++	+	N	N	N	N	N	++	+	N	
1	G. Lamont	32	2 yrs.	++++	++++	+++	++++	+	++	++++	+	+	N	N	At hom. W.D.
					(Had similar attack 11-28-38)										
0	B.D.Brooks	27	5 yrs.	++	++	+	N	N	N	+	N	N	N	N	P.100
8	A.C.Niblock	26	2 yrs.	++	++	+	+	+	N	N	N	N	N	N	
7	H.Stevenson	25	2 yrs.	++	++	+	N	+	+	++	++	++	+	N	P.100
35	H.A.Waterman	33	2 yrs.	++	++	+	N	N	N	+	+	+		+	
03	H.Taylor Foreman	34	8 yrs.	++	++	+	N	++		+				+	
18	R.O.McCurdy	44	8 yrs. All oper.	N	N	N	N	+	N	N	N	+	N		(Frequent colds)
43	J.Sullivan		2 yrs.	++++	++++	+++	++			+++	++				
51	H. Stokely		2 yrs.	+	+			+							
54	J. Byrnes	26	3 mo.	+++	+++	+	N	N	N	+	N	+	N	N	P.95
											Marked aft. beer				
	E.Crispin		1 yr.	+	+	N	N	N	N	N	+	N	N	N	
				17	13	9	4	6	3	8	7	6	2	2	

000003

Moderate
Mild

NAME	AGE	TIME IN COMPANY	SHORTNESS OF BRE ON EXERTION	SUB-STERNAL OR PRECORDIAL PAIN OR TIGHTNESS ON EXERTION	DIZZY ON EXPO-SURE TO FUMES	DIZZY FREQUENTLY	HEADACHE	NERVOUSNESS	WEAKNESS-TIREDNESS	PALPITATION ON EXERTION	INDIGESTION	NAUSEA & VOMITING	MUSCLE PAINS	
							ROUTINE EXAMINATIONS							
.B.Smith	49	3 mo.	N	N	N	N	N	N	N	N	N	N	N	
.A.Ingeman	38	5 yrs.	+	+	N	N	N	+	N	+	N	N	N	
.A.Peterson	32	5 yrs.	+++	+++	N	+++	N	N	+++	++	N	N	N	
				Taken off job for time										
.D.St.George	50	8 yrs. All oper.	++	++	+	N	N	N	N	N	++	+	N	
. Lamont	32	2 yrs.	++++	++++	+++	++++	+	++	++++	+	+	N	N	At home W.D.
				(Had similar attack 11-28-38)										
.D.Brooks	27	5 yrs.	++	++	+	N	N	N	+	N	N	N	N	P.100
.C.Niblock	26	2 yrs.	++	++	+	+	+	N	N	N	N	N	N	
.Stevenson	25	2 yrs.	++	++	+	N	+	+	++	++	++	+	N	P.100
.A.Waterman	33	2 yrs.	++	++	+	N	N	N	+	+	+		+	
.Taylor	34	8 yrs.	++	++	+	N	++		+				+	
Foreman .O.McCurdy	44	8 yrs. All oper.	N	N	N	N	+	N	N	N	+	N	(Frequent colds)	
.Sullivan		2 yrs.	++++	++++	+++	++			+++	++				
. Stokely		2 yrs.	+	+			+							
. Byrnes	26	3 mo.	+++	+++	+	N	N	N	+	N	+	N	N	P.95
Crispin		1 yr.	+	+	N	N	N	N	N	Marked aft. beer +	N	N	N	
POSITIVE		(15 mo)	13	13	9	4	6	3	8	7	6	2	2	

000004

LABORATORY FINDINGS

		ICTERUS INDEX & UROBILOGEN				URINALYSIS
4331	George Lemont	Aug.'38	Nov.'38	Feb.'39	Mar.'40	
		15	15	13.6		Neg.
		1/50	1/20	1/20	1/10	
4354	J. Byrnes	Mar.'40				
		15				Neg.
		1/20				
4337	H. Stevenson	Mar.'40				
		10 ✓				Neg.
		1/10				
4303	H. Taylor	Jan.'39	Mar.'40			
		7.5 ✓	10.5			Neg.
		1/10	1/10			
Sal.	E. Hawkins	Aug.'38	Jan.'39	Mar.'40		
		11.5	8.8 ✓			Neg.
		1/50	1/20	1/10		
4335	H. Waterman	Apr.'39	Mar.'40			
			14.5			Neg.
		1/10	1/10			
4353	E. Crispin	Jan.'39	Mar.'40			
		15.5	25			Neg.
		1/10	1/10			
4318	H. Mc Curdy	Feb.'39	Mar.'40			Alb. F. Tr.
		5.1		✓		WBC. occ
		1/100	1/10			RBC. occ
						6 casts
4310	B. Brooks	Aug.'38	Feb.'39	Mar.'40		
		18	6.25	25		
		1/10	1/5	1/10		
4308	A. Niblock	Mar.'40				
		37.5				WBC ++++
		1/110				
4350	H. Peterson	Mar.'40				
		9	✓			Neg.
		1/10				
4351	H. Stokely	Aug.'38	Jan.'39	Mar.'40		
		11	10	21		Neg.
		1/20	1/40	1/10		
4307	C. Ingemar	Apr.'39	Feb.'40			
						Neg.
		1/10	1/10			
4301	W. Smith	Feb.'40		✓		
		7.5				Neg.
		1/10				

CRALPTON VALUES

CHLOROPRENE (C.D.) WORKERS

		3-8-40		3-11-40								BL C.
1354	J. Byrnes	+15	+25	+10	+10							
4335	H. Waterman	+75	+85									
		3-4-40		3-5-40		3-6-40		3-7-40		3-9-40	3-12-40	
4350	H. Peterson	L25	R35	60	10	-10	30	+5	+30	+30	+10	-5	+30
				3-13-40								
4351	G. Lamont	-75	-40	0	+10							
4343	J. Sullivan											
4353	E. Crispin	+65	+55									
4337	H. Stevenson	+60	+60									
				3-11-40								
4336	T. Hancock	-15	-15	40	35	(Before work)						
4312	W. Sparks	+45	+45									

PRELIMINARY STUDY OF TOXIC EFFECTS WITH CHLOROPRENE

Recently two of the operators working in the CD Building have been unable to work because of extreme shortness of breath and sub-sternal and precordial pain on even slight exertion. On questioning it was learned that this complaint was common among the men working in the CD building, so a study was begun to determine if these symptoms were caused by chemicals handled by this group of men. In this building the only probable toxic materials are Chloroprene and Mono Vinyl Acetylene. The fifteen men working in CD were examined and the accompanying chart indicates that of the fifteen men thirteen were suffering from shortness of breath, sub-sternal and precordial pain on examination; nine said they occasionally became dizzy on exposure to the fumes; four were dizzy very frequently; six had fairly severe headaches which were not present before working in CD; three complained of nervousness; eight of weakness and tiredness fairly marked; seven of palpitation on exertion; six of indigestion; two of nausea and vomiting; two of muscle pains. In three of the men a persistently high pulse was noticed. X-ray's were taken unless they had been taken within the last year, and showed nothing remarkable. Cardiograms were taken on five individuals in the group and showed nothing abnormal. Physical examination was normal in most of the men with the exception of one of those who is unable to work at present. This individual showed a deep sighing res-

piration, was dizzy on standing, had marked muscular pains, and the left ankle jerk was absent. Laboratory studies which had been started by Dr. Rankin, were continued in the present series and the accompanying chart indicates some evidence of early liver damage in nearly all cases. Urine analysis were normal with few exceptions. Blood counts were normal except for occasional shift towards premature cells.

The above train of symptoms suggested that the primary trouble with all of these men was referable to the circulatory system and the Crampton test, as recommended by Dr. Folger and Dr. Fleming, of the Haskell Laboratory, was started. To date nine men in this group have been checked and of these six show very marked circulatory disturbance. One shows slight disturbance with normal limits; four of these men show sufficient circulatory disturbances that they should not be working on any toxic chemical. The Index in two of these men was so low as to indicate the possibility of complete collapse at any time.

A study of the properties of Chloroprene was carried out by Dr. Von Oettingen several years ago and his conclusions were that circulatory disturbances would probably be the first finding in the men handling Chloroprene. Marked blood pressure drops in the animals were observed and explained on the basis of the marked vasodilation particularly involving the abnormal vessels. Secondary changes were experienced in the liver, kidney, and the heart, particularly, and to a larger extent through all tissues of the body due to the passive chronic congestion of these organs, and

to a less extent the direct toxic effect of the Chloroprene. We found by inhalation, one part in a million of Chloroprene exerted a toxic effect upon laboratory animals, and suggested that methods be devised to determine the concentration in the work room of men handling this material and that frequent checks be made. The very close similarity of these symptoms in laboratory animals, to those which are developing in the group of men exposed to Chloroprene ~~with these from the experimenting laboratory is so close that it~~ leaves no doubt that these workers are suffering from the accumulative effects of Chloroprene which is an exceedingly toxic material. We have noticed that even after being away from work for two or three days the Crampton Index still remains low indicating that these cumulative effects are not easily thrown off by the body. The men who are away from work at present on wage disability, complained of gradual increase in the precordial pain and shortness of breath on exertion over a period of many months until they became completely incapacitated. In the Crampton Index, we have an ideal test to indicate when a man should be removed from exposure to this chemical, as the circulatory changes are the first evidence ~~in this chemical~~ of toxic effects from this chemical. The present study should be continued to include the men working in the Polymerization Building who get considerable exposure to Chloroprene. It is ~~of value~~ felt that all of this group should have a Crampton test at least once every three weeks. An effort should be made to improve the working conditions by improved ventilation and great care in the handling of this chemical. The men should be

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told that it is a toxic chemical and should be handled accordingly. An effort should be made to determine the concentration of CD anywhere in the building, whereever desired. It will be interesting to determine whether Vitamin C, which improves tissue respiration will be of value in treating cases of chronic intoxication, but of course the major problem is to improve working conditions, make frequent examinations ~~possible and~~ If, in spite of all improvements toxic symptoms still exist, very frequent checks will have to be made to determine when a man should be removed from this operation.

Submitted by
W. D. Norwood, M.D.

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Contractor	—				21				

Doc Type	• RI • UP • HEASD 8D, S.U H.S F.N 22
Doc Title	VISIT TO THE U.S.S.R. ON BETA-CHLOROPRENE 23

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

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VISIT TO THE U.S.S.R. ON B-CHLOROPRENE

Report by: Sidney Pell
Sidney Pell, Ph.D.

Robert M. Prosser

Henry J. Trochimowicz
Henry J. Trochimowicz, Sc.D.

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3. Akopyan Institute of Public Hygiene & Occupational Diseases
4. Institute of Roentgenology and Experimental Oncology
5. Ministry of Health

APPENDIX

Outline of Dr. Sidney Pell's Presentation in the USSR

Dr. Trochimowicz's Presentation in the USSR

1. General Toxicity Considerations
2. Animal Carcinogenicity Studies
3. Embryotoxicity/Teratogenicity Studies
4. In Vitro Mutagenicity Studies
5. In Vivo Mutagenicity Studies
6. Male Reproduction Studies

Summaries on Chronic DCB and TCB Inhalation Toxicity Studies

SUMMARY

SUMMARY

Three Du Pont scientists visited the USSR from February 26 to March 8, 1978. They are listed in Exhibit A. Persons and places visited are listed in Exhibit B. At a "wrap-up" meeting at the Ministry of Health, Du Pont provided a three-page handout, which is shown in Exhibit C. This includes Du Pont's conclusions about the visit. A detailed write-up of findings on epidemiology is presented in Exhibit D, and on animal toxicology in Exhibit E.

The group's findings are listed below by subject matter.

Mutagenicity

Both Du Pont and Soviet scientists find chloroprene mutagenic to Salmonella bacteria.

Carcinogenicity

Both Du Pont and Soviet scientists agree that chloroprene is not a carcinogen in animals or man. The Soviet animal data is published. The animal studies at the Central Institute for Food and Nutrition Research (jointly sponsored by Bayer, BPCL, Denka and Du Pont) are not yet complete.

Du Pont's epidemiological study has been published. The Soviets informed us about a new epidemiology study of chloroprene workers, not yet published. This study covers approximately 7,000 workers of whom 600 are workers with up to 40 years of chloroprene exposure. The period covered was 1966-1975 and the chloroprene workers were compared to machine shop workers with no chemical exposure and with the plant as a whole. The Soviets stated that their preliminary results agree with those of Dr. Pell of Du Pont that chloroprene does not cause cancer.

Embryotoxicity, Teratogenicity and Male Reproduction

Du Pont scientists find no reproduction effects in rats at chloroprene levels significantly higher than those reported by Soviet scientists to produce effects. No resolution of these differences was found. The Soviets asked if we were interested in cooperative research, and we said we would answer that question.

The Soviet scientists questioned the validity of the Haskell Lab study on embryotoxicity and teratogenicity. It was agreed that their specific questions would be answered.

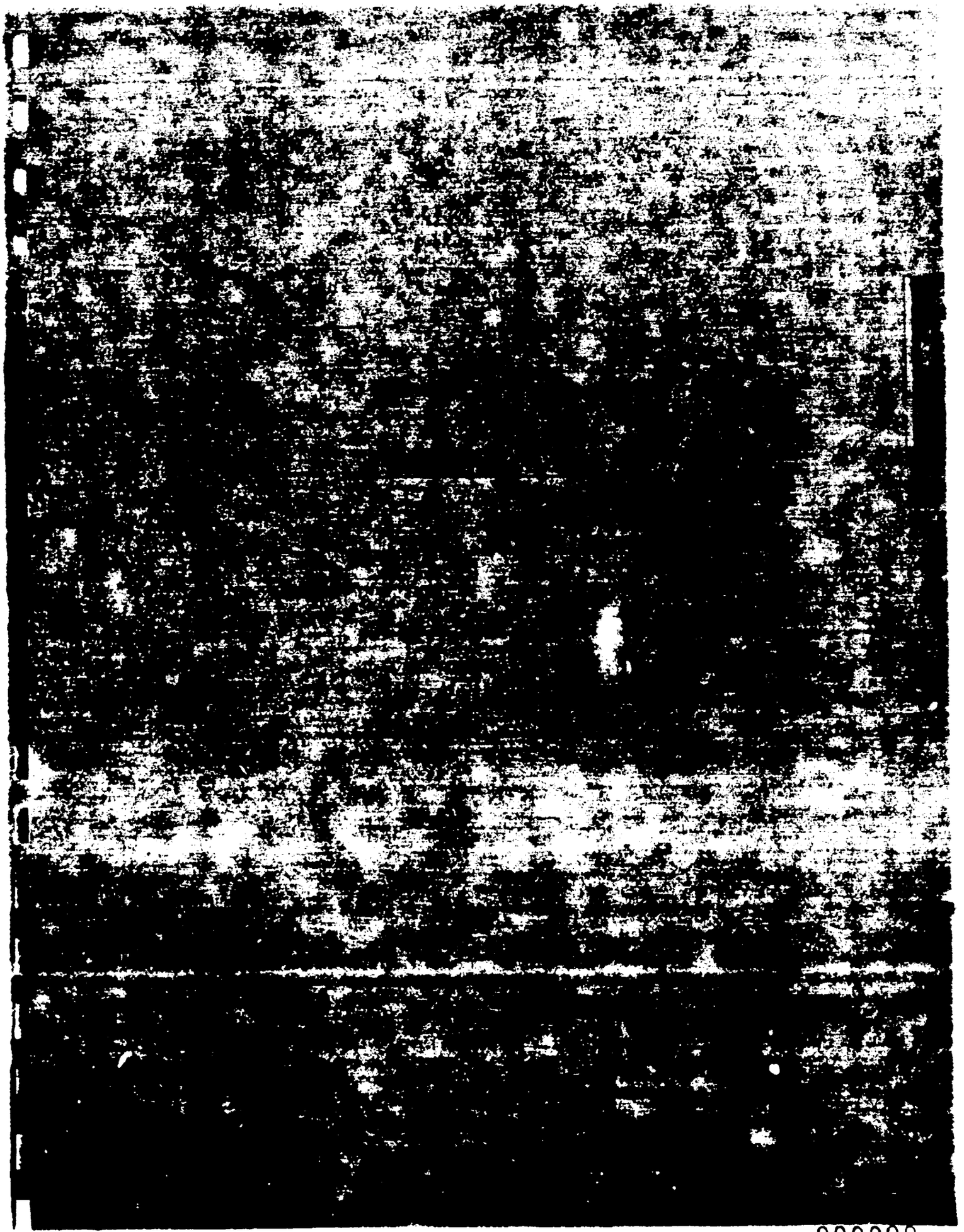
Industrial Hygiene

The delegation was not permitted to visit the Soviet polychloroprene plant. The questions which were to be asked at the plant were left with the Ministry of Health. They are on the third page of Exhibit C.

Other Items of Interest

IARC will present the results of a chronic feeding study on chloroprene at a meeting in Helsinki this year.

The Ministry of Health coordinates in the US with Dr. Rall of NIEHS.

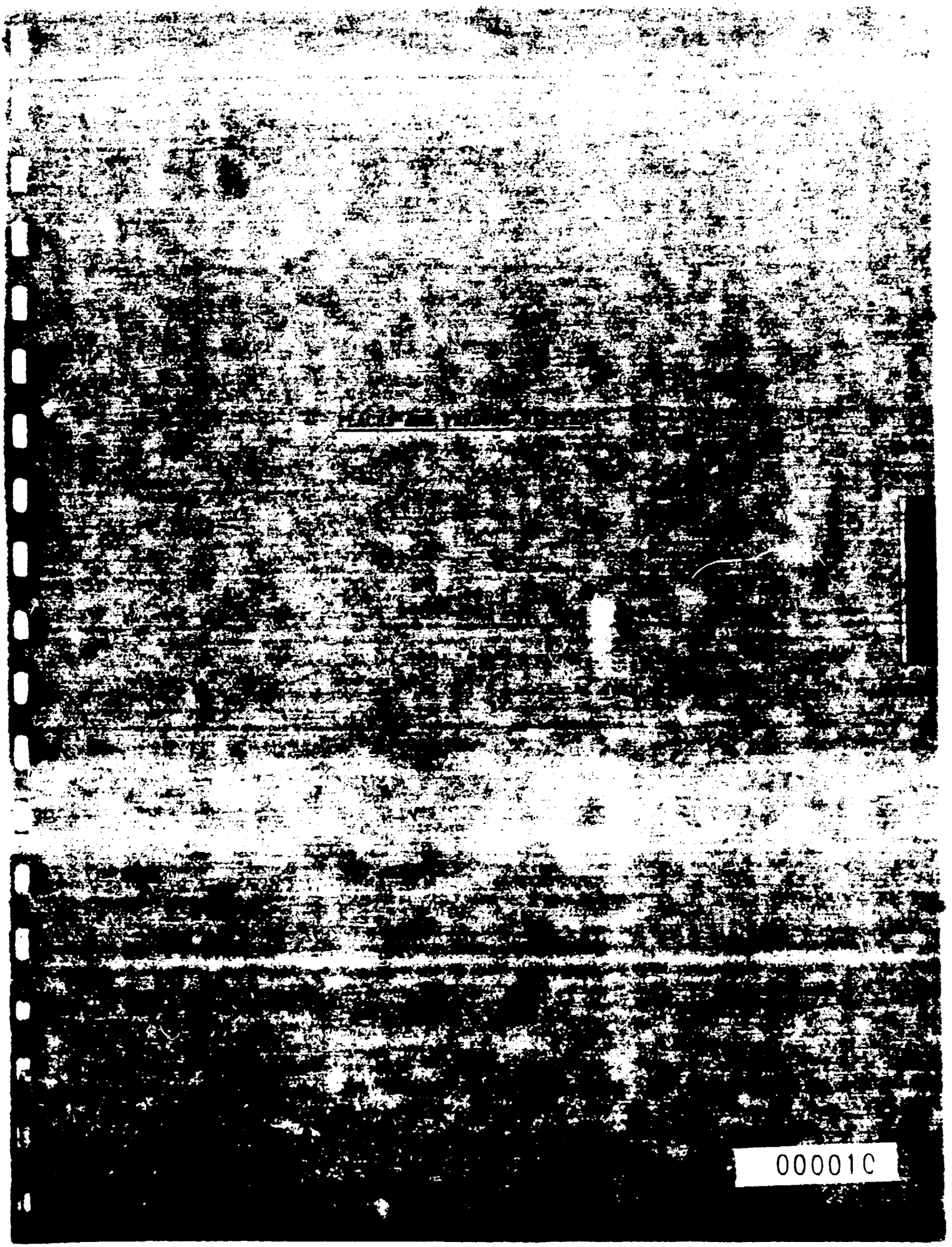


DU PONT VISITORS TO THE U.S.S.R.

Dr. Sidney Pell
Epidemiologist
Medical Division
Employee Relations Department

Mr. Robert M. Prosser
Research Manager, Chloroprene
Elastomer Chemicals Department

Dr. Henry J. Trochimowicz
Chief, Inhalation Toxicology
Haskell Laboratory
Central Research and Development Department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PLACES AND PERSONS VISITED

Oncological Research Institute - Moscow

Dr. Shabad - Chief of Institute
Dr. Turusov - Deputy Chief

Institute for Industrial Hygiene and Occupational Diseases of the Academy of Medical Sciences of USSR - Moscow

Dr. Izmerov - Director
Dr. Kasparov - Deputy Director
Dr. Sanotskii - Head, Toxicology Department
Dr. Fomenko
Dr. Salnikova — Toxicologists
Dr. Katasova
plus 6 other toxicologists, plus
Dr. Babian
Dr. Davtyan — from Armenia
Dr. Gizlarjian

Akopyan Institute of Public Hygiene and Occupational Diseases - Yerevan

Dr. Nairimonov - Director
Dr. Kogon - Deputy Director
Dr. Babian - Head, Toxicology Department
Dr. Davtyan - Toxicologist
plus 6 other toxicologists

Institute of Radiology and Oncology - Yerevan

Dr. Zilfian - Deputy Director
Dr. Fichidzhian
Dr. Gizlarjian — Toxicologists
plus 4 other toxicologists

Hosts for Visit to Yerevan

Dr. Pogosian - Deputy Minister of Health, Armenia
Dr. Tsekonian - Foreign Relations, Ministry of Health, Armenia
Dr. Arutiunian - Head of Radiobiology, Institute of Radiology and Oncology

Ministry of Health - Moscow

Dr. Borisov - Assistant to the Minister for Foreign Relations
Dr. Sklerov - Head, Industrial Hygiene
Dr. Sanotskii - Head, Toxicology Department, Institute mentioned above

PURPOSE OF DU PONT VISIT TO THE USSR

COMPARE INFORMATION ON THE POTENTIAL CARCINOGENICITY OF CHLOROPRENE.

DISCUSS AND SEEK AN EXPLANATION FOR THE GREAT DIFFERENCES BETWEEN SOVIET AND U.S. TOXICITY DATA ON CHLOROPRENE IN THE AREAS OF EMBRYOTOXICITY, TERATOLOGY AND REPRODUCTION.

VISIT YEREVAN CHLOROPRENE PLANT TO COMPARE SOVIET AND DU PONT INDUSTRIAL HYGIENE PRACTICES AND PROCEDURES.

DU PONT'S CONCLUSIONS OF VISIT

Very useful exchange of information.

Established valuable scientific contacts for future exchange.

Areas of agreement:

- Soviet and Du Pont scientists agree that evidence to date indicates chloroprene is not a carcinogen,
- and that chloroprene is mutagenic to Salmonella bacteria.

Areas of difference:

- Soviet scientists report that inhaled chloroprene produces embryotoxic and teratogenic effects in rats. Du Pont scientists find no such effects at levels 100 to 200 times higher.
- Soviet scientists report effects on male rat reproduction. Du Pont scientists find no effect on male reproduction at levels 100 to 200 times higher.
- There was no resolution of the cause of these large differences.

No conclusions on comparison of industrial hygiene practices were made. We were not able to visit the Yerevan plant.

DU PONT'S QUESTIONS CONCERNING POLYCHLOROPRENE PLANT

How is chloroprene exposure monitored at Yerevan plant?

Sampling
Analytical Methods

At Yerevan, what are the average and maximum levels of chloroprene to which workers in Dr. Zilfian's epidemiology study were exposed (1966-75, unpublished)?

Where is exposure in the process? Do workers have different symptoms depending on where they are exposed?

What controls are used?

What are the frequency and content of physical examinations for workers?

What other chemicals are in use at Yerevan?

How is vinyl chloride controlled?

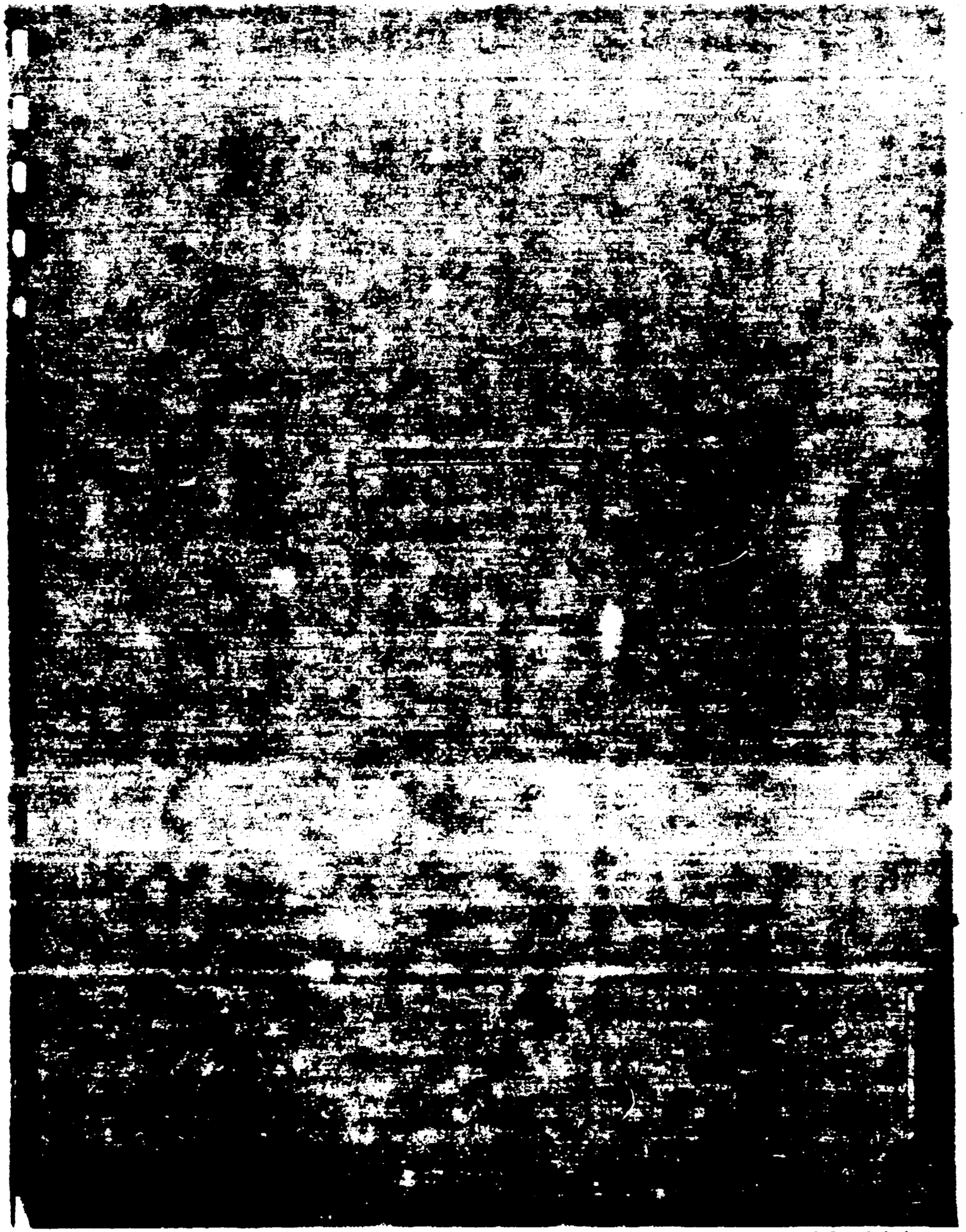
At Yerevan, what type of antioxidant (and concentration) is used for B-chloroprene?

Are chloroprene and polychloroprene operations indoors or outdoors?

For indoor operations, what is routine and emergency air changes per minute?

Is spot ventilation used?

What is threshold odor limit?



EPIDEMIOLOGY

Epidemiological studies of chloroprene were discussed with Drs. Shabad and Turusov at the Oncological Research Institute in Moscow. Dr. Turusov had just returned from the International Agency for Research on Cancer (IARC) in Lyon where he was chairman of the committee selected to prepare a monograph on the carcinogenicity of chloroprene. He told us that at the meeting American epidemiologists (unnamed) wanted to include for consideration the studies done by Khachatryan and to give it equal status with the Pell study. Dr. Turusov objected, telling the group that at their meeting two years ago, the Khachatryan papers were considered inadequate and wanted to know why they should now be given consideration. He said that although both studies had limitations, those of the Pell study were acknowledged and stated in the paper. He told the committee that the Pell study should be considered as at a higher level than those of Khachatryan, and his view prevailed.

Both Drs. Shabad and Turusov were strongly critical of Khachatryan's studies. They believed that evidence currently available indicates that chloroprene is not a carcinogen.

At the meeting of the Institute for Industrial Hygiene and Occupational Diseases in Moscow, Dr. Pell presented the results of his studies of workers exposed to chloroprene at the Chambers Works and Louisville Works. Copies of the tables were distributed to the attendees. The questions that were raised after the presentation dealt with the statistics that were used to compare the exposed workers with that of the control population. The Soviet scientists were apparently not familiar with the method of computing expected numbers and comparing them with observed numbers. They were looking for death rates. Dr. Pell, using a blackboard, described the method that was used to compute expected numbers.

Dr. Pell asked Dr. Fomenko questions dealing with the methodology of her study of reproductive function of male workers. This study was cited by Dr. Sanotskii in the paper he presented on the toxicology of chloroprene at a conference at Research Triangle Park, North Carolina sponsored by the National Institute of Environmental Health Sciences and published in Environmental Health Prespectives (Oct. 1976). A translation of a paper by Dr. Fomenko and others in which this study was described was obtained by Haskell Laboratory. This translation does not specify the numbers of subjects in the exposed and control groups, or the methods by which the data were obtained, although the numbers of subjects were stated by Sanotskii in his paper.

Dr. Pell's questions dealt with the methods by which the exposed and control subjects were selected, the methods by which the occurrence of spontaneous abortions was obtained in each group, and the exposure levels. In Dr. Fomenko's reply, she stated that she selected all 143 men who worked in the chloroprene synthesis and 118 controls in another plant. There was no indication of any attempt to match the controls to the exposed workers by any characteristic.

The occurrence of spontaneous abortions was obtained by a questionnaire, but a copy of the questionnaire was not available at that time. Dr. Fomenko agreed that it is difficult to determine accurately the incidence of spontaneous abortions.

With regard to exposure levels, Dr. Fomenko showed Dr. Pell a publication that contained a table showing the range of air concentrations. These data did not appear in the translated paper obtained by Haskell Laboratory, suggesting that the papers abailable in the U.S. may not be complete, and that complete, detailed papers published in the U.S.S.R. are not always accessible in the U.S.

At the meeting at the Akopyan Institute of Public Hygiene and Occupational Disease in Yerevan, Dr. Babian suggested to Dr. Pell that he discuss studies other than cancer because their views and ours on the carcinogenicity of chloroprene are the same. However, time did not permit Dr. Pell to make any presentation.

Dr. Babian described a theory he has proposed. He said that the literature indicates that peroxides reduce tumor growth. Since chloroprene is a peroxide, it may inhibit tumor growth in large doses, even though it may possibly induce tumors in small doses. He said oncologists believe his theory has merit, but they agree with Dr. Pell and other Soviet scientists that chloroprene is not a carcinogen.

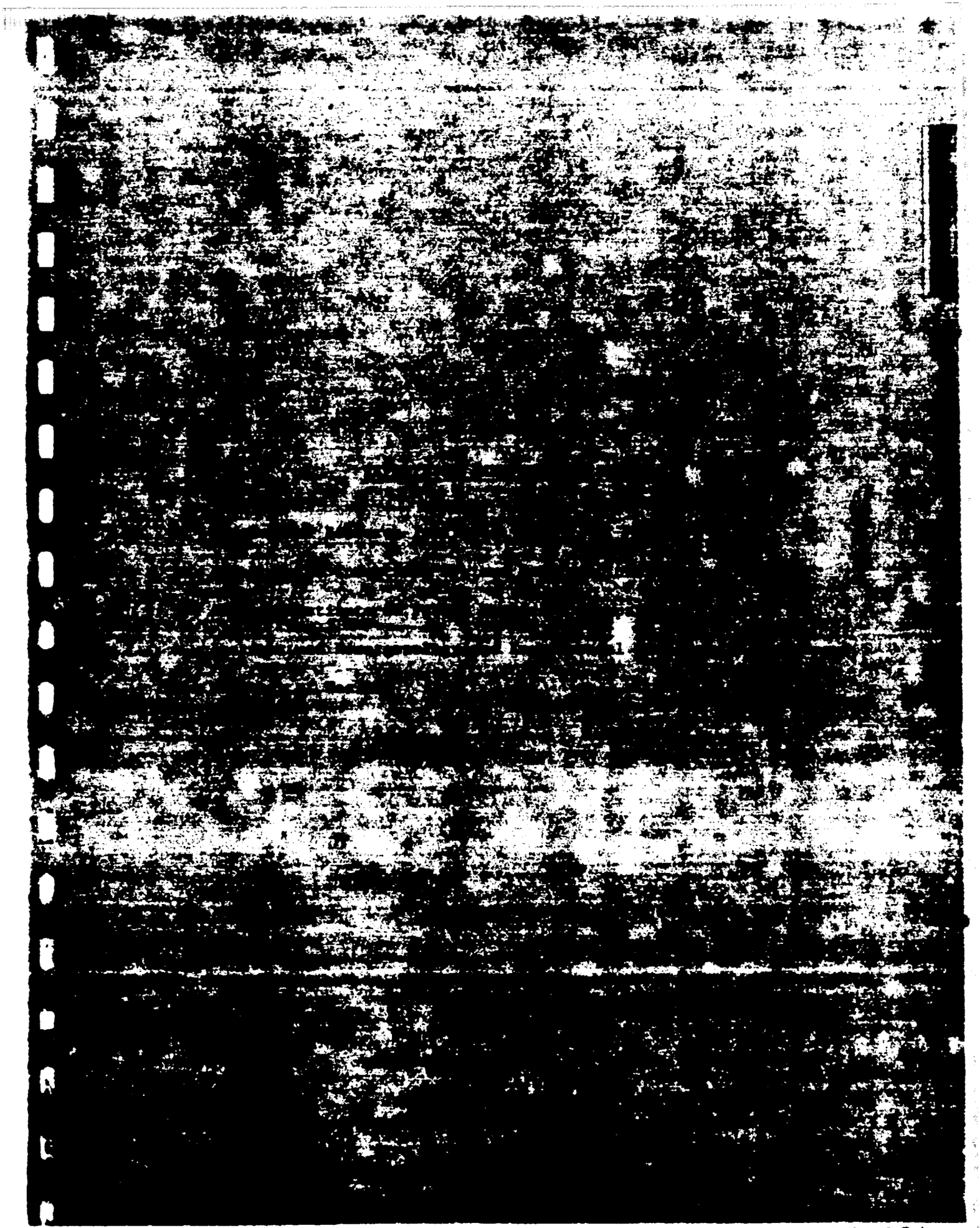
At the Institute of Radiology and Oncology in Yerevan, Dr. Pell presented the results of his cancer epidemiological studies. After the presentation, Dr. Zilfian stated that there are "no significant differences between your data and our data." He said that they have been doing an epidemiological study that covers the ten-year period, 1966 through 1975. They are studying cancer incidence in about 600 men in chloroprene shops; in the plant as a whole; and in men in a machinery shop who had no chemical exposure. There are a total of about 7,000 men in the study. Preliminary results show no significant differences in incidence of lung cancer among the three groups.

In response to questions by Dr. Pell, Dr. Zilfian stated that although the study period begins in 1966, exposure to chloroprene started before that date, and could have been up to 40 years before the start of the study, indicating that there was sufficient latency to detect any cancer incidence that might be related to chloroprene exposure.

In response to other questions, Dr. Zilfian said that all the persons in the study were men, since women were removed from chloroprene exposure in 1946. Regarding exposure levels, he said they would have to be obtained from the industrial hygienists. Lung cancer cases are found by periodic health examinations. All workers at the plant are given chest x-rays annually at a polyclinic near the plant, and persons with suspicious findings are sent to the Institute for follow-up. Retired workers are examined at the polyclinic every six months, and can use the services of the clinic at any time. Losses to follow-up are not a problem because "Armenians prefer to stay in Armenia."

These responses by Dr. Zilfian indicate that their methodology is sound. A complete evaluation of their study cannot be made until they publish a final report.

At the final meeting at the Ministry of Health in Moscow, Dr. Pell requested of Dr. Sanotskii a description of the methodology for Dr. Fomenko's study of spontaneous abortions, which he agreed to furnish. Dr. Sanotskii then requested of Dr. Pell the results of his morbidity studies, which Dr. Pell agreed to furnish as soon as they are completed.



MEETING ON B-CHLOROPRENE (CD)

at the
Oncological Research Institute
Moscow, U.S.S.R.
February 27, 1978

ATTENDEES:

Oncological Research Institute	Du Pont Company
Dr. L. Shabad, Academician	Mr. R. M. Prosser
Dr. Turusov, Deputy Director	Dr. Sidney Pell
	Dr. H. J. Trochimowicz

The meeting took place in Dr. Shabad's office and began with a brief introduction by R. Prosser on the reasons for our visit. No interpreter was needed at this meeting since both scientists spoke English very well.

Dr. Shabad then limited the scope of further discussions by stating the main function of his institute - fundamental research in cancer. He then turned the meeting over to Dr. Turusov who had just recently returned from Lyon, France, where he had been Chairman of IARC Monograph Committee on Chloroprene (Dr. Loser of Bayer represented the Joint Interindustry Group on Chloroprene at the same meeting). He then reviewed pertinent items from that meeting:

1. Animals Studies by Zilfian (1975 & 1977; Armenia SSR)

   The IARC group accepted these studies as being "fairly sound." The conclusion was that CD is probably not carcinogenic by the skin, intratracheal, subcutaneous, or oral routes to mice or rats. The IARC group did point out some limitations in these studies - like not specifying the sex of the test animals - but felt that they were inconsequential.

Only published studies were considered for the IARC Monograph. Thus, our chronic inhalation studies (at CIVO; Zeist, Netherlands) were not considered.

2. Chronic Feeding Studies at IARC

These studies in rats have completed the experimental phase but are not yet published. Thus, they probably will not be included in the IARC Monograph on Chloroprene. Dr. Turusov did say, however, that the results of these CD studies will be presented at a meeting in Helsinki this year (Du Pont will send a representative; probably J. Stam; dates not known).

The IARC group felt that results of these rat feeding studies were ambiguous relative to compound-related tumors (kind? malignant? benign? - unknown to Turusov). If the data were analyzed by Chi Square without Yates' correction, results were statistically significant. With Yates' correction added, results were not statistically significant.

3. Khachatryan and Pell Epidemiology Studies

Two years ago at an IARC meeting on Chloroprene, the group felt that the Khachatryan studies were "inadequate." Now (February 1978), certain epidemiologists in the group (J. Wagner of OSHA was present) want to "elevate the Khachatryan work." "What happened over two years? Like good wine, they must have improved with age," said Dr. Turusov.

Dr. Turusov then mentioned that "some epidemiologists" in the Group wanted to put both the Khachatryan and Pell studies on the same level because, supposedly, both had many inadequacies. However,

RESULTS OF CARCINOGENICITY STUDIES

Reference	Species	Lowest Effect Level	Type of Effect
1. Trochimowicz (unpublished)	Rat	None at 180 mg/m^3 (gross results at 24 months)	Not carcinogenic
	Hamster	None at 180 mg/m^3 (gross results at 18 months)	Not carcinogenic
2. Zilfian (1975)	Mice & rats	None at: 200 mg/kg (oral) 200 mg/kg (subcutaneous) 50% (skin)	Not carcinogenic
3. Zilfian (1977)	Mice & rats	None at: 200 mg/kg (oral) 200 mg/kg (subcutaneous) 200 mg/kg (intratracheal) undiluted (skin)	Not carcinogenic
4. Khachatryan (1974)	Mice & rats	N.R. (skin) 0.6 ml latex (subcutaneous) 0.5 ml latex (intratracheal)	Carcinogenic
5. Khachatryan (1975)	Mice & rats	0.5 ml latex (oral) 0.6 ml latex (subcutaneous) N.R. (skin)	Carcinogenic
6. Garibyan (1977)	Mice & rats	N.R.	No blastomogenic activity in sebaceous gland test.

RJT:vls
2/23/78

Additional Items

Drs. Shabad and Turusov were very interested in the carcinogenicity potential of acrylonitrile (AN) and vinylidene chloride (VDC). Dr. Turusov told us that the IARC group concluded that AN was a human carcinogen. He asked Dr. Pell's opinion. Dr. Pell stated that the data on AN "strongly suggest" that it is a human carcinogen.

Relative to VDC, the IARC group felt that "VDC should be treated as if it were a human carcinogen." This opinion was based on positive animal data alone.

The entire meeting lasted approximately two hours. Dr. Pell left a copy of updated tables for his mortality study. Dr. Trochimowicz left a summary of animal carcinogenicity data on CD. Both are found in the appendices.

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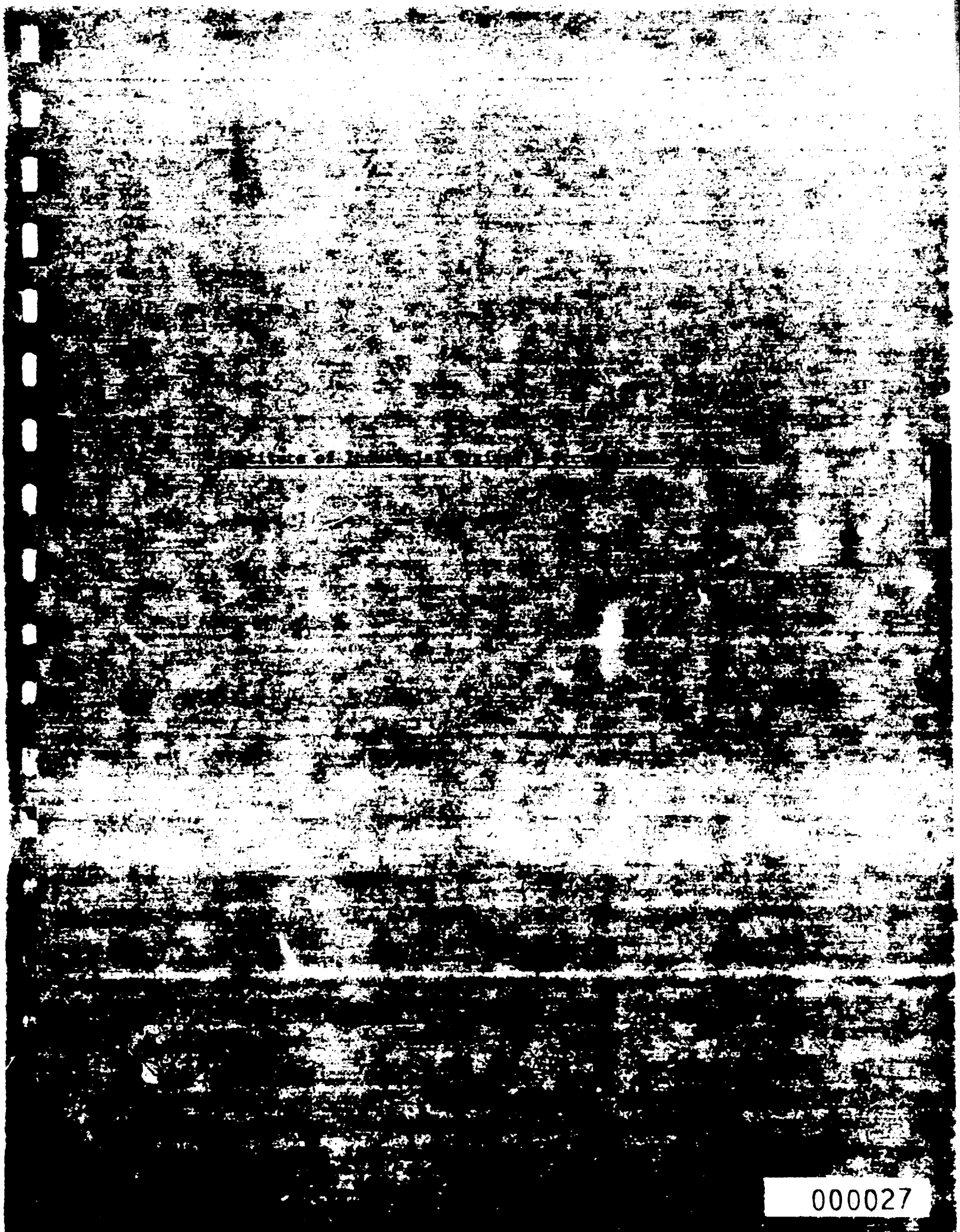

MEETING ON B-CHLOROPRENE (CD)

at the
Institute of Industrial Hygiene & Occupational Disease
Moscow, U.S.S.R.
February 28, 1978

ATTENDEES:

Dr. N. F. Izmerov, Director
Dr. A. A. Kasparov, Deputy Director
Dr. I. V. Sanotskii, Head of Toxicology Laboratory

TOXICOLOGISTS AT INSTITUTE:

Dr. V. N. Fomenko
Dr. L. D. Katosova
Dr. L. S. Salnikova
Dr. Z. A. Volkova
Four others

INSTITUTE OF PUBLIC HYGIENE AND OCCUPATIONAL DISEASES
YEREVAN, ARMENIA SSR

Dr. Babian
Dr. Davtyan

Dr. Gizlarjan

Two interpreters from Institute

DU PONT COMPANY

Mr. R. M. Prosser
Dr. Sidney Pell
Dr. H. J. Trochimowicz

The meeting began with introductions of the above participants. Mr. Prosser then reviewed Du Pont's historic interest in chloroprene, past and current methods of production, exposure history to CD, and the many precautions taken in the industrial synthesis, handling, and storing of B-chloroprene. The Soviets were particularly interested in the individual monitoring devices which Mr. Prosser had described and they questioned him extensively.

ochimowicz then reviewed the experimental toxicology data on
ne. Briefly, this presentation involved the areas of animal
city, in vivo and in vitro mutagenicity, embryotoxicity, terato-
nd male reproduction studies. Each of the preceding topics was
n terms of:
rtinent Soviet and Du Pont references
mparison of Soviet and Du Pont results
ssible reasons (general) for differences
re specific items for discussion relative to our understanding
the Soviet studies
ls of Dr. Trochimowicz's presentation are given in the Appendix of
report.

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and Comments to Dr. Trochimowicz

Soviet scientists did not immediately comment or reply to questions
the preceding presentation. That came toward the end of the day.
oint, Dr. Sanotskii and colleagues proceded to question Dr. Trochimowicz
is technical and philosophical aspects of our toxicological studies
Haskell Laboratory or at CIVO (Netherlands). The Soviet scientists
iously received copies of Dr. Culik's paper and pre-publication
f general review papers by J. J. Clary, et. al.
e of the questioning dealt with our chronic study at CIVO. Dr. Sanotskii
ed that B-chloroprene could be inhaled by rats and hamsters for almost
s at 180 mg/m^3 without producing mortality or other adverse effects.
oned that most chlorinated hydrocarbons are cumulatively toxic,
lly at a concentration of 1/10 the LC50 (Dr. Sanotskii must have been
g of a Soviet LC50 for CD; an approximate LC50 for CD in rats is
/m^3 reported by CIVO). He then mentioned that Soviet animal studies

anomalies
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Soviet Replies to Dr. Trochimowicz's Question

Dr. Sanotskii started by saying that one difference between Soviet and Du Pont studies is that Du Pont uses "abstract chloroprene" whereas they use "natural chloroprene." In response to our question on a definition for the latter, he said, "This is the kind of chloroprene that occurs in plant air."

Dr. V. Fomenko then summarized three kinds of experimental studies that had been conducted with B-chloroprene:

1. Chloroprene Latex

   In these experiments, the latex (containing CD and ammonia) was prepared daily. Rats were exposed to CD at a concentration of 14 mg/m^3 (NH_3 concentration was 4 x lower than MPC). Under these conditions, embryotrophic effects were produced in rats (subcutaneous swelling at gross pathologic examination). CD was monitored analytically in chamber air via spectrophotometry.

   In another experiment, pregnant rats were exposed to NH_3 at 60 mg/m^3 (3 x MPC). The result was no embryotrophic effects.

2. Pure B-Chloroprene

   CD was distilled daily, kept in a dark glass container and kept on ice during exposure of animals. The CD was stabilized with 1.0% phenylbetanaphthylamine.

   When not in use, CD was kept refrigerated. Daily, one fresh portion was taken for use.

   Rats were then exposed to CD at 3-4 mg/m^3 (spectrophometrically determined). No embryotrophic effects were seen.

3. B-Chloroprene and Dimers

This work is being done by a combined effort of Russian and Armenian scientists.

"Daily-fresh" CD and fresh dimer samples were used and compared by intraperitoneal injection in small animals. Cytogenetic indices were measured. The result was that "more cytogenetic damage was seen with CD than with dimers."

As far as general philosophies of testing, these are shown schematically in Figure 1 (Embryotoxicity/Teratogenicity), Figure 2 (Gonadotropic Action), and Figure 3 (Mutagenicity). These testing schemes are thorough and generally the same as ours in terms of philosophy.

In reply to some specific questions on techniques, the Soviets gave the following answers:

1. Embryotoxicity/Teratogenicity

   a. In all experiments (although rarely reported in their papers), controls exposed to air alone are used. Except for exposure to the compound, controls are handled and treated in an identical manner to test animals.

   b. Soviet embryotoxicity studies often show ~15% "total embryonic death" on the average (maximum of 30%) as a normal background variation.

   c. Also as a normal occurrence in such studies, "embryo swelling" is not uncommon. Whether this is an embryotoxic or teratogenic effect is not known. Soviets say they used the methods of Wilson but are thinking of switching to those of Dr. Staples (NIEHS).

As an example of "embryo swelling" as well as other effects of chloroprene on fetuses (hemorrhage, hydrocephaly), the Soviets gave us some photographs of sectioned rat fetuses. Whether or not these photographs are of fetuses from CD-exposed mothers is not known.

d. As far as when and how pregnant animals are sacrificed, this is usually done (standard study) on the 20th day of gestation. One animal from each test and control group are sacrificed almost simultaneously until all groups are sacrificed. Sacrifice takes place by quickly breaking the animal's neck.

e. Embryotoxicity and/or teratogenicity <u>data is collected individually for each pregnant female</u>:

$$\text{example: } \frac{B - A}{B} \times 100 = \% \text{ for each pregnant female rat}$$

where B = no. of corpora lutea
A = no. of live fetuses

f. As to whether or not the position of the fetus in the uterine horn is noted, the answer was "no." They stressed that damage to the fetus is independent of its place in the uterus. And, certainly, resorptions are not dependent on uterine position either.

g. As far as the kind of statistics used to evaluate such studies, the Soviets presented us with two books (in Russian). Statistical methods, as well as other procedures for testing, were reported to be contained in these books. However, they reported that their statistical methods were the same as those of Dr. Culik.

2. Cytogenetics

Relative to techniques used for treated and untreated groups, the evaluator reads all slides "blind." Confirmation is then achieved by re-examination on the same "blind" basis.

Some of the basic techniques in this area reportedly are described in the books above.

3. Male Reproduction

Dr. Davtyan (Armenia SSR) had published apparently contradictory results:

a. CD produces effects on male fertility and sperm motility at 0.15 mg/m^3 (1972).

b. CD produces no effects on fertility or sperm motility at 39 mg/m^3 CD.

He responded by saying that study (a) (1972) involved a 5 1/2 month exposure, whereas study (b) was only a 48-day exposure. Although we were aware of the exposure difference, this alone cannot account for the large difference.

4. Carcinogenicity

Dr. Sanotskii was not interested in discussing animal carcinogenicity. He said that there was no concern over the potential carcinogenicity of B-chloroprene in the USSR. Other concerns such as mutagenicity, embryotoxicity, etc., were of far greater importance.

Dr. Pell's Presentation

Dr. Pell presented his updated results of his studies of CD workers at the Chambers Works and Louisville sites. Copies of updated tables (see Appendix) were distributed to the Soviet Scientists. From the types of questions raised, it appeared that the Soviet scientists were not familiar with many basic statistical and epidemiologic techniques used in the USA.

Dr. Pell then questioned Dr. Fomenko about her study on the reproductive function of male workers. Specifics can be found in Dr. Pell's epidemiology overview in Exhibit D. One interesting item on this study was that apparently no attempts were ever made to match controls to exposed workers by any characteristics or to take into account other characteristics in the analysis of their data. This appears to be true for every USSR human study (male reproduction, cytogenetics, etc.) evaluated by Haskell Laboratory.

Other Items of Interest

1. Lunchtime Comments

   a. 10 ppm TLV recommendation

      Dr. Sanotskii asked why we were recommending a TLV of 10 ppm (v/v) rather than 25 ppm. We answered that 10 ppm provides a beter margin of safety relative to basic toxicity indices. For example, 50 ppm in our chronic rat study has produced a slight weight loss in the test animals as well as some evidence of slight intermittent respiratory irritation.

   b. Ames Test

      When asked about how he views Ames Test results, Dr. Sanotskii said "they are worthless." He added further that no correlation to animals or human can be made from work with bacteria that had been stripped of all natural barrier defenses.

c. Visit to NCTR

On a visit there about a year ago, Dr. Sanotskii was very impressed with Arkansas facility, staff, and equipment.

d. Metabolism Studies with CD

In response to his question, we informed Dr. Sanotskii that we have not conducted metabolism studies on CD since radioactive CD readily polymerizes.

2. Dr. Sanotskii's Position in the USSR

Not only does Dr. Sanotskii head the Toxicology Laboratory at the Institute, he is also responsible for proposing (like NIOSH) and promulgating (like OSHA) industrial Maximum Permissible Concentrations for the USSR.

The PR booklet we received from the Institute (in English, incidently) more fully describes the functions of the Institute in general and Dr. Sanotskii's Toxicology Laboratory in particular.

3. Tour of Inhalation Toxicology Laboratory

Dr. Sanotskii gave us a "five-minute" tour of part of his laboratory. Although the laboratories were not very clean by our standards, his inhalation chambers (painted on inside and outside) and generating equipment were simple but adequate. His analytical equipment was very up to date (Miran IRs, German and French GCs, etc.)

4. Cooperative Research

Dr. Sanotskii suggested that one way we might resolve the differences between Soviet and Du Pont studies is by some cooperative research programs. Possibly, we could use the same indices of effect, the same CD samples, etc. We agreed to consider this possibility.

MEETING ON B-CHLOROPRENE (CD)

at the
Akopyan Institute of Public Hygiene & Occupational Diseases
Yerevan, Armenia SSR
March 2, 1978

ATTENDEES:

Akopyan Institute	Du Pont Company
Dr. Z. M. Nairimonov, Director	Mr. R. Prosser
Dr. Kogan, Deputy Director	Dr. S. Pell
Prof. Babian, Head of Toxicology Laboratory	Dr. H. Trochimowicz
Dr. Davtyan, Toxicologist	
Six other Institute Toxicologists	
Dr. U. G. Pogosian Deputy Minister of Health Sanitary Doctor of the Republic Former employee of Institute	
Mr. R. V. Tsekonian Foreign Relations Deputy Ministry of Health	
Dr. Arutiunian Head of the Radiobiology Laboratory Institute of Roentgenology & Experimental Oncology Armenia SSR	
Interpreter (from Intourist)	

The meeting began with an introduction by Director Dr. Nairimonov. He introduced his staff and outlined the functions of the Institute. The Institute stresses preventive medicine as well as solving everyday problems. He said that growth of the chemical industry is important but people's health is even more important. Thus, in order to assure the health of the people, everything (air, water, etc.) must be under the strict control of the State.

The Institute deals mainly with the chemical and metalurgical industries in Armenia. "The chemical industry is our major polluter. Now, we're in the chemical era, just as we were in the era of microbes decades ago. One can see the results of the Institute's work over the past few-decades."

Mr. Prosser's Presentation

Mr. Prosser introduced the Du Pont people and then reviewed our historical interest in chloroprene, past and present methods of manufacture, handling precautions, and the various physical and chemical properties of B-chloroprene.

Dr. Trochimowicz's Presentation

Dr. Trochimowicz reviewed the animal toxicology data on CD in the areas of animal carcinogenicity, in vivo and in vitro mutagenicity, embryotoxicity, teratogenicity, and male reproduction studies. Each of the preceding toxicological areas was reviewed in terms of:

- Pertinent Du Pont and Soviet References
- Comparison of Soviet and Du Pont Results
- Possible Reasons (General) for Differences
- Specific Items or Questions for Discussion

Details of the preceding presentation are given in the Appendix.

Questions to Dr. Trochimowicz

As occurred in the previous Institute of Industrial Hygiene and Occupational Diseases (Moscow), Dr. Trochimowicz's questions were not to be answered until much later. Dr. Babian and his staff then proceeded to ask numerous questions on philosophy of testing in general, criteria used for setting TLVs, criteria for evaluating gonadotripic actions, teratogenicity, embryotoxicity, mutagenicity, and carcinogenicity. Questions were also asked specifically on laboratory procedures used for embryotoxicity/teratogenicity studies. As in Moscow, most of these questions were answered in Dr. Culik's paper.

It is interesting to note that Dr. Babian (Head Toxicologist) and Dr. Davtyan were present at the February 27 Moscow meeting. Practically the identical questions asked in Moscow and answered were asked here again for the edification of the rest of the staff in attendance.

Also, questions raised in Moscow (February 27) about Dr. Culik's paper were asked here again. Dr. Trochimowicz replied that Dr. Culik's reply to Dr. Fomenko would also be sent to Dr. Davtyan at this Institute.

After two hours of such questioning, Dr. Babian concluded incorrectly that the differences between Soviet and Du Pont results are attributable to differences in sensitivity of test methods. This, of course, was not true and we said so. The effects reported by the Soviets at levels as low as 0.15 mg/m^3 are gross effects like embryo death, hemorrhage, hydrocephaly, etc. These indices are by no means 'sensitive' parameters but represent very gross indices of effect. We see no such effects at levels of CD 200-300x higher.

After more discussion on methods and philosophy, Dr. Babian said that "We are very different. Our system works for us and your system works for you."

Answers to Dr. Trochimowicz's Questions

1. Chloroprene and handling

Dr. Babian said that the B-chloroprene used in their animal experiments is 99.7 - 99.9% pure. It is "fresh chloroprene" taken directly from the plant in bottles cooled by dry ice (~5°C)

CD is kept refrigerated at approximately -10°C. Air was bubbled through the CD to generate chamber atmospheres but "fresh CD" was used daily. "CD was never stored but used immediately."

2. Analytical Measurements

During exposures, many measurements are taken (~10/4-hour exposure) to assure uniformity. Spectrophotometry was the main analytical method used in these studies and is described in a paper by Apoyan (Gig. Hyg. & Sanit. 9, around 1970).

However, since 1973, Dr. Babian reported that gas chromatography (GC) was the main method used to analyze CD in air. In a paper by Babina (~1973), GC, spectrophotometric, and wet chemistry methods for CD were compared. The most sensitive method was spectrophotometry but GC was most specific. In all comparisons, GC was either lower or equivalent to spectrophotometry. Wet chemistry methods were not as good and often differed from spectrophotometric results by as much as 25%.

As an example, using both GC and spectrophotometry over two months on a long term study, the average CD concentration using spectrophotometry was 1.02 mg/m^3 while GC gave 0.99 mg/m^3.

3. Stabilizer for Chloroprene

Dr. Babian mentioned that the "fresh chloroprene" they use from the plant contained no stabilizer. Mr. Prosser asked where in the plant can you get a CD sample without stabilizer. Dr. Babian said from "the hydrocarbon phase." When Mr. Prosser said that he knew of no place in our CD plant where one could get CD without stabilizer, Dr. Babian corrected his earlier statement. He said that an antioxidant is present in their chloroprene sample to the extent of 0.02 - 0.03% but what it is is not known. However, he knew that it was not phenylbetanaphthylamine (as was mentioned in the February 27 meeting in Moscow).

4. Cytogenetic Methods

As to which methods they use, Dr. Babian said that he and Dr. Fomenko (Moscow) use the methods of Ford and Wolan (Exper. Cell Research, 32 (2): 320-326, 1963).

Most cell work is evaluated in the metaphase stage (Method of Hangenford).

In addition, like in Great Britain and Hungary, we also measure fetal gamma agglutinin as another index of effect.

"We have defined cytogenetic effects of CD in man (in vivo and in vitro)."

Thus, the methodology for cytogenetic work is the same in the USSR, Great Britain, and other countries.

Although we were happy to get the general references above, Dr. Trochimowicz's specific questions in his initial presentation were not answered (see Appendix on "Animal Mutagenicity").

5. Procedures on Embryotoxicity/Teratogenicity Studies

"Some of the discrepancies between Soviet studies (Mnatsakanyan, for example) and yours may result from whole life sacrificing." Dr. Babian said that sacrifices are conducted on pregnant mothers over the whole pregnancy period (day 1 - day 20). The final sacrifice is conducted on the 20th day of pregnancy. On any one sacrifice day, control and test animals are sacrificed one from each group until all animals are done (within a 4-hour period).

Dr. Babian said that they look at all aspects of embryotoxicity (preimplantation, post-implantation, fetal stage) and thus, "our methods are much more sensitive and thorough than yours." Thus, the obvious implication was that this could account for the differences between Du Pont and Soviet studies. This is simply not true!!!

Dr. Pell's Presentation

Dr. Babian apologized to Dr. Pell because there was no time for any presentation on his part. However, he added that he didn't care to discuss Dr. Pell's cancer studies because "our views are the same."

Other Important Items

1. Carcinogenicity

Dr. Babian stated that there was "no serious concern over carcinogenicity in our country." He then, however, espoused his own theory. Peroxides lessen the growth of tumors and this is the basis for radiotherapy. Possibly, at low doses, CD cannot induce peroxides. Oncologists always want to test substances at doses that don't induce peroxides since high doses of CD, for example, induce peroxide formation.

"However, there is no data in the literature to say that chloroprene is a carcinogen and we agree with Dr. Pell," said Dr. Babian. He continued, "Therefore in our opinion and in the opinion of other Soviet scientists, chloroprene is not a carcinogen."

2. Dimers

Dr. Babian had asked what studies had been done on dimers. We mentioned that we had gotten negative results on Ames Tests with dimers. He said that "we agree for a change." His laboratory, too, had tested dimers in bacteria and had found no mutagenic effects.

In response to our question on publication of the above results, Dr. Babian replied that "we will publish the results in an American journal if you like."

3. DCB

Dr. Babian asked whether or not we had publications on DCB toxicity. We answered that we were doing studies but had not yet published.

4. Reprints

Dr. Trochimowicz, at Dr. Babian's request, left him copies of Dr. Culik's paper on embryotoxicity/teratogenicity studies on CD and Dr. Pell's paper on CD workers.

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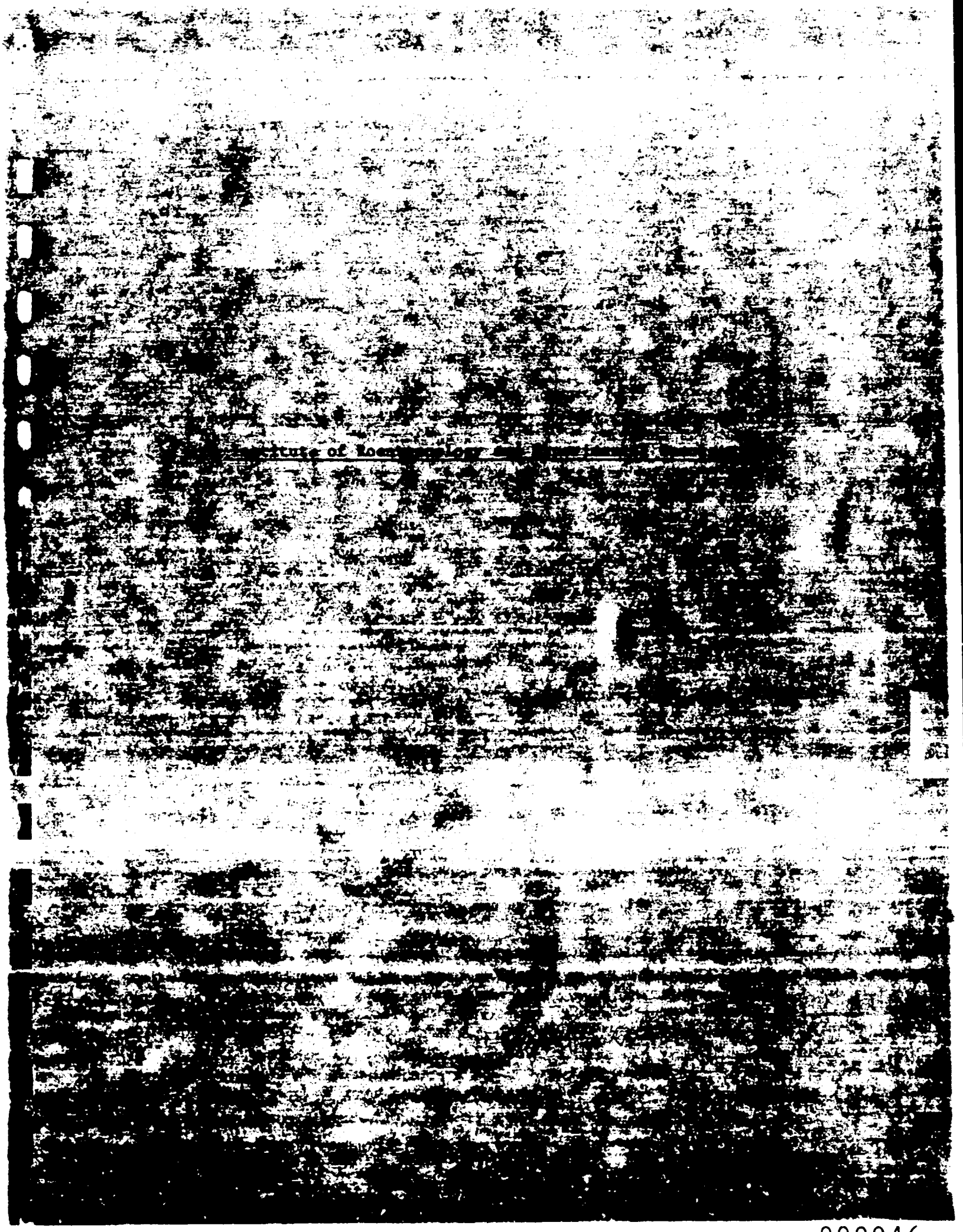


MEETING ON B-CHLOROPRENE (CD)

at the
Institute of Roentgenology and Experimental Oncology
Yerevan, Armenia SSR
March 3, 1978

ATTENDEES:

Institute	Du Pont Company
Dr. V. N. Zilfian, Deputy Director	Mr. R. Prosser
Dr. Arutiunian, Director of Radiobiology Laboratory	Dr. S. Pell
Dr. B. S. Fidchidzhian, Toxicologist	Dr. H. J. Trochimowicz
Dr. Gizlarjan, Toxicologist	
Four other toxicologists	
Dr. T. G. Pogosian, Deputy Minister of Health for Armenia	
Mr. R. V. Tsekonian, Foreign Relations Officer of the Ministry of Health, Armenia	
Interpreter (In-tourist)	

Dr. Zilfian introduced his staff and pointed out that his institute was mainly concerned with carcinogenicity research.

Mr. Prosser then gave a brief summary of our historical interest in B-chloroprene and then introduced the other Du Pont people.

Dr. Trochimowicz's Presentation

Dr. Trochimowicz reviewed only the "Animal Carcinogenicity" portion of his presentation contained in the Appendix. After reviewing the protocol for our ongoing chronic study at CIVO, and the negative results to date, Dr. Zilfian was glad to hear that our animal studies are confirming his findings (Zilfian, 1977) that chloroprene is not carcinogenic.

Dr. Pell's Presentation

Dr. Pell presented updated results from his cancer epidemiology studies at Chambers Works and Louisville and left copies of his presentation with Dr. Zilfian. Dr. Zilfian then said that he was happy to hear that there are "no significant differences between your data and our data."

Dr. Zilfian's Epidemiology Study

Dr. Zilfian reported that his Institute has conducted an epidemiology study on chloroprene workers. The study covers a 10-year period (1966-1975) and involves workers who started before 1966 and some workers with as much as 40 years' exposure to CD. The whole study involves about 5,000-6,000 workers:

1. 600 men in chloroprene shops,
2. machinery shop workers who "never had exposure to CD or any other chemicals,"
3. workers in the plant as a whole.

Preliminary results show no significant differences in the incidence of lung cancer among the three above groups.

Dr. Zilfian then added that both epidemiological and experimental data show that chloroprene has no carcinogenic effect.

In response to questions by Dr. Pell about details of the preceding study, Dr. Zilfian offered the following points:

1. When the study is ready for publication, we will get a copy.
2. "Our chloroprene plant has been in existence since 1932" said Dr. Zilfian.
3. The levels to which workers in the study were exposed were not known. Industrial hygiene people at the plant would know, however.

4. Relative to case-finding, all workers receive annual X-rays at the Polyclinic. If lung scans are suspicious, patients are sent to the Institute for further testing and treatment (if necessary).
5. Retired workers are examined every six months at the Polyclinic.
6. Losses to follow-up are uncommon since Armenians tend to stay on the same jobs without moving around. "Armenians are very patriotic" added Dr. Zilfian.
7. No women were involved in the study since women were removed from chloroprene exposure in 1946.

From what we heard, Dr. Pell felt that the preceding epidemiology was sound. However, he would not make a final evaluation until he receives a final detailed report.

Other Important Items

1. Chemical Exposure Other Than CD

In response to a question from Dr. Gizlarjan, Mr. Prosser replied that when we used the acetylene process, workers were also exposed to acetylene, monovinyl acetylene, divinyl acetylene, 1,3-DCB-2, vinyl chloride, TCB, dichlorobutadiene (as a comonomer), phenothiazine, phenylalphanaphylamine, tertiary butyl catechols, etc.

Dr. Gazlarjan then asked what we knew about 1,3-DCB-2. Mr. Prosser stated that we knew very little since little testing has been done. However, in all operations that Dr. Pell studied (CD workers), 1,3-DCB-2 was present. Mr. Prosser then mentioned two older papers (by Nystrom and by a Czech author) that had toxicity information about 1,3-DCB-2. Dr. Gizlarjan had never read either of the two papers.

2. TCB and DCB

Dr. Gizlarjan asked if any of the other chemicals that CD workers encounter could be carcinogens. Mr. Prosser mentioned vinyl chloride, TCB and 1,4-DCB. Dr. Trochimowicz then briefly reviewed our ongoing chronic studies on DCB (Haskell) and TCB (CIVO). A short written summary on DCB (see Appendix) was given to Dr. Gizlarjan. The attached TCB summary (Appendix) was only summarized verbally.

Dr. Gizlarjan then said that the Institute was doing preliminary toxicity work on 1,4-DCB:

LC50 Skin Irritation

LD50 Skin Sensitization

Eye Irritation

When reports are available, he will send us the information.

3. Mutagenic Work on CD

Dr. Fidchidzhian was happy to hear that our Ames Test work on CD (Haskell, IARC) confirmed his positive findings in Salmonella.

He said what other types of mutagenicity studies would be run on CD. Dr. Trochimowicz mentioned the work started, or ready to start, at CIVO:

a. Dominant lethal studies in rats (100 ppm) and mice (10 & 100 ppm).

b. Reproduction study in rats (10, 33 & 100 ppm)

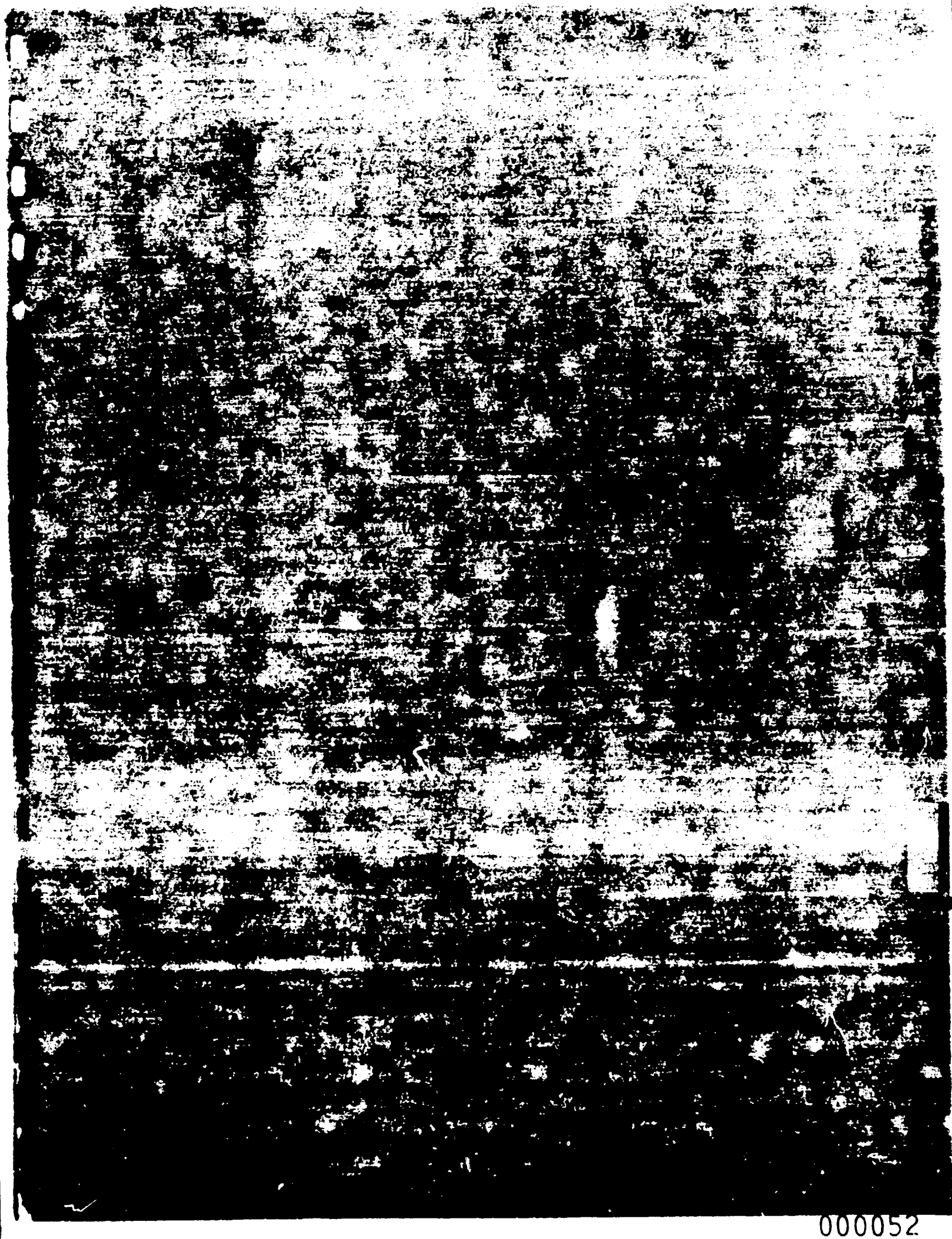
c. Micronucleus test (rats)

d. Sperm head morphology and sperm motility in rats.

4. Visit to the Yerevan Chloroprene Plant

At the previous Institute, on March 2, we had told Mr. Tsekonian (Foreign Relations Officer) that our March 6 plane reservation (originally 2:30 p.m.) had been moved back to 8:00 a.m. Therefore we could not tour the Yerevan Plant on that day, so could we arrange a visit for the afternoon of March 2 or 3, or even on March 4 or 5 (Saturday or Sunday). He said we will make arrangements on March 3. We asked again on March 3 (since our meeting ended at ~1:00 p.m.) and were told that the plant was undergoing "major construction" and that no visit was possible. Mr. Prosser then asked if we could see the plant from a distance to get an idea of the layout, etc. The answer was that there was also "major road construction" around the plant and a view from a distance would not be possible either.

As a result, we were not able to get any of our industrial hygiene questions on CD answered at this time.



MEETING ON B-CHLOROPRENE (CD)

at the
Ministry of Health
Moscow, USSR
March 7, 1978

ATTENDEES:

Ministry of Health	Du Pont Company
Dr. M. V. Borisov Assistant to the Minister for Foreign Relations	Mr. R. Prosser
Dr. Sklerov Industrial Hygiene	Dr. S. Pell
Dr. Sanotskii Head Toxicologist Institute of Industrial Hygiene and Occupational Diseases	Dr. H. J. Trochimowicz
	Mr. P. Meshkoff
	Ms. I. Bougasheva

Dr. Borisov began by apologizing for the absence of the Deputy Chief Doctor of the USSR who had planned to be there but got called away suddenly. He then introduced the other Soviet scientists and referred to Dr. Sanotskii as "our Chief Toxicologist." He was happy to have cooperation between the USSR and Du Pont in terms of CD toxicology and mentioned that the Ministry of Health was cooperating with the United States in many scientific research areas - cardiology, radiology, pollution control, industrial hygiene, and environmental health, for example. In fact, he said he expected Dr. Califano (HEW) to visit this year.

Mr. Prosser's Presentation

Mr Prosser presented to the Soviet scientists an evaluation of our visit to date. This summary is found in Exhibit C and was given to the attendees at this meeting. Briefly, it states the purpose of our visit, our conclusions,

and a list of industrial hygiene questions (since we didn't get to see the CD plant).

Dr. Borisov assured us that our questions on industrial hygiene would be answered. He also mentioned that the USSR Ministry of Health is cooperating with NIEHS (Dr. Rall) on industrial hygiene matters.

Other Items

1. DCB and TCB

We informed that Ministry of Health that we were able to furnish the Yerevan scientists with new information on the carcinogenicity of DCB and TCB in animals. Both Dr. Borisov and Dr. Sanotskii were very interested in these compounds and we briefly summarized our findings to date from Haskell Laboratory (DCB) and CIVO (TCB).

2. Cooperative Research on CD

Again, Dr. Sanotskii mentioned that he would be interested in conducting some type of cooperative research program to help resolve the differences between Soviet and Du Pont animal toxicity studies. We replied that we would certainly consider the possibility.

Dr. Sanotskii again mentioned that he would have expected chloroprene to be much more cumulatively toxic than is indicated by our chronic studies at CIVO.

3. Pell Requests to Dr. Sanotskii

Dr. Pell, following up on his earlier requests (February 27) to Dr. Fomenko, asked that Dr. Sanotskii send him the methods they use to determine spontaneous abortions in women, as well as the questionnaire used in Dr. Fomenko's study of reproduction in male workers.

In response to Dr. Sanotskii's request, Dr. Pell would send copies "as soon as available" of his morbidity study of Du Pont workers.

4. Meshkoff Request for Meeting on Lannate®

Mr. Meshkoff introduced the topic of meetings on Lannate® toxicology with the Ministry of Health. Dr. Borisov was interested in setting up such meetings. He said he was unaware of Dr. Reinhardt's problems on his last visit.

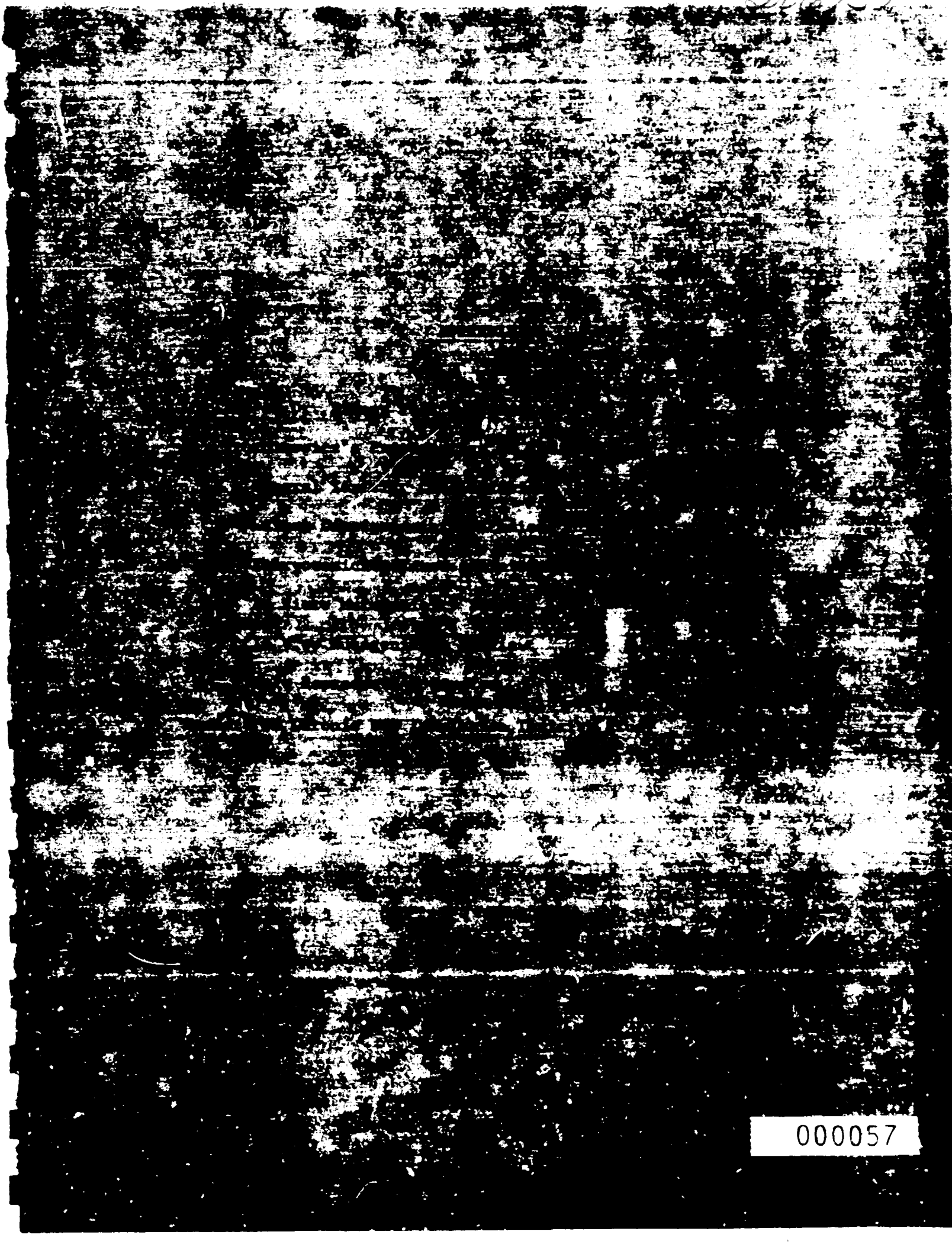
APPENDIX

Outline of Dr. Sidney Pell's Presentation in the USSR

Dr. Trochimowicz's Presentation in the USSR

1. General Toxicity Considerations
2. Animal Carcinogenicity Studies
3. Embryotoxicity/Teratogenicity Studies
4. In Vitro Mutagenicity Studies
5. In Vivo Mutagenicity Studies
6. Male Reproduction Studies

Summary on Chronic DCB Inhalation Toxicity Study



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Outline of Dr. Sidney Pell's Presentation in the USSR

DU PONT COMPANY EPIDEMIOLOGICAL STUDIES

Cohort Mortality Studies

STUDY POPULATION

- Chambers Works
- Louisville Works

METHODOLOGY

- Study Design
- Sources of Data
- Follow-up Procedures

STATISTICAL ANALYSIS

- Computation of person-years at risk, by age
- Comparison population
- Computation of expected numbers
- Allowance for latency
- Tests of significance

RESULTS

- Total mortality
- Cancer mortality
- Other causes of death

Planned Morbidity Studies

STUDY OF SICKNESS ABSENCES

- Study Design
- Sources of Data
  - Study population
  - Medical
- Statistical Analysis

BLOOD CHEMISTRY AND HEMATOLOGY STUDY AT THE LOUISVILLE WORKS

Study Design

Selection of Exposed and Control Groups

Statistical Analysis

Planned Reproduction Studies

FERTILITY RATES OF WIVES OF CHLOROPRENE WORKERS

Study Design

Selection of Subjects

Required Data and their Source

Comparison Population

Statistical Analysis

INCIDENCE OF SPONTANEOUS ABORTIONS AND FETAL DEATHS

Study Design

Sources of Data

Health insurance records
Interview questionnaire

Comparison Population

Statistical Analysis

QUESTIONS FOR USSR SCIENTISTS

SELECTION OF SUBJECTS FOR HUMAN STUDIES

Chloroprene Exposed Workers

Were all the exposed workers at a plant site selected for the study?

If not, what proportion of the total was selected?

What were the criteria for selecting exposed workers for the study and excluding others from the study?

Controls

What were the procedures for selecting controls? (At random? Matching by age, sex, or other characteristics?)

MISSING INFORMATION IN PUBLISHED PAPERS

In some papers, certain information concerning the study design, methodology, and results is not included. Can this information be obtained from the investigation? Types of missing information are as follows:

- Level and duration of exposure
- Numbers used to calculate percentages and ratios
- Age distribution and sex of the subjects

OTHER QUESTIONS

How were exposures to substances other than chloroprene taken into consideration?

Were subjects in studies followed up after employment termination, and, if so, how was this done?

In reproduction studies:

What specific questions were asked and what procedures were used to get information on reproductive functions?

What procedures were used to determine the occurrence of spontaneous abortions in the early stages of pregnancy?

Are death rates by age and sex for various causes of death available for Yerevan or Armenia?

CANCER EPIDEMIOLOGICAL STUDIES BY KHACHATRYAN

1. The data cover the period, 1956 to 1970. Were all subjects observed from the beginning of the study period in 1956, or was the start of observation for some subjects at some time after 1956?

2. Were any steps taken to follow-up subjects who left their place of employment during the study period to retire or to work in another part of the country?

3. What was the age and sex distribution of the subjects in the study?

4. What procedures were used to identify cancer cases? How were the diagnoses verified?

5. The lung cancer incidence in control group I and II were much greater than in control group III. Neither group I or II were exposed to chloroprene and group II came from non-chemical industries. What explanation is there for the higher rates in these two groups?

CHLOROPRENE STUDY COHORTS

Location	Study Population	Year First Exposed	Number of Subjects
Chambers Works, New Jersey	Neoprene Area Personnel	1931-1948	243
	Maintenance Mechanics	1931-1948	36
Louisville Works, Kentucky	Male Wage Roll Employees on Plant as of June 30, 1957*	1942-1957	1,576

*Includes maintenance mechanics

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OBSERVED AND EXPECTED NUMBERS OF DEATHS
FOR SELECTED CAUSES, 1957-1974

Cause of Death	Observed Number	Expected Number Based on Mortality in: Du Pont Company	Expected Number Based on Mortality in: United States
All causes	193	194.7	279.8
Cancer - all sites	51	44.7	52.8
buccal cavity and pharynx	1	1.2	2.0
digestive organs	19	13.3	14.9
respiratory system	18	15.5	18.3
genital organs	2	2.4	2.6
urinary organs	2	2.7	2.7
lymphatic and hematopoietic tissues	7	4.5	5.5
all other sites	2	5.1	6.8
Heart disease - all forms	71	89.9	113.5
Ischemic heart disease	66	83.1	98.7
Cerebrovascular disease	18	14.5	17.1
Accidents	16	12.0	21.9
All other causes	37	33.6	74.5

OBSERVED AND EXPECTED NUMBERS OF DEATHS
FROM CANCER OF THE TRACHEA, BRONCHUS, AND LUNG:
TOTAL COHORT AND HIGH EXPOSURE OCCUPATIONS

Occupation Category	Observed Numbers	Expected Numbers Based on Mortality in:	
		Du Pont Company	United States
Total Cohort	16	14.6	17.1
Maintenance Mechanics	4	3.5	3.8
Chemical Operators	3	2.5	3.2
Other High Exposure Occupations	2	1.3	1.6

OBSERVED AND EXPECTED NUMBERS OF LUNG CANCER DEATHS, BY LATENCY

Total Cohort

Number of Years Since First Exposure	Observed/ Expected*	Ratio: Observed/ Expected
Less than 15	2/1.6	1.25
15 to 19	3/3.0	1.00
20 or more	11/10.0	1.10

*Based on Du Pont Company mortality

OBSERVED AND EXPECTED NUMBERS
OF LUNG CANCER DEATHS, BY LATENCY

High Exposure Occupations

Number of Years Since First Exposure	Observed/ Expected*	Ratio: Observed/ Expected
Less than 15	1/0.9	1.11
15 to 19	2/1.6	1.25
20 or more	6/4.8	1.25

*Based on Du Pont Company mortality

PERCENT DISTRIBUTION OF LUNG CANCER
CASES BY OCCUPATION

	Population		Lung Cancer Cases, 1957-1974*	
	No.	Pct.	No.	Pct.
Maintenance mechanics	263	16.7	8	40.0
Chemical operators	458	29.0	3	15.0
Other high exposure occupations	124	7.9	2	10.0
Miscellaneous	731	46.4	7	35.0
Total	1,576	100.0	20	100.0

*Male wage employees, living and deceased.

OBSERVED AND EXPECTED NUMBERS OF DEATHS
FOR SELECTED CAUSES: CHAMBERS WORKS COHORT

Cause of Death	Observed Number	Expected Numbers Based on Mortality in: Du Pont Company	United States
All causes	39	37.7	48.6
Cancer - all sites	12	8.7	9.7
digestive organs	3	2.7	3.0
respiratory system	4	3.0	3.3
trachea, bronchus, and lung	3	2.7	3.0
urinary organs	5*	0.6	0.5
other sites	0	2.4	2.9
Ischemic heart disease	14	16.1	18.6
Cerebrovascular disease	2	3.2	3.7
Accidents	2	1.2	2.3
Other and unknown	9	8.5	14.3

*Significantly greater than both expected numbers ($P < 0.01$).

ıral Toxicity Considerations

USSR PRESENTATION
ANIMAL TOXICITY STUDIES ON B-CHLOROPRENE

A. General Toxicity

B. Carcinogenicity

C. Embryotoxicity/Teratogenicity

D. Mutagenicity - in vitro and in vivo

F. Male Reproduction

Each topic above was discussed in terms of:

1. Pertinent References

2. Summary of Results (Du Pont vs. USSR)

3. Possible Reasons for Differences (General)

4. Items for Discussion (Specific)

GENERAL TOXICITY CONSIDERATIONS

PHYSICAL AND CHEMICAL PROPERTIES OF THE B-CHLOROPRENE USED IN DU PONT-SPONSORED TOXICITY STUDIES

Common Name:	B-Chloroprene
Chemical Name:	2-chloro-1,3-butadiene
Structure:	$CH_2=\overset{Cl}{\overset{\vert}{C}}-CH=CH_2$
Appearance:	colorless liquid
Molecular Weight:	88.5
Boiling Point:	59.4°C
Specific Gravity (20/20°C):	0.9583
Density:	0.931
Vapor Pressure (20°C):	188 mm Hg
Flash Point (open cup):	-20°C
Solubility:	soluble in alcohol; slightly soluble in water
Conversion Factor:	1 mg/m^3 = 0.278 ppm (v/v)
Purity:	99.9+% B-Chloroprene < 0.05% Dimers 0.01% Phenothiazine inhibitor

HJT:vls
2/20/78

PRECAUTIONS TAKEN WITH B-CHLOROPRENE
USED IN DU PONT-SPONSORED TOXICITY STUDIES

Storage:	Immediately after distillation, fresh samples were put under N_2 at -20°C in individual bottles holding one day's supply.
Handling:	A fresh sample from cold storage was used for each day's exposure.
Analysis of Liquid Sample:	Before and after exposure.
Analysis of Chamber Atmosphere:	At one-half hour intervals during exposure using gas chromatography (FID).
Generation of Test Atmosphere:	Vaporization by syringe into air stream or by bubbling N_2 through liquid.

HJT:vls
2/20/78

CHLOROPRENE AIR ANALYSIS

Sample Size:	1 ml syringe sample of chamber atmosphere.
Instrument:	Gas Chromatograph
Model:	Hewlett Packard 5710
Detector:	Flame Ionization
Column:	Poropak® T 1/8" diameter 5' long stainless steel
Calibration:	Standard gas samples prepared daily.
Sensitivity:	~0.4 mg/m^3 with 1 ml sample.

HJT:vls
2/23/78

VAPOR GENERATION

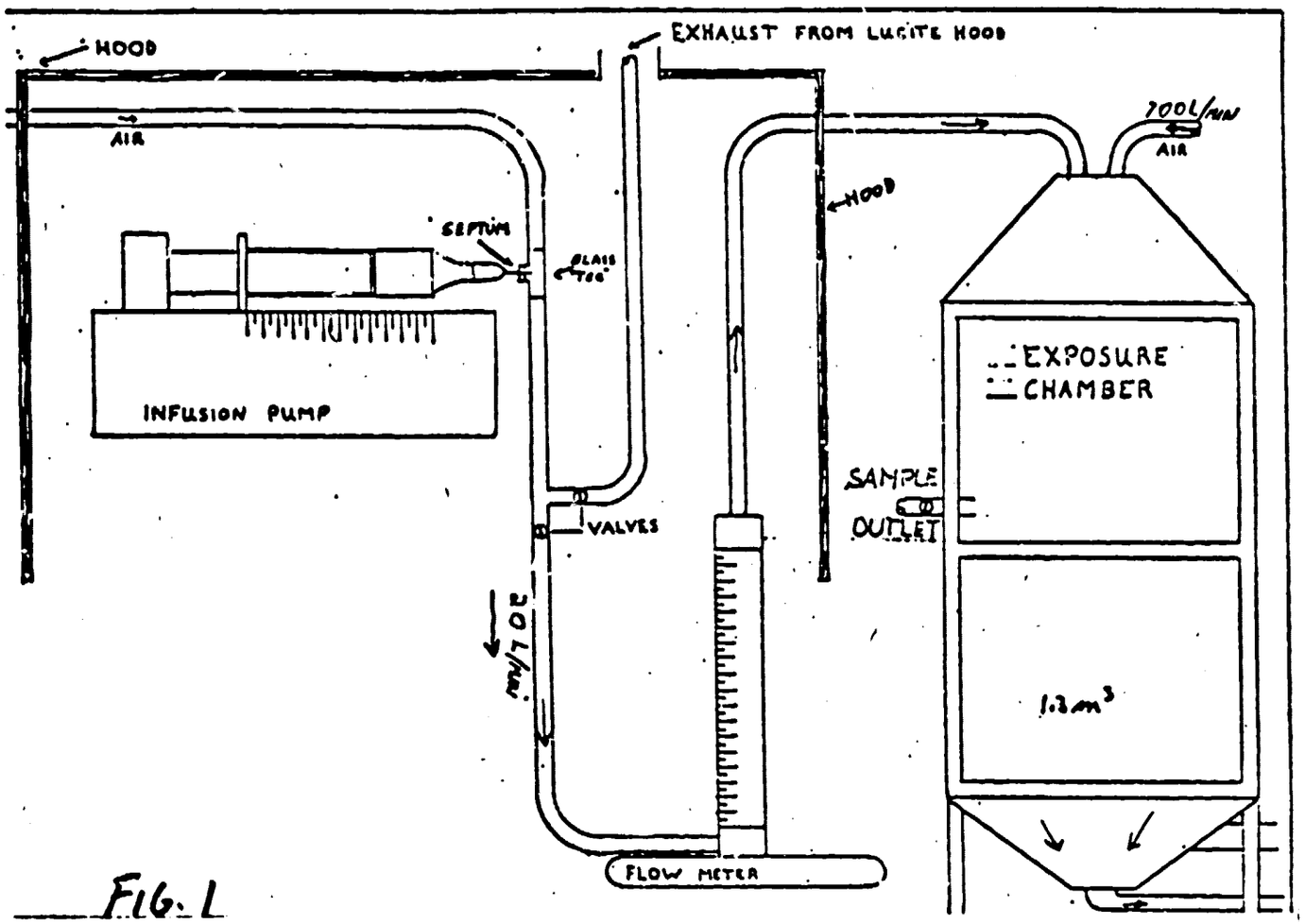

FIG. 1

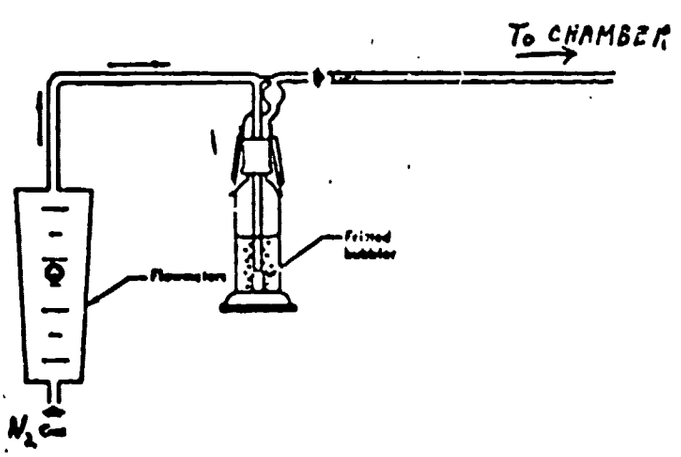

FIG. 2

Animal Carcinogenicity Studies

REFERENCES FOR ANIMAL CARCINOGENICITY STUDIES

1. Trochimcwicz, H. J., et al. Ongoing Studies at CIVO Laboratories, Zeist, Netherlands. Sponsored by the Joint Interindustry Group on Chloroprene. 1978.

2. Zilfian, V. N., et al. Laboratory of Experimental Oncology of the Institute of Radiology and Oncology, Academy of Science, Armenian Soviet Socialist Republic. Journal of Exp. and Clin. Medicine XU (No. 3), 1975.

3. Zilfian, V. N., et al. Laboratory of Experimental Oncology of the Institute of Radiology and Oncology, Academy of Science, Armenian Soviet Socialist Republic. Voprosy Oncologii 23:61, 1977.

4. Khachatryan, E. A. Oncological Dispensary of the Republic, Erevan. Trudy Leningradskogo Nauchnogo Obshchestva Patologoanatomov 15:174-177, 1974.

5. Khachatryan, E. A. Oncological Dispensary of the Republic, Erevan. Gig. Sanit. Vol. 9:119-120, 1975.

6. Garibyan, D. Kh., et al. Institute of Roentgenology and Oncology of the Armenian SSR Ministry of Public Health, Erevan. Gig. Sanit. Vol. 8:pp. 74-76, 1977.

HJT:vls
2/24/78

RESULTS OF CARCINOGENICITY STUDIES

Reference	Species	Lowest Effect Level	Type of Effect
1. Trochimowicz (unpublished)	Rat	None at 180 mg/m^3 (gross results at 24 months)	Not carcinogenic
	Hamster	None at 180 mg/m^3 (gross results at 18 months)	Not carcinogenic
2. Zilfian (1975)	Mice & rats	None at: 200 mg/kg (oral) 200 mg/kg (subcutaneous) 50% (skin)	Not carcinogenic
3. Zilfian (1977)	Mice & rats	None at: 200 mg/kg (oral) 200 mg/kg (subcutaneous) 200 mg/kg (intratracheal) undiluted (skin)	Not carcinogenic
4. Khachatryan (1974)	Mice & rats	N.R. (skin) 0.6 ml latex (subcutaneous) 0.5 ml latex (intratracheal)	Carcinogenic
5. Khachatryan (1975)	Mice & rats	0.5 ml latex (oral) 0.6 ml latex (subcutaneous) N.R. (skin)	Carcinogenic
6. Garibyan (1977)	Mice & rats	N.R.	No blastomogenic activity in sebaceous gland test.

RJT:vls
2/23/78

PROTOCOL FOR
CHRONIC INHALATION TOXICITY STUDY* ON B-CHLOROPRENE

Objective:	To assess chronic toxicity of B-chloroprene.
Exposure:	Six hours/day, 5 days a week, for 78 weeks or longer.
Exposure Level:	0, 36, and 180 mg/m^3 B-chloroprene.
Animals:	Rats and hamsters, 100/sex/level.
Evaluation:	1. Histopathology, 35 tissues from each rat. 2. Body weights. 3. Clinical chemistry.
Started:	Rats - February 1976. Hamsters - September 1976.

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* Ref. #

Trochimowicz, H. J., et al. E. I. du Pont de Nemours and Company. Study in progress at CIVO Laboratories, Zeist, Netherlands. Sponsored by Joint Interindustry Group on Chloroprene, 1978.

HJT:vls
2/24/78

SAMPLE SPECIFICATIONS FOR B-CHLOROPRENE USED IN ANIMAL CARCINOGENICITY STUDIES

Ref. #	Purity	Storage & Handling	Analytical Evaluation Before & After Use
1. Trochimowicz, et al. (1978)	99.9+%	Fresh samples from storage at -20°C under N_2.	Yes
2. Zilfian, et al. (1975)	N.R.	N.R.	N.R.
3. Zilfian, et al. (1977)	N.R.	N.R.	N.R.
4. Khachatryan (1974)	99.88%: Latex	N.R.	N.R.
5. Khachatryan (1975)	Latex	N.R.	N.R.
6. Garibyan, et al. (1977)	N.R.	N.R.	N.R.

N.R. = Not Reported.

HJT:vls
2/24/78

SPECIFIC QUESTIONS ON
USSR ANIMAL CARCINOGENICITY STUDIES

Ref. #	Item
2 & 3 (Zilfian)	Protocol Items: • Number of animals for all groups. • Number of skin applications of undiluted B-chloroprene. • Amount (ml) of B-chloroprene painted on skin at each application. • Details on survival rates.
4 & 5 (Khachatryan)	Protocol Items: • Number of animals/group. • Doses, duration, exposure regimen. • Are wooden cages routinely used for animal housing?
6 (Garibyan)	Protocol Item: • Duration of skin application.

HJT:vls
2/24/78

Embryotoxicity/Teratogenicity Studies

REFERENCES FOR
EMBRYOTOXICITY/TERATOGENICITY STUDIES ON CHLOROPRENE

1. Culik, R., et al. E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine. Accepted for Publication in Toxicology and Applied Pharmacology, 1978.

2. Salnikova, L. S. Institute of Industrial Hygiene and Occupational Diseases. Toksikologiya novykh promyshlennykh khimicheskish veshchestv No. 11:pp. 106-111, 1968.

3. Salnikova, L. S., et al. Institute of Industrial Hygiene and Occupational Diseases. Gig. Tr. Prof. Zabol No. 8: pp. 23-26, 1973.

4. Salnikova, L. S. et al. Institute of Industrial Hygiene and Occupational Diseases. Gig. Tr. Prof. Zabol. No. 7: pp. 30-33, 1975.

5. Mnatsakanyan, A. V., et al. Akopyan Institute of Epidemiology and Hygiene. Hyg. Sanit 36(7):pp. 140-141, 1971.

6. Mnatsakanyan, A. V., et al. Akopyan Institute of Epidemiology and Hygiene. Trudy Erevanskogo Gosudarstvennogo Instituta Usovershenstvovaniia Vrachei Vol. 5:pp. 155-158, 1972.

7. Sanotskii, I. V. Institute of Industrial Hygiene and Occupational Diseases (Moscow). Enviromental Health Perspectives Vol. 17:pp. 85-93, 1976 (Review Paper).

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2/20/78

RESULTS OF EMBRYOTOXICITY STUDIES

Reference #	Species	Lowest Effect Level	Type of Effect
1. Culik (1976)	Rat	None at 90 mg/m^3	None
2. Salnikova (1968)	Rat	14.8 mg/m^3	Pre- and post-implantation deaths
	Mice	14.8 mg/m^3	Pre- and post-implantation deaths
3. Salnikova (1973)	Rat	0.13 mg/m^3	Prenatal mortality
4. Salnikova (1975)	Rat	4 mg/m^3 or 0.5 mg/kg oral	Pre- and post-implantation deaths
5. Mnatsakanyan (1971)	Rat	0.5 mg/m^3	Pre- and post-implantation deaths
6. Mnatsakanyan (1972)	Rat	0.73 mg/m^3	Embryonal death

HJT:vls
2/21/78

RESULTS OF TERATOGENICITY STUDIES

Reference #	Species	Lowest Effect Level	Type Of Effect
1. Culik, et al. (1976)	Rat	None up to 90 mg/m^3	None
2. Salnikova (1968)	Rat	14.8 mg/m^3	Hematomas
3. Salnikova (1973)	Rat	3.0 mg/m^3	Vascular irregularities
4. Salnikova (1975)	Rat	4 mg/m^3 or 0.5 mg/kg oral	Hemorrhages, hydrocephaly
5. Mnatsakanyan (1971)	Rat	0.5 mg/m^3	Hematomas
6. Mnatsakanyan (1972)	Rat	0.7 mg/m^3	Hydrocephaly, altered weight, biochemical changes

HJT:vls
2/21/78

SAMPLE SPECIFICATIONS FOR B-CHLOROPRENE USED IN EMBRYOTOXICITY/TERATOGENICITY STUDIES

Ref. #	Purity	Storage & Handling	Analytical Evaluation Before & After Use
1. Culik (1978)	99.9+%	Fresh samples from storage at -20°C under N_2.	Yes
2. Salnikova (1968)	Vapor from Latex	N.R.	N.R.
3. Salnikova (1973)	N.R.	N.R.	N.R.
4. Salnikova (1975)	N.R.	N.R.	N.R.
5. Mnatsakanyan (1971)	Plant Air	-	-
6. Mnatsakanian (1972)	Plant Air	-	-
7. Sanotskii (1976)	N.R.	N.R.	N.R.

N.R. = Not Reported.

HJT:vls
2/21/78

EMBRYOTOXICITY STUDY
Ref. #1 - Culik, et al., (1978)

Experimental Design

1. Levels of 0, 3.6, 36, and 90 mg/m^3 B-chloroprene.
2. 50 Pregnant rats per level.
3. Exposed day 1 through 12 of gestation 4 hrs./day.
4. Sacrificed on day 17.
5. Resorptions, pre- and post-implant losses measured.
6. Viability of fetuses established by gross examination.

TERATOGENICITY STUDY
Ref. #1 - Culik, et al., (1978)

Experimental Design

1. Levels of 0, 3.6, 36, and 90 mg/m^3 B-chloroprene.
2. 25 Pregnant rats per level.
3. Exposed day 3 through 20 of gestation 4 hrs./day.
4. Sacrificed on day 21.
5. Resorptions, pre- and post-implant losses measured.
6. Fetuses thoroughly examined for teratogenic effects.

PERTINENT ITEMS
RELATED TO USSR EMBRYOTOXICITY STUDIES

- Criteria for classification of "dead embryos," distinction made between early and late embryo death.
- Control animals always run concurrently?
- Time period between first and last animal sacrifice in each particular experimental group.
- Holding rooms for control and treated animals.
- Background variation for the following:
  - Corpora lutea/female
  - Implantation sites/female
  - Resorption sites/female
  - Fetuses/female
  - % non-viable fetuses at term

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2/23/78

SPECIFIC ITEMS FOR DISCUSSION

Teratology Studies

- Specific handli of animals during exposure:
    - Frequency of weighing?
    - How are animals identified or physically marked?
    - Individual or group housing?
    - Ancillary studies (collection of urine or blood samples, restraining) requiring handling?

- On what basis are dose levels selected?

- Is there a distinction between growth retardation and developmental malformations?
    - What type of findings are considered 'delayed development?'
    - What type of findings are considered teratogenic?
    - Incidence of above findings in control populations?

- Are sacrifices of pregnant animals from all test levels conducted on the same da ?
    - What is the time (in hours) from examination of first to last animal in a particular group(s)?

- Are data collected by litter with uterine position noted?

- Statistical treatment of teratogenic data on a routine basis? Recommended standard methods?

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2/24/78

In Vitro Mutagenicity Studies

IN VITkO MUTAGENICITY STUDIES

	Ref. #1	Ref. #2	Ref. #3
S. typhimurium			✓
TA 100	✓	✓	
TA 1535	✓	✓	✓
Metabolic Activation	Rat liver	Rat liver	CBA Mouse Peritoneal Cavity
Chloroprene Form	Gas (48-hour incubation)	Liquid (48-hour incubation)	Liquid in oil (injected i.m.)
Results	+ (±activation)	+ (±activation)	+ (in vivo activation)
+ Dose-Response	✓	✓	✓

Conclusion: Chloroprene is mutagenic to S. typhimurium under certain experimental conditions.

1. E. I. du Pont de Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine. HLR No. 570-77, 1977 (Unpublished).

2. E. I. du Pont de Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine. HLR No. 571-77, 1977 (Unpublished).

3. Fichidzhian, B. S., et al., Zhurnal eksperimental'-noi i klinicheskoi meditsiny, Akademiia Nauk Armianskoi SSR Vol. 16 (No. 5):pp. 39-41, 1976.

HJT:vls
2/20/78

In Vivo Mutagenicity Studies

REFERENCES FOR
IN VIVO MUTAGENICITY STUDIES

1. Culik, R., et al. E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine. Accepted for Publication in Toxicology and Applied Pharmacology:1978.

2. Trochimowicz, H., et al. Joint Interindustry Group study at CIVO Laboratories, Zeist, Netherlands. 1977 (Unpublished).

3. Davtyan, R. M. Institute of Occupational Hygiene and Occupational Diseases of the AMN SSR (Moscow). Toxsikol. Gig. Prod. Neftekhim. Proisvod., Vses Konf. (Dokl.) Vol. 1971: pp. 95-97, 1972.

4. Davtyan, R. M., et al. Institute of Occupational Hygiene and Occupational Diseases of the AMN SSR (Moscow). Toxsikol. Nov. Prom. Khim. Veschestv. (13):pp. 58-62, 1973.

5. Volkova, Z. A., et al. Institute of Industrial Hygiene and Occupational Diseases (Moscow). Gig. Tr. Prof. Zabol. No. 3: pp. 31-36, 1976.

6. Sanotskii, I. V. Institute of Industrial Hygiene and Occupational Diseases. Environmental Health Perspectives Vol. 17:pp. 85-93, 1976.

7. Fomenko, V. N., et al. Institute of Industrial Hygiene and Occupational Diseases (Moscow). Vses. Nauch. Konf. Lab. Zziv v. Med. Issled. Moscow:pp 44-46, 1974.

8. Bagramyan, S. B., et al. Institute of Public Hygiene and Occupational Diseases, Ministry of Health, Armenian SSR. Biol. Zh. Arm. 27 (No. 6):pp. 102-103, 1974.

HJT:vls
2/23/78

RESULTS OF IN VIVO MUTAGENICITY STUDIES

	Species	Lowest Effect Level	Type of Effect
1. Culik, et al. (1976)	Rats	None at 90 mg/m^3	No dominant lethal effect.
2. Trochimowicz (unpublished)	Rats	None at 180 mg/m^3	No dominant lethal effect.
3. Davtyan (1972)	Rats	0.15 mg/m^3	Dominant lethal-preimplantation deaths.
4. Davtyan, et al. (1973)	Rats	3.8 mg/m^3	Chromosomal aberrations dominant lethal pre- and post-implantation deaths.
5. Volkova, et al. (1976)	Mice	1.85 mg/m^3 0.13 mg/m^3	Dominant lethal. Chromosomal aberrations.
6. Sanotskii (1976)	Rats Mice	0.14 mg/m^3 0.13 mg/m^3	Dominant lethal. Chromosomal aberrations dominant lethal.
7. Fomenko, et al. (1974)	Mice	1.85 mg/m^3	Chromosomal aberrations.
8. Bagramyan (1976)	Rats	0.54 mg/m^3	Chromosomal rearrangements.

HJT:vls
2/23/78

SAMPLE SPECIFICATIONS FOR B-CHLOROPRENE USED IN IN VIVO MUTAGENICITY STUDIES

Ref. #	Purity	Storage & Handling	Analytical Evaluation Before & After Use
1. Culik, et al. (1978)	99.9+%	Fresh samples from storage at -20°C under N_2.	Yes
2. Trochimowicz, et al. (1977)	99.9+%	Fresh samples from storage at -20°C under N_2.	Yes
3. Davtyan (1972)	N.R.	N.R.	N.R.
4. Davtyan, et al. (1973)	N.R.	N.R.	N.R.
5. Volkova, et al. (1976)	N.R.	N.R.	N.R.
6. Sanotskii (1976)	N.R.	N.R.	N.R.
7. Fomenko, et al. (1974)	N.R.	N.R.	N.R.
8. Bagramyan (1974)	N.R.	N.R.	N.R.

N.R. = Not Reported.

HJT:vls
2/24/78

SPECIFIC ITEMS FOR DISCUSSION

Mutagenicity In Vivo

- Is there a correlation between reported chromosomal changes and induced dominant lethal effect?
- Time-specificity for induced dominant lethal effects:
    - Post-meiotic?
    - Meiotic?
    - Pre-meiotic?
- Background variation of early resorptions?

Cytogenetics

- Techniques used for treated and untreated groups:
    - Collected concurrently?
    - Evaluated blind?
    - Confirmatory evaluations?
- What are the criteria for acceptance/rejection of chromosome spreads, anaphase plates, etc?
- Is there a variation in frequency/type of change in any one individual when studied serially?
- Is there a correlation between chromosome gaps and grossly abnormal chromosomes?
- Definition of criteria for chromosome classification.

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2/24/78

MALE REPRODUCTION STUDIES ON B-CHLOROPRENE

1. Culik, R., et al. E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine. Accepted for Publication in Toxicology and Applied Pharmacology:1978.

2. Davtyan, R. M. Institute of Occupational Hygiene and Occupational Diseases of the AMN SSR (Moscow). Toxsikol. Gig. Prod. Neftakhim. Proizuod., Vses. Konf. (Dokl.) Vol. 1971: pp. 95-97, 1972.

3. Davtyan, R. M., et al. Institute of Occupational Hygiene and Occupational Diseases of the AMN SSR (Moscow). Toxsikol. Nov. Prom. Khim. Veschestv. (13):pp. 58-62, 1973.

4. Volkova, Z. A., et al. Institute of Industrial Hygiene and Occupational Diseases (Moscow). Gig. Tr. Prof. Zabol. No. 3: pp. 31-36, 1976.

5. Sanotski, I. V. Institute of Industrial Hygiene and Occupational Diseases. Environmental Health Perspectives Vol. 17:pp. 85-93, 1976.

HJT:vls
2/20/78

RESULTS OF MALE REPRODUCTION STUDIES

	Species	Lowest Effect Level	Type of Effect
1. Culik, et al. (1976)	Rats	None at 90 mg/m^3	Fertility not impaired.
2. Davtyan (1972)	Rats	0.15 mg/m^3	Testicular atrophy, decreased sperm viability and motility.
3. Davtyan (1973)	Rats	None at 39 mg/m^3	No effect on fertility or sperm motility.
4. Volkova, et al. (1976)	Rats, Mice	0.15 mg/m^3	Testicular atrophy, decreased sperm viability and motility.
5. Sanotskii (1972)	Rats	0.15 mg/m^3	Testicular atrophy, decreased sperm viability and motility.
	Mice	0.32 mg/m^3	Desquamation of tubular epithelium in testes.

HJT:vls
2/23/78

RESULTS OF MALE REPRODUCTION STUDY
Ref. #1 - Culik, et al. (1978)

Dose:	0 and 90 mg/m^3
Exposure:	4 hours/day for 22 days
No. of Animals:	5 male rats/level
Breeding Schedule:	Each male bred to three females for one week. Repeat with new females for eight weeks.
Parameters Evaluated:	Mating Index Pups/Litter Viability Index Lactation Index Weanling Body Weights

HJT:vls
2/24/78

SAMPLE SPECIFICATIONS FOR B-CHLOROPRENE USED IN MALE REPRODUCTION STUDIES

Ref. #	Purity	Storage & Handling	Analytical Evaluation Before & After Use
1. Culik (1978)	99.9+% CD .01% inhibition	Fresh sample daily from storage under N_2 at -20°C.	Yes
2. Davtyan (1972)	N.R.	N.R.	N.R.
3. Davtyan (1973)	N.R.	N.R.	N.R.
4. Volkova (1976)	N.R.	N.R.	N.R.
5. Sanotskii (1976)	N.R.	N.R.	N.R.

N.R. = Not Reported.

HJT:vls
2/21/78

SPECIFIC ITEMS FOR DISCUSSION

Male Reproduction Studies

- Methods for determination of sperm motility and viability? References?
- What variation exists in the above parameters among:
    - Individuals?
    - Populations?
- Is reproductive capability measured in other ways (mating of exposed males, for example)?
- Do you usually conduct morphologic evaluation of germinal tissues?
    - Light microscope?
    - Electron microscope?
    - Special techniques?

HJT:vls
2/24/78

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898
CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT

HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

February 24, 1978

TO: Chloroprene Joint Interindustry Group

FROM: Henry J. Trochimowicz
DCB Study Director
Member - Joint Interindustry Group

INTERIM REPORT ON TWO-YEAR INHALATION STUDY
WITH 1,4-DICHLOROBUTENE-2

- Starting Date

November 1, 1976

- Exposure Conditions

Groups of Charles River Cesarean Derived rats, 140/sex/level, are exposed 6 hours/day, 5 days/week (except holidays) to design levels of 0, 0.5 (2.6 mg/m^3), and 2.5* ppm (12.8 mg/m^3) DCB vapor. (*The original design level of 5.0 ppm (25.6 mg/m^3) was reduced to 2.5 ppm during the 28th week of exposure. The 2.5 ppm exposures were continued for an additional 24 weeks then terminated and the animals put on recovery.)

Body weights are taken biweekly. Mortality and clinical signs are taken daily on the exposed and recovery animals.

- Clinical Laboratory and Pathologic Examinations

Hematology, urinalysis, and blood chemistry on 10/sex/level at 90 days and at 1 year, respectively. No compound-related effects.

- Clinical Signs and Observations

Prior to reduction of the high level concentration, animals at that level exhibited excessive weight loss (> 10% compared to control), greater than expected mortality, and an increased incidence of respiratory problems (pneumonitis, for example).

BETTER THINGS FOR BETTER LIVING . . . THROUGH CHEMISTRY

- Clinical Signs and Observations (Continued)

In the 41st week of the study, the first nasal tumor was observed (high level male); the first nasal tumor in a female high level was observed in the 43rd week.

To date (2/22/78), 82 nasal tumors# have been observed at the high level (46 male, 36 female). Some are squamous cell carcinomas. Others appear to be of neural origin but remain classified as undifferentiated carcinomas at this time.

- Mortality (Found dead or sacrificed in extremis)*

Control	Male - 18/140	Female - 8/140
Low (0.5 ppm)	Male - 9/140	Female - 7/140
High (2.5 ppm)	Male - 75/140	Female - 52/140

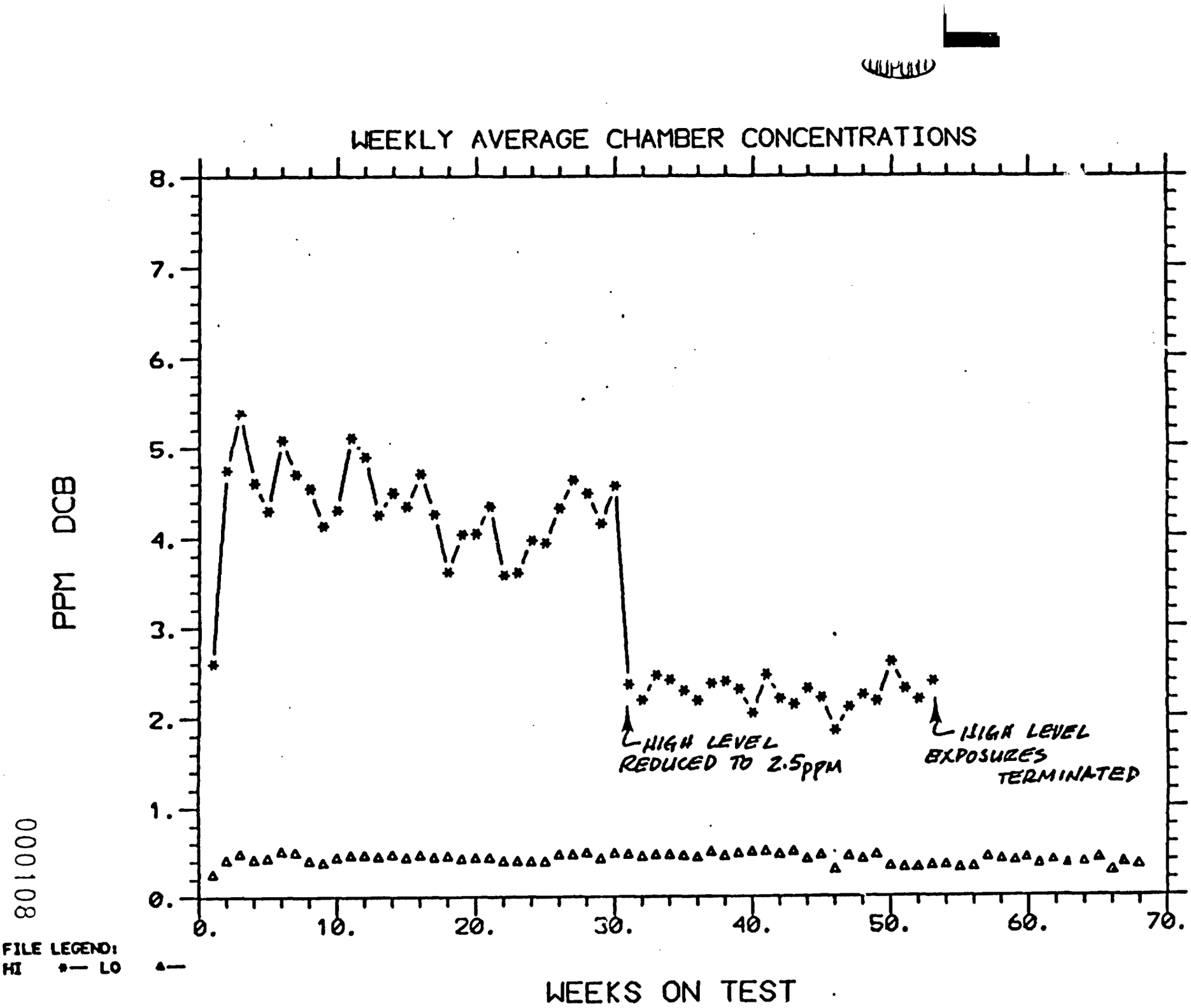
* Does not include 10 rats/sex/level that were sacrificed by design at 90-days and at one year on test.

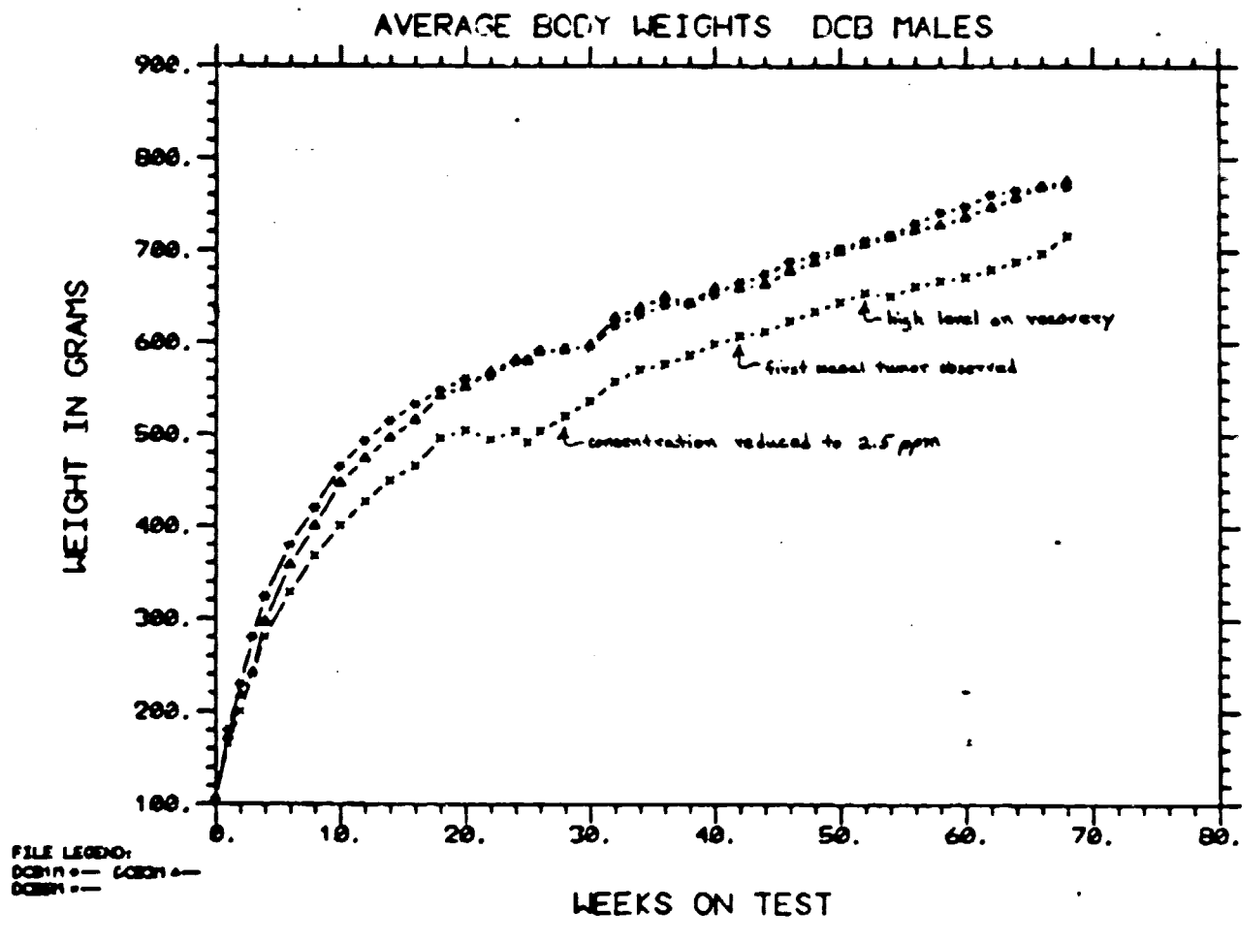
See attached photograph.

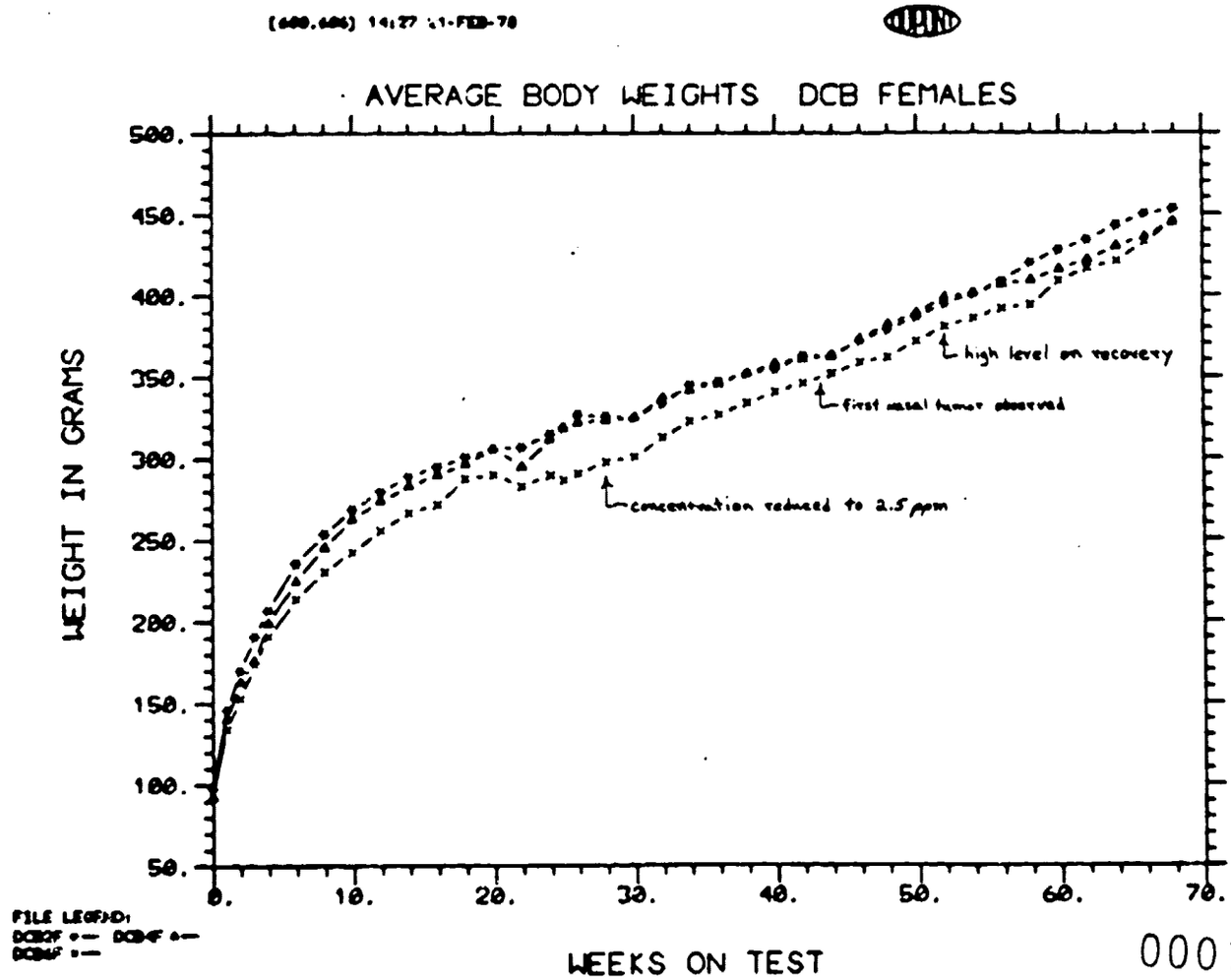
HJT:vls

Attachments

The preceding study is presently underway at the Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, U.S.A. The study is sponsored by the Joint Interindustry Group on Chloroprene and Intermediates (BP, Du Pont, Bayer, and Denki).


WEEKLY AVERAGE CHAMBER CONCENTRATIONS
PPM DCB
8.
7.
6.
5.
4.
3.
2.
1.
0.
0.
10.
20.
30.
40.
50.
60.
70.
WEEKS ON TEST
HIGH LEVEL REDUCED TO 2.5ppm
HIGH LEVEL EXPOSURES TERMINATED
801000
FILE LEGEND:
HI *— LO ▲—


AVERAGE BODY WEIGHTS DCB MALES
WEIGHT IN GRAMS
900.
800.
700.
600.
500.
400.
300.
200.
100.
0.
10.
20.
30.
40.
50.
60.
70.
80.
WEEKS ON TEST
high level on recovery
first nasal tumor observed
concentration reduced to 2.5 ppm
FILE LEGEND:


AVERAGE BODY WEIGHTS DCB FEMALES
WEIGHT IN GRAMS
500.
450.
400.
350.
300.
250.
200.
150.
100.
50.
0.
10.
20.
30.
40.
50.
60.
70.
WEEKS ON TEST
high level on recovery
first nasal tumor observed
concentration reduced to 2.5 ppm
FILE LEGEND:

*Actual photographs given to Russians.

CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

February 24, 1978

TO: Haskell Laboratory Central File

FROM: H. J. Trochimowicz

INTERIM REPORT ON TWO-YEAR INHALATION STUDY
WITH 2,3,4-TRICHLOROBUTENE-1 (TCB)

- Starting Date

December 20, 1976

- Exposure Conditions

Groups of "Wistar-Derived" albino rats, 110/sex/level, were exposed to TCB for 6 hours/day, 5 days/week (except holidays) to design levels of 0, 0.2 ppm (1.3 mg/m^3) and 2.0 ppm (13.0 mg/m^3). After approximately three months of exposure, the high level had to be reduced from 2.0 ppm to 1.5 ppm (9.8 mg/m^3). The high-level animals are still being exposed (2-24-78) but will probably be terminated by 18 months.

Body weights are taken every two weeks. Mortality and clinical signs are taken daily during exposure.

- Clinical Laboratory and Pathologic Examinations

Hematology, urinalysis, and blood chemistry on 10/sex/level at 90 days and at 1 year, respectively. No compound-related effects.

- Clinical Signs and Observations

Prior to reduction of the high-level concentration, animals at that level exhibited excessive weight loss (> 10% compared to control) and an increased incidence of respiratory problems (pneumonitis, for example).

In the 50th week of the study, the first nasal tumors were observed (3 cases). By week 55, two more cases appeared. At present (2-22-78), two of the above five rats have been autopsied, examined microscopically, and found to have highly malignant nasal tumors which appear to be of squamous cell origin.

No malignant tumors have yet been observed at the low level.

878215018

- Clinical Signs and Observations (Continued)

Body weight retardation was observed in rats at the high TCB level. Mean body weights at end of 52 weeks are shown below:

	Males	Females
Controls	460	254
0.2 ppm Group	452	262
2/1.5 ppm Group	411**	243*

* $p < 0.01$ ** $p < 0.001$; Student's T-Test

- Mortality (Found Dead or Sacrificed in Extremis) After 55 Weeks

Control	Male - 1/110	Female - 1/110
Low (0.2 ppm)	Male - 2/110	Female - 1/110
High (1.5 ppm)	Male - 5/110	Female - 4/110

*** Does not include 10 rats/sex/level that were sacrificed by design one year on test.

This preceding study is currently underway at CIVO laboratories, Zeist, Netherlands, and is sponsored by the Joint Industry Group on Chloroprene and Intermediates (Du Pont, Bayer, BP, and Denki).

HJT/aph

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) • OTS Ø2Ø6752	1	No. of Pages	2
Doc I.D. 878215Ø19	3	Old Doc I.D. 8DS	4
Case No.(s) OTS 84ØØ3 A II			5
Date Produced (6) ØØ6473	6	Date Rec'd (6) Ø22285	7
Conf. Code • N	8		

Check One: ☐ Publication ☐ Internally Generated ☑ Externally Generated

Pub/Journal Name	9
	9
Author(s)	10
Organ. Name E I DUPONT DENEMOURS & CO INC	11
Dept/Div	12
P.O. Box	13
Street No./Name	14
City WILMINGTON	15
State DE	16
Zip 19898	17
Country	18
MID No. (7)	19
D & B NO. (11)	20
Contractor	21
Doc Type • R.I • U.P • H.E.A.S.D 8D. S.U H.S F.N	22
Doc Title SOLUBILITY AND REMOVAL OF ORGANIC MATERIALS FROM WASTE BRINE	23

Chemical Name (300 per name)	25	CAS No. (10)	24
2-CHLORO-1-3-BUTADIENE		126-99-8	

1A

3/11/85 P.S.

WATER SOLUBILITY OF CHLOROPRENE

(31)

8732150?9

E. I. DU PONT DE NEMOURS AND COMPANY
ELASTOMER CHEMICALS DEPARTMENT
EXPERIMENTAL STATION
JUNE 4, 1973

BIWEEKLY SUMMARY NO. 73-5

P. R. Johnson

Solubility and Removal of Organic Materials from Waste Brine
(C. A. Stewart, Jr.)

In order to evaluate various methods of removal of chloroprene and other organic materials from waste dehydrochlorination brine and to consider possible environmental effects of dichlorobutene handling, it is necessary to have some understanding of the behavior of these materials as dilute solutions in water, with and without various concentrations of salt, etc. The first property of interest is the solubility of these species -- the concentration in aqueous solution saturated with respect to the pure liquid of interest. The technique we are using to estimate solubility is to equilibrate an aqueous solution with various mixtures of a pair of compounds, at least one of which is significantly more dense than the water phase, coalesce the heavy organic phase by gentle agitation, settle, or centrifuge if necessary, and sample and analyze the aqueous phase by extraction with a third component (ortho-dichlorobenzene) and chromatographic analysis of the extract. The apparent solubility is then determined by dividing the aqueous concentration by organic phase concentration and the true solubility estimated by extrapolating to pure component on a plot of apparent solubility vs. concentration in the organic phase. This circuitous procedure avoids the experimental difficulties in obtaining legitimate samples when the organic phase is lighter than the aqueous phase, allows measurements above the boiling point of low boiling liquids, and permits

discarding of spurious values which do not fall on the plot if incomplete phase separation is obtained. Even with these precautions, the results obtained are not highly precise, but the accuracy should be adequate for the desired purpose. Some typical values are given below:

Solubility, ppm by Volume

	Chloroprene	Toluene	CCl_4	"Perclene"
Water, 25°	1270	490	400	155
" , 60°	1310	720	420	175
Brine*, 25°	230	54	48	24
60°	330	140	105	44

* 18% NaCl, 1.5% NaOH

Solubility at 25°C, ppm by Volume

	3,4-DCB	1,4-DCB
Water	620	665
4.8% NaCl	410	420
9.1% "	330	370
13.0% "	200	190

As a first approximation, extraction efficiency can be predicted on the basis that the equilibrium concentration in water will be equal to the saturation value times the mole fraction of solute in the extracting phase. This is quite adequate in the experiments described above; apparent solubility does not vary appreciably from the estimated true solubility for solutions containing 30-100% of solute. It is untrustworthy for very low concentrations and applies, of course, only when the solute and extractant are miscible. If a thousand volumes of hot brine containing 100 ppm of chloroprene is extracted with two volumes of toluene, the resulting aqueous phase should contain only 14 ppm of dissolved chloroprene, but now contains 130 ppm of dissolved

toluene and an undefined amount of the 4% chloroprene in toluene extract suspended in the brine if decantation is not complete. Recent experiments (J. F. Englert, A. T. Harris, BWS 5/2/73) suggest that complete equilibration and complete separation of the extract are not easily obtained.

The vapor pressure of a poorly soluble species may similarly be approximated as pure component vapor pressure times. fractional saturation. For brine at 90°C using estimated values of 400 ppm solubility of chloroprene, 1860 mm vapor pressure for CD, 400 mm for water, calculation shows that at 100 ppm of chloroprene, the vapor in equilibrium should be 34 mole % (96 wt%) chloroprene, and at 1 ppm, the equilibrium vapor should be 1.1 mole % (6 wt %). About 0.2% of the water should need to be vaporized to remove 99% of the chloroprene. Experimentally, using batch rather than continuous distillation, chloroprene was reduced from 1000 to about 0.3 ppm by distillation of 1.7% of the water. This is significantly poorer than the predicted efficiency, but should be satisfactory for practical removal.

CAS/rke

E. I. DU PONT DE NEMOURS & CO. (INC.)

124 Page No. TITLE Soly CD, "Perclene"/Water, Brine 25, 60°C DATE 5/17/73

E 2444 Book No. PURPOSE See p 123

As p. 123 but C_2Cl_4 instead of CCl_4, chloroprene instead of toluene

Arm/10³ A DCB	CD	C_2Cl_4			
5) A (20/10)	18.81	11.20	CD 0.940, 0.894/ml, Fv: 1.09		
B (10/20)	8.94	21.9	C_2Cl_4 1.12, 1.10, 1.17 Fv: 0.88		
C (-/30)	-	35.1			
1) A	38.5	3.66	S_{CD} = 1260	S_{PC} 193	Ave W, 25° 1270 CD, 155 PC
B	19.6	5.8	1280	145	
C	—	7.37	-	128	
2) A	(11.6)	(4.34)	(390)	(229)	
B	3.51	0.86	230	23	B, 25° 230, 24
C	-	1.35	-	24	
3 A	38.7	4.08	1270	215	
B	20.6	6.42	1350	170	W, 60° 1310, 175
C	-	8.03	-	140	
4 A	(12.5)	3.54	(410)	(187)	
B	5.05	2.03	330	54	B, 60° 330, 44
C	-	1.92	-	34	

As above (5/22/73)

A) 2 ml 3,4 DCB (Pembelly), 5 ml 1,4 DCB (UTCM)

B) 5 " " 2 " "

10) 10 µl A / 1 ml DDCB } Sensitivity 3,4 DCB: 0.274, 0.276 [ht ± atten / ht DDCB (6 x)]

10 µl B / " " } 1,4 " " 0.129, 0.117

6) 100 H_2O + 2 ml A S_{2x} = 640 ppm (ovl) S_{4x} = 682

B 600 (620 ave) 644 (665 ave)

7) 100 H_2O, 5 salt, 2 ml A	410	420	4.8
8) " 10 " "	330	370	9.1
9) " 15 " "	200	190	13.0

% NaCl

Corr fit 3,4 DCB ≈ 625 - 33 x % salt

1,4 ≈ 645 - 35 x "

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EXPERIMENTER

E. I. DU PONT DE NEMOURS & CO. (INC.)

TITLE Soly CCl_4, Tol / water, Brine, Temp. DATE 5/15/73 Page No. 123

PURPOSE Book No. E 2444

Make up A: 10 ml Tol / 5 ml CCl_4, B - 5 Tol / 10 CCl_4 C = CCl_4

⑤ VPC 30 µL each / 1 ml ODCB

① 2 ml A, B, or C + 100 H_2O @ RT, Shin to equilibrate, 50 ml AQ sample extract with 1 ml ODCB + VPC

② Same but 18/1.5 Brine (see p 122) instead of water [2A ORG lighter than water]
③ As ① but 60°C Equil
④ " ② " " " [4A " " " "]

Area / 1000 ODCB as per p 122

⑤ A CCl_4 12.13/10 Tol 19.58/20 CCl_4 Equil = 0.82, 0.77, 0.67 Ave 0.75
B 26.23/20 10.58/10 Tol 1.02, 0.94 Ave 0.98
C 45.13/30 –

1) A 10.02 CCl_4 20.41 Tol CCl_4 10.02×3, 16.92×25, 21.5: 30, 25, 22 µL in cone $\frac{.026}{.050}$ × .75 = 400 ppm
B 16.92 6.15 " Tol 31, 18, – 25/.05 × .98 = 490 "
C) 21.5 –

2) 2.84? 4.73?, CCl_4 (8) 2.9, 3.5 3.2 → 48 ppm
1.93 0.90 Tol (7) 2.7, – 54 "
3.49 –

3) 10.1 24.0 30, 28, 26 28 → 420 ppm
18.9 11.8 36, 35, – 36 " 730 ppm
25.5 –

4) 2.39 4.52 7.2, 7.6, 6.0 7.0 → 1050 ppm
5.10 2.33 6.8, 7.0, – 7.0 → 140 ppm
1.04 –

Temp effects 60/25 CCl_4/H_2O = 1.05 Tol/w = 1.5
CCl_4 Brine = 2.2 Tol/Brine = 2.6
Brine effects CCl_4/25 0.12 Tol/25 = 0.11
AQ 0.24 " 0.19

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EXPERIMENTER [signature] DATE

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E. I. DU PONT DE NEMOURS & CO. (INC.)

878215019

122 Page No. TITLE Brine, Toluene Solns. DATE 5/14/23

E 2462 Book No. PURPOSE VPC Calibration

Make up 90g NaCl, 15g 50% NaOH, 395 (H_2O) = 18% NaCl, 1.5% NaOH

A) 1, 2.5, 6 µL Toluene/10 µL hypo to 1ml ODCB (Pipette)
15, 30, 60 " " /100 " " " " " "

B) 25ml water + 0, 2.5, 20, 50 µL Tol., shake well ≈ 1ml ODCB

C) 25ml 18/1.5 Brine + 0, 2.5, 20, 50 " " " " " "

D) 1ml CCl_4 + 1ml Tol, 20 µL of this to 1ml ODCB

VPC 130° 8'×10% SE52 5µL for 1-10 µL/ml, 1µL for 20-60

Area (HPW = 0.16cm / Tol @ 1.8min, 0.125/CCl_4 @ 1.35, 0.425 ODCB (1µL) 5.5min
5µL ODCB variable, 79-83 cm ~ 6min

Area Tol/1000,000 ODCB / ppm by volume

A) 717/1000, 1510/2500 4290/6000 14930/15000, 25,700/30000, 62,300/60000
1.40 1.65 1.40 1.00 1.17 0.96

B) 814/2000, 2400/5000 18,300/20000 42,700/50000
Fvol = 2.46 2.08 1.10 1.17

C) 810/2000 1776/5000 19,250/20000 48,170/50000
Fvol = 2.47 2.82 1.04 1.04

D) 12,480/10,000 CCl_4 10,060/10000 Tol
Fvol = 0.802 0.994

Ave. Fvol ≈ 1.0 ± 0.1, lower values = evap, incomplete mixing on?
water, brine soln is not enough to bother within exptl error

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