Environmental Health Perspectives
Vol. 31, pp. 171-182, 1979

Carcinogenicity of Chloroform

by Melvin Dwaine Reuber*

Chloroform is carcinogenic in rats, mice, and probably in dogs. Chloroform induced carcinomas of the liver and kidney and malignant tumors in other organs in rats and mice. Liver neoplasms have been described in three strains of mice. Carcinomas of the kidney were found in a first study in mice and in the repeat of that study. Dogs given chloroform developed neoplasms of the liver as well as in other organs.

Rats given chloroform also developed toxic changes, particularly male rats, as a result of treatment. These lesions included interstitial fibrosis of the kidney; polyarteritis of the mesenteric, pancreatic, and other arterioles and arteries; and atrophy of the testes. These toxic changes may have interfered with the development of neoplasms in male rats.

Introduction

Chloroform ($CHCl_3$, trichloromethane) is used chiefly in the manufacture of fluorocarbons for refrigerants, propellants, and plastics. However, there are many other uses (*1*): chloroform is used as a solvent in photographic processing and in dry cleaning, as well as in the preparation of antibiotics, dyes, drugs, and pesticides. It is added to some toothpastes, cough medicines, linaments, and salves.

This review includes, to the best of our knowledge, every study on the carcinogenicity of chloroform in animals. The results and conclusions for the NCI chloroform-rat study and NCI chloroform-mouse study are based on my examinations of the histological sections. The results of the other studies are my own analyses of the data which are, in most cases, raw data. Other studies reviewed were: Huntington Research Center chloroform studies, Eschenbrenner study, Rudali study, and Roe study.

Statistical tests of significance were *p* (probability) values obtained with Fisher's exact test and tests for positive linear trend and departure from linear trend, which are described in detail (*2*).

NCI Chloroform-Rat Study

Chloroform was administered orally to male and female Osborne-Mendel rats, 50 of each sex, at two dose levels (*1*). Chloroform was given in corn oil by gavage five times per week for 78 weeks. Rats were started at 52 days of age and killed after 111 weeks.

The high dose concentrations of chloroform were intended to be the maximal amount that the animals could tolerate for 78 weeks; and the low doses were one-half of the high doses. These doses were initially chosen on the basis of survival, weight gain, and clinical observations during a trial 6-week study. As the full-scale study progressed, it was decided to reduce the dose given to the female rats.

The dose levels for male rats were 90 or 180 mg/kg of body weight. Female rats were given 125 or 250 mg/kg for 22 weeks, at which time the doses were changed to 90 or 180 mg/kg (with time weighted average of 100 or 200 mg/kg for the study) (Table 1). Groups of 20 rats of each sex received the corn oil vehicle and other groups of 20 rats received no treatment.

Necropsy was done on all rats. Complete histopathologic examinations were performed on killed rats and whenever possible on those found dead.

Average survival times for male rats given the high dose chloroform was 80 weeks, and 83.6 weeks for the females (Table 2). Control males survived for 80.5 weeks and control females for 95.6 weeks.

The results are based on my examination of the histological sections. Results are presented for the following: tumors of the liver, tumors of the kidney, tumors of the thyroid, malignant tumors in other organs, malignant tumors in all organs, and lesions other than tumors, and summary.

*Chemical Carcinogenesis Program, NCI Frederick Cancer Research Center, Frederick, Maryland 21701.

Table 1. Average dose levels of chloroform for males and females (NCI rat study).

	Males	Females
Low dose, mg/kg	90	100
High dose, mg/kg	180	200

Tumors of the Liver

Cholangiofibromas and cholangiocarcinomas were present in the liver of 11 of 39 females (28%) ($p = 0.00599$) given the high dose of chloroform and in 3 of 39 females (8%) given the low dose, whereas control rats did not develop such tumors (Table 3). There were fewer lesions in male rats. Cholangiocarcinomas, which were well-differentiated histologically, invaded the adjacent hepatic tissue. The first cholangiofibroma was found at 28 weeks.

Hyperplastic nodules and hepatocellular carcinomas developed in 36% (14 of 39) females given the high dose ($p = 0.03105$) and 23% (9 of 39) females receiving the low dose of chloroform, compared to 8% (3 of 40) controls (Table 4) (*3*). Fewer such lesions were seen in male rats (Table 5). If time of survival is taken into consideration (i.e., time of appearance of first nodule or carcinoma, 32 weeks), then 14 of 32 high dose females (44%) and 9 of 29 low dose females (31%) had hyperplastic nodules or hepatocellular carcinomas. Hepatocellular carcinomas were well-differentiated histologically.

If numbers of rats with tumors of the liver is taken into consideration, then 51% (20 of 39) females receiving the high dose ($p = 0.00161$) and 26% (10 of 39) females given the low dose of chloroform had such tumors compared to 8% of the controls (Table 6). Twelve of 49 (24%) high dose male rats, compared to 5% controls, had tumors of the liver (0.04877 test for positive trend).

Some male and female rats given the low or high doses of chloroform had focal areas of hyperplastic parenchymal cells of the liver. Necrosis of the liver was often present in rats dying early; however, cirrhosis was not observed in rats given chloroform.

Table 2. Survival times for males and females given chloroform (NCI Rat Study)[a]

Treatment	Survival time, weeks: Females, average (range)	Survival time, weeks: Males, average (range)
0	95.6 (61-110)[b]	80.5 (54-110)[c]
Vehicle	101.2 (29-111)	101.0 (67-110)
Low dose	91.1 (40-111)	97.5 (45-111)
High dose	83.6 (28-111)	80.0 (30-111)

[a]Based on time of development of first tumor of the liver at 28 weeks.
[b]Five rats were killed at 61 weeks.
[c]Four rats were killed at 61 weeks.

In summary, chloroform was carcinogenic for liver of rats. Female rats were much more susceptible than were male rats to the induction of tumors of both parenchymal cells and bile duct cells. Rats given chloroform did not develop cirrhosis of the liver.

Tumors of the Kidney

Carcinomas of the kidney were present in 12 of 49 male rats (25%) given the high dose ($p = 0.004861$) and 12% (6 of 50) receiving the low dose of chloroform compared to 0% in the control rats (Table 7) (*4*). A few rats had both carcinomas of the cortex of the kidney and the renal pelvis. Administration of chloroform did not cause a significant increase in tumors of the kidney in female rats. The only hepatic lesion associated with carcinoma of the kidney was a cholangiocarcinoma in one rat.

Carcinomas of the kidney were well-differentiated, poorly differentiated, or undifferentiated. Carcinomas invaded and replaced the adjacent renal parenchyma and metastasized to other organs in two male rats and one female rat. The carcinomas in females were undifferentiated.

Carcinomas of the kidney generally were associated with chronic interstitial nephritis. Approximately 75% of the carcinomas were associated

Table 3. Cholangiofibromas and cholangiocarcinomas of the liver in females given chloroform (NCI rat study)[a]

Treatment	Cholangiofibroma	Cholangiocarcinoma	Total
0	0/20 (0%)	0/20 (0%)[b]	0/20 (0%)
Vehicle	0/20 (0%)	0/20 (0%)	0/20 (0%)
Low dose	1/39 (3%)	2/39 (5%)	3/39 (8%)
High dose	3/39 (8%)	8/39 (20%), $p = 0.02774$ $p = 0.0042$[c]	11/39 (28%), $p = 0.00599$ $p = 0.00067$

[a]Statistics using vehicle control unless otherwise stated.
[b]Colony control was given as 0/98 (0%).
[c]Test for positive trend unless otherwise specified.

Table 4. Hyperplastic nodules and hepatocellular carcinomas in females given chloroform (NCI Rat Study).[a]

Treatment	Hyperplastic nodules	Hepatocellular carcinomas	Total
0	1/20 (5%)	0/20 (0%)[b]	1/20 (5%)
Vehicle	2/20 (10%)	0/20 (0%)	2/20 (10%)
Low dose	7/39 (18%)	2/39 (5%)	9/39 (23%)
High dose	12/39 (31%)	2/39 (5%)	14/39 (36%), $p = 0.03105$
	$p = 0.03617$		$p = 0.01886$

[a]Based on time of development of first tumor of the liver at 28 weeks.
[b]Colony control was given as 0/98 (0%).

with chronic renal disease, with 25% each being mild, moderate, or severe in degree.

In summary, chloroform was carcinogenic for kidney in male rats and not for female rats. By contrast, female rats were more susceptible to the development of hepatic tumors than male rats. Apparently, chloroform was not as readily metabolized in the liver of male rats and more chemical(s) reached the kidney.

Tumors of the Thyroid

There were increased incidences of tumors of the thyroid gland in females given the low or high doses of chloroform (Table 8). Tumors of the thyroid were present in 11 of 39 (28%) low dose females ($p = 0.03687$) and 12 of 39 (31%) high dose female rats ($p = 0.02089$). Male rats receiving the corn oil vehicle had more tumors of the thyroid than either the control rats not given corn oil or the chloroform-treated male rats (Table 9). Tumors in chloroform-treated rats usually were larger than in the control rats and sometimes invaded the adjacent tissue.

Tumors of the thyroid, liver, and/or kidney generally were not found, with a few exceptions, in the same animal. One carcinoma of the thyroid was present in a high dose female rat with cholangiocarcinoma, and one adenoma of the thyroid with hepatocellular carcinoma in a high dose male rat. However, rats with tumors of the thyroid generally did not have tumors of the liver, or if they did, the tumors were hyperplastic nodules (as in the case of four low dose male rats). Three high dose male rats with thyroid tumors also had renal cell carcinomas.

In summary, chloroform administration resulted in a significant increase in tumors of the thyroid in female rats. Rats with thyroid tumors generally did not have tumors of the liver and kidney; however, they rarely had hyperplastic nodules of the liver. The latter finding suggests that thyroid tumors may have interfered with the development of hepatic carcinomas.

Table 5. Hyperplastic nodules and hepatocellular carcinomas in males given chloroform (NCI Rat Study).

Treatment	Hyperplastic nodules	Hepatocellular carcinomas	Total
0	0/20 (0%)	0/20 (0%)[a]	0/20 (0%)
Vehicle	1/19 (5%)	0/19 (0%)	1/19 (5%)
Low dose	5/50 (10%)	0/50 (0%)	5/50 (10%)
High dose	8/49 (16%)	2/49 (4%)	10/49 (20%)
			$p = 0.04502$

[a]Colony control was given as 1/99 (1%).

Malignant Tumors in Organs Other Than Liver, Kidney, and Thyroid

The incidences of malignant tumors in organs other than liver, kidney, and thyroid in chloroform-treated rats were not increased, with one exception (Tables 10 and 11). Of 50 low dose male rats, 24 (48%) had malignant tumors compared with 16% of the controls ($p = 0.00639$). There were four splenic, three subcutaneous, and one ostogenic sarcomas. The tumors were mostly sarcomas in rats that did not have other tumors, in rats without chronic renal disease, and in rats that survived over 100 weeks.

Table 6. Tumors of the liver in males and females given chloroform (NCI Rat Study).[a]

Treatment	Males	Females
0	0/20 (0%)	1/20 (5%)
Vehicle	2/19 (11%)	2/20 (10%)
Low dose	5/50 (10%)	10/39 (26%)[b]
High dose	12/49 (24%)[c]	20/39 (51%)[d], $p = 0.00161$
	$p = 0.04877$	$p = 0.0004352$

[a]Combining all cholangiofibromas, hyperplastic nodules, and carcinomas of the liver.
[b]Two rats had both parenchymal cell and bile duct cell tumors; $p = 0.02937$ when compared with pooled controls.
[c]One rat had both a parenchymal cell and bile duct cell tumors; $p = 0.01233$ when compared with pooled controls.
[d]Five rats had both parenchymal cell and bile duct cell tumors.

Table 7. Tumors of the kidney in males given chloroform (NCI Rat Study).[a]

Treatment	Renal cortex	Renal pelvis	Total
0	0/20 (0%)	0/20 (0%)	0/20 (0%)[a]
Vehicle	0/19 (0%)	0/19 (0%)	0/19 (0%)
Low dose	6/50 (12%)	2/50 (4%)	6/50 (12%)[b]
High dose	12/49 (24%), $p = 0.01267$ $p = 0.004861$	2/49 (4%)	12/49 (25%)[b], $p = 0.004861$ $p = 0.004861$

[a]Colony control was given as 0/99 (0%).
[b]Two male rats at each dose had both tumors.

Malignant Tumors in All Organs

There were slightly increased incidences of malignant tumors in male and female rats given chloroform in both the low and high dose groups (Tables 12 and 13). Malignant tumors were found in 25 of 50 (50%) low dose male rats given chloroform ($p = 0.03070$).

The incidences of high dose male and female and low dose male and female rats are even more significant when corrections are made for survival time, i.e., taking into consideration the survival time of the first rat to develop a malignant tumor (42 weeks) (Tables 14 and 15). Malignant tumors were found in 72% of low dose female rats ($p = 0.00969$), 66% of high dose female rats ($p = 0.02816$); 58% of low dose male rats ($p = 0.00383$), and 50% of high dose male rats ($p = 0.03726$). Control rats of both sexes had approximately 30%.

Carcinomas in high dose chloroform-treated female rats were in the liver, thyroid, and mammary gland, and in the mammary gland and endocrine organs in low dose female rats. Carcinomas in male rats were in the kidney and endocrine organs. Sarcomas were observed in the spleen, lung, bone, and subcutaneously. Carcinomas in control females were in the mammary gland and endocrine organs. Carcinomas in control male rats were in endocrine organs.

Hyperplasia was occasionally seen in the pancreas, both of the acinar and islet cells, and a few animals had nodules.

Lesions Other Than Tumors

Lesions other than tumors were observed more often in chloroform-treated rats, particularly in males, than in the controls. These lesions were interstitial fibrosis of the kidney; polyarteritis of the mesenteric, pancreatic, and other arteries and arterioles; and atrophy of the testes.

Polyarteritis developed in approximately one-fourth of the male rats given chloroform. Polyarteritis was present in 22% low dose male rats ($p = 0.0064$), and 25% high dose male rats ($p = 0.00322$). The lesion involved predominantly the mesenteric and pancreatic arteries, as well as small arteries and arterioles throughout the body in treated rats, whereas in control rats polyarteritis was limited to the mesenteric artery (Table 16). Thrombosis was also present in the mesenteric artery of treated rats. The mesenteric artery was examined only when the arteritis was severe; and the incidence, particularly of less advanced arteritis, would probably have been higher if the mesenteric artery were routinely sectioned histologically. The lesion was generally associated with severe atrophy of the testes, severe interstitial fibrosis of the kidney, and carcinoma of the kidney.

Table 8. Tumors of the thyroid gland in females given chloroform (NCI Rat Study).[a]

Treatment	Adenomas	Carcinomas	Total
0	1/20 (5%) 1/40 (2.5%)[c]	2/20 (10%) 3/40 (7.5%)[c]	3/20 (15%)[b] 4/40 (10%)[c]
Vehicle	0/20 (0%)	1/20 (5%)	1/20 (5%)
Low dose	8/39 (21%), $p = 0.01299$	3/39 (7%)	11/39 (28%), $p = 0.03687$
High dose	7/39 (18%), $p = 0.025494$ $p = 0.03186$	5/39 (13%)	12/39 (31%), $p = 0.02089$ $p = 0.01914$

[a]Approximately one-half of the tumors were F-cell and one-half C-cell adenomas and carcinomas.
[b]Colony control was given as 1/98 (1%).
[c]Statistics based on combined controls.

Interstitial fibrosis of the kidney was seen in male rats more often than female rats, and the lesion was more severe in male rats. It was usually associated with carcinomas of the kidney.

Atrophy of the testes was present in the chloroform-treated male rats and was extremely rare in the control male rats (about 2%). The lesion was observed in one-third of the chloroform-treated male rats.

Summary

Chloroform is carcinogenic in rats. Chloroform not only induced malignant tumors of the liver in female rats and of the kidney in male rats, but in other organs as well. There also are toxic changes, particularly in male rats, as a result of the treatment. These toxic changes may have interfered with the development of tumors in male rats.

Table 9. Tumors of the thyroid gland in males given chloroform (NCI Rat Study).[a]

Treatment	Adenomas	Carcinomas	Total
0	0/20 (0%)	1/25 (4%)	1/20 (5%)[b]
Vehicle	2/19 (11%)	2/19 (11%)	4/19 (21%)
Low dose	3/50 (6%)	0/50 (0%), $p = 0.07289$[c]	3/50 (6%), $p = 0.08491$[c]
High dose	4/49 (8%)	3/49 (6%)	7/49 (14%)

[a]Tumors generally were F-cell adenomas or carcinomas.
[b]Colony control was given as 8/99 (8%).
[c]Two-tail.

Table 10 Carcinomas and sarcomas in other organs in males given chloroform[a]

Treatment	Carcinomas	Sarcomas	Total
0	2/20 (11%)	3/30 (15%)	5/20 (25%)
	3/39 (8%)[b]	5/39 (13%)	8/39 (21%)
Vehicle	1/19 (5%)	2/19 (11%)	3/19 (16%)
Low dose	11/50 (22%), $p = 0.05844$	13/50 (26%)	24/50 (48%), $p = 0.00639$
High dose	5/49 (10%)	8/49 (16%)	13/49 (27%)
			$p = 0.0033$

[a]Colony control incidences are not available.
[b]Statistics based on combined controls.

Table 11. Carcinomas and sarcomas in other organs in females given chloroform.[a]

Treatment	Carcinomas	Sarcomas	Total
0	4/20 (20%)	0/20 (0%)	4/20 (20%)
Vehicle	4/20 (20%)	1/20 (5%)	5/20 (25%)
Low dose	10/39 (26%)	3/39 (8%)	13/39 (33%), $p = 0.07071$
High dose	5/39 (13%)	5/39 (13%)	12/39 (31%), $p = 0.07071$
		$p = 0.07113$	

[a]Colony control incidences are not available.

Table 12. Carcinoma and sarcoma incidences of male rats ingesting chloroform (NCI Rat Study).[a,b]

Treatment	Carcinomas	Sarcomas	Total
0	3/20 (15%)	3/30 (15%)	6/20 (30%)
	6/39 (15%)[c]	5/39 (13%)[c]	11/39 (28%)[c]
Vehicle	3/19 (16%)	2/19 (11%)	5/19 (26%)
Low dose	15/50 (30%)	13/50 (26%)	25/50 (50%)[d], $p = 0.03070$
High dose	16/49 (32%)	8/49 (16%)	21/49 (43%)[d]

[a]Colony control incidences are not available.
[b]One rat was counted only once, regardless of the number of tumors.
[c]Statistics based on combined controls.
[d]Some rats had both carcinomas and sarcomas.

Table 13. Carcinoma and sarcoma incidences of female rats ingesting chloroform (NCI Rat Study)[a, b]

Treatment	Carcinomas	Sarcomas	Total
0	5/20 (25%)	0/20 (0%)	5/20 (25%)
Vehicle	6/20 (30%)	0/20 (0%)	6/20 (30%)
Low dose	20/39 (51%)	3/39 (8%)	21/39 (54%),[c] $p = 0.07071$
High dose	19/39 (50%)	5/39 (13%) $p = 0.07113$	21/39 (54%),[c] $p = 0.07071$

[a]Colony control incidences are not available.
[b]One rat was counted only once regardless of the number of tumors.
[c]Some rats had both carcinomas and sarcomas.

Table 14. Carcinoma and sarcoma incidences (corrected for survival) of male rats ingesting chloroform (NCI Rat Study).

Treatment	Carcinomas	Sarcomas	Total
0	3/20 (15%) 6/39 (15%)[a]	3/20 (15%) 5/39 (13%)[a]	6/20 (30%) 11/39 (28%)[a]
Vehicle	3/19 (16%)	2/20 (11%)	5/20 (26%)
Low dose	15/43 (35%)	13/43 (30%), $p = 0.04974$	25/43 (58%),[b] $p = 0.00583$
High dose	16/42 (38%) $p = 0.07780$	8/42 (19%) $p = 0.0626$	21/42 (50%),[b] $p = 0.03726$ $p = 0.03526$ $p = 0.0422$[c]

[a]Statistics based on combined controls.
[b]Some rats had both carcinomas and sarcomas.
[c]Departure from trend.

Table 15. Carcinomas and sarcoma incidences (corrected for survival) of female rats ingesting chloroform (NCI Rat Study).

Treatment	Carcinomas	Sarcomas	Total
0	5/20 (25%)	0/20 (0%)	5/20 (25%)
Vehicle	6/18 (33%)	0/18 (0%)	6/18 (33%)
Low dose	20/29 (69%), $p = 0.01815$	3/29 (10%)	21/29 (72%),[a] $p = 0.00969$
High dose	19/32 (59%) $p = 0.0531$[b]	5/32 (13%) $p = 0.06691$	21/32 (66%),[a] $p = 0.02816$ $p = 0.03832$ $p = 0.462$[b]

[a]Some rats had both carcinomas and sarcomas.
[b]Departure from trend.

NCI Mouse-Chloroform Study

Chloroform was administered orally to four groups each of B6C3 F_1 mice (*1*). The groups consisted of 50 males and 50 females each at two doses. The mice were started on the chemical at the age of 35 days. Chloroform was given in corn oil by gavage daily five times a week for 78 weeks, and the mice were killed at 92 weeks. Time and weighted average doses are given in Table 17.

Complete necropsies were performed on all mice. Histopathologic examinations were done on killed mice and whenever possible on those found dead.

There was very little difference in the growth curves for male and female mice given chloroform and those not receiving the chemical. Food consumption was also comparable.

Abdominal distention, apparently because of hepatic tumors, was observed in female mice given the high dose of chloroform after 42 weeks and in the high dose male mice after 78 weeks. All of the high dose female mice developed abdominal distention, compared with one-half of the high dose male mice. Carcinoma of the liver at autopsy was first observed at 54 weeks.

Chloroform-treated mice survived well (Table 18). There were no significant differences between the control or chloroform-treated mice.

Results are based on my examination of the histological section.

Table 16. Polyarteritis in males and females given chloroform (NCI Rat Study).

Treatment	Males	Females
0	1/20 (5%) 1/39 (3%)	0/20 (0%)
Vehicle	0/19 (0%)	1/20 (5%)
Low dose	11/50 (22%) p = 0.00664	3/29 (8%)
High dose	12/49 (25%) p = 0.00322 p = 0.006036	4/39 (13%)

[a]Statistics based on combined controls.

Table 17. Average dose levels of chloroform for males and females (NCI Mouse Study).

	Males	Females
Low dose, mg/kg	138	238
High dose, mg/kg	277	477

Table 18. Survival times for males and females given chloroform (NCI Mouse Study).[a]

	Survival time, weeks	
Dose	Females, average (range)	Males, average (range)
0	90.5 (70-92)	85.0 (75-91)
Vehicle	89.9 (68-92)	86.7 (72-92)
Low dose	90.0 (65-93)	89.0 (61-92)
High dose	85.4 (67-93)	88.3 (54-92)

[a]Based on time of development of first tumor of the liver at 54 weeks.

Tumors of the Liver in Female Mice

Female mice given chloroform developed tumors of the liver, and most of the tumors were carcinomas (Table 19). Every female mouse (40/40 given the high dose (p = 0.00001), and 40 of 45 female mice (90%) (p = 0.00001) receiving the low dose developed carcinomas of the liver.

Female mice on the high dose of chloroform had two or three carcinomas per liver, and the carcinomas were well-differentiated, poorly differentiated, or undifferentiated hepatocellular carcinomas. Two mice also had cholangiocarcinomas. The carcinomas in female mice on the low dose of chloroform were usually solitary, well-differentiated carcinomas. There was invasion of adjacent hepatic tissue by the carcinomas, and metastases to the lungs were found in two high dose female mice. Many of the carcinomas in high dose female mice were completely necrotic. Some of the pedunculated necrotic (infarcted) tumors had twisted on their small pedicles and obstructed their blood supply; however, the cause of necrosis of most carcinomas was not apparent. Cirrhosis was not noted in the liver even though some mice had bile duct proliferation and focal necrosis. One of the possible causes for necrosis of these carcinomas of the liver would be hepatic vein thrombosis.

Tumors of the Liver in Male Mice

Male mice administered chloroform also developed high incidences of hepatic tumors (Table 20). Every male mouse (44/44) (p = 0.00001) receiving the high dose and 20 of 46 male mice (43%) (p = 0.00001) on the low dos3 of chloroform had carcinomas of the liver. There were hyperplastic nodules in an additional 11 male mice (24%) on the low dose. There were fewer carcinomas per liver in male mice, and the carcinomas tended to be smaller and not as malignant as in the female mice given chloroform. Two male mice had metastases to the lungs from hepatic carcinomas. Necrosis of carcinomas of the liver was rare in male mice. Cirrhosis of the liver was not present in these mice.

Malignant Lymphomas

Malignant lymphomas were observed in 14 of 46 male mice (30%) receiving the low dose (p = 0.00642) and in 10 of 44 male mice (23%) given the high dose (p

Table 19. Incidences of hepatic lesions in females given chloroform (NCI Mouse Study).

Dose	Hyperplastic nodules	Small carcinomas	Large carcinomas	Total
0	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)
Vehicle	0/19 (0%)	0/19 (0%)	0/19 (0%)	0/19 (0%)
Low dose	1/45 (2%)	3/45 (7%)	37/45 (82%), p = <0.00001	41/45 (91%), p = <0.00001
High dose	0/40 (0%)	1/40 (2%)	39/40 (98%), p = <0.00001 $p = 3.914 \times 10^{-15}$ p = 0.0002[b]	40/40 (100%),[a] p = <0.00001 $p = 2.168 \times 10^{-18}$ p = <0.00001[b]

[a]Two mice had metastases to the lungs.
[b]Departure from trend.

Table 20. Incidences of hepatic lesions in males given chloroform (NCI Mouse Study).

Dose	Hyperplastic nodules	Small carcinomas	Large carcinomas	Total
0	1/17 (6%)	1/17 (6%)	1/17 (6%)	3/17 (18%)
			1/34 (3%)[a]	5/34 (15%)
Vehicle	2/17 (12%)	0/17 (0%)	0/17 (0%)	2/17 (12%)
Low dose	11/46 (24%)	3/46 (7%)	17/46 (37%), $p = 0.00018$	31/46 (67%),[b] $p = <0.00001$
High dose	0/44 (0%)	3/44 (7%)	41/44 (93%), $p = <0.00001$ $p = 1.313 \times 10^{-18}$	44/44 (100%), $p = <0.00001$ $p = 5.794 \times 10^{-17}$

[a]Statistics based on combined controls.
[b]Two mice had metastases to the lungs.

= 0.02751) of chloroform (Table 21). Lymphomas were not present in male mice given the vehicle or in control mice. The incidence of lymphomas was significantly increased in chloroform-treated female mice given the low dose (9 of 45) ($p = 0.03217$).

Tumors Other Than Liver and Lymphomas

Malignant tumors, other than carcinomas of the liver and lymphomas, were slightly increased in the low dose male and female mice (Table 22). Such tumors were seen in 11 of 45 female mice (24%) ($p = 0.06747$) compared to 1 of 12 vehicle control mice. Carcinomas present mainly in the digestive system (5 of 45 mice), but also in the endocrine organs, lung, and kidney. Four unusual carcinomas occurred at the anal-rectal junction — three sebaceous carcinomas and one mixed sebaceous and adenocarcinoma. There was an adenocarcinoma of the stomach at the squamous glandular junction. The incidence of lesions at the anal-rectal junction may well have been higher, because this area was examined histologically only when a tumor was observed grossly. Malignant tumors, other than liver or lymphoma, generally occurred in the low dose male mice without carcinomas of the liver; however, an occasional mouse had a small solitary hepatic carcinoma. By contrast, malignant tumors in the high dose male mice occurred in a high incidence only in the liver.

There was an increased incidence of glandular hyperplasia of the endometrium in low dose and control female mice. There was focal adenocarcinoma, which was present slightly more often in the chloroform-treated female mice. Stromal cell hyperplasia of the endometrium was more common in the low dose treated females.

All Malignant Tumors

The incidence of total malignant tumors was also increased in male mice given the low dose of chloroform; however, most of the increase was carcinomas of the liver. The incidence in the mice on chloroform was 52% (24/46), compared with none in the vehicle control male mice.

Tumors of the kidney were seen in 3 of 44 male mice (7%) on the high dose of chloroform, as well as in one male mouse in each of the low dose group, the vehicle control group, and the untreated group. The renal tumors in the high dose group were much larger and more malignant than in the other groups.

Lesions Other Than Tumors

Thrombosis of the atrium of the heart was present in 9 of 40 female mice (23%) ($p = 0.0013$) on the high dose of chloroform and not in any other female or male mice (positive trend 0.0001; departure from trend 0.0189). Those nine mice also had carcinomas of the liver. Thrombosis of the heart is undoubtedly

Table 21. Incidences of malignant lymphomas in male and female mice given chloroform.[a]

Dose	Males	Females
0	0/17	0/20 (0%)
Vehicle	0/17 (0%)	0/19 (0%)
Low dose	14/46 (30%), $p = 0.00642$ ($p = 0.00016$)	9/45 (20%), $p = 0.03217$ ($p = 0.00241$)
High dose	10/44 (23%) $p = 0.02751$ $p = 0.0257$[b]	4/40 (10%) ($p = 0.06082$) $p = 0.0262$[b]

[a]p values in parentheses are for treated rats compared to pooled controls.
[b]Departure from trend.

Table 22. Incidences of malignant tumors (other than liver and lymphoma) in male and female mice given chloroform.[a]

Dose	Males	Females
0	0/17 (0%)	0/20 (0%)
Vehicle	1/17 (6%)	1/19 (5%)
Low dose	11/46 (24%), $p = 0.09956$[b]	11/45 (24%)[c], $p = 0.06747$
High dose	4/44 (9%)	3/40 (8%)[d]
	$p = 0.00248$[e]	$p = 0.0117$[e]

[a]Carcinomas of lung, kidney, adrenal (cortical and malignant pheochromocytoma), anal (adenocarcinoma and sebaceous), and stomach (adenocarcinoma); subcutaneous leiomyosarcoma with metastases to liver and lungs.
[b]$p = 0.00817$ when treated rats are compared with the pooled controls.
[c]Eight mice with stomal cell sarcomas of the uterus.
[d]Two mice with stromal cell sarcomas of the uterus; $p = 0.00376$ when the treated rats are compared with the pooled controls.
[e]Departure from trend.

related to chloroform treatment, because of the extremely rare occurrence of this lesion in control mice and in large numbers of mice given several other organochlorine chemicals.

Summary

Chloroform is carcinogenic in mice. Female and male mice developed high incidences, 100% in the high dose groups, of carcinomas of the liver. Female mice were more susceptible than male mice. Female mice also had an extremely rare lesion, thrombosis of the heart.

Huntington Research Center Chloroform Studies

In a preliminary report Roe described studies in which chloroform was administered to four strains of mice. Sprague-Dawley rats, and beagles (5-7).

ICI-Swiss Mouse Studies

ICI-Swiss male and female mice, 52 in each group, were given 17 or 60 mg/kg/day of chloroform for 80 weeks. The chemical was given to 3 to 10 week old mice in a toothpaste base by gavage six days a week. Animals were killed after 96 weeks.

Eight of 37 (22%) male mice given the high dose ($p = 0.00012$) of chloroform developed tumors of the kidney compared to none in the controls (Table 23). Some of the tumors which were described as "adenomatous" were large and some mice had multiple tumors. Some of the tumors of the kidney had previously been described as malignant tumors, i.e., carcinomas by Roe (6). There was also a slight increase in the incidence of hepatic tumors in males on both doses of chloroform. There was no increase in renal or hepatic tumors in female mice.

There was a significant increase in the incidence of "hepatic degeneration" ($p = <0.05$) in male mice given 60 mg/kg chloroform.

In summary, chloroform is carcinogenic for the kidney of ICI-Swiss male mice. In addition, there was significant "hepatic degeneration."

Other Mouse Studies

The carcinogenicity of chloroform was studied in four strains of specific pathogen free mice. Male ICI-Swiss, C57BL, CBA, and CF1 mice 3 to 10 weeks of age were given 60 mg/kg-day of chloroform for 80 weeks and killed after 93 to 104 weeks. The chemical was given in toothpaste base (with essential oils and 3.6% chloroform) by gavage six times per week. There were 52 mice in each group.

The incidences of renal or hepatic tumors were not increased in C57BL, CBA, or CF1 mice given chloroform (Table 24). ICI-Swiss male mice (5 of 47) given chloroform developed an increased incidence of renal tumors; however, 1 of 49 control mice also had a renal tumor. Chronic renal disease was a "prominent feature" in chloroform-treated and control mice of the ICI-Swiss strain. The presence or

Table 23. Incidence of hepatic and renal tumors in male ICI-swiss mice given chloroform.

Dose, mg/kg	Hepatic tumors	Renal tumors	Total tumors
0	5/70 (7%)	0/70 (0%)	5/70 (7%)
17	5/35 (14%)	0/35 (0%)	5/35 (14%)
60	5/37 (14%)	8/37 (22%), $p = 0.00012$	13/37 (35%), $p = 0.00042$
		$p = 0.1151 \times 10^{-4}$	$p = 0.214 \times 10^{-3}$

absence of chronic renal disease with tumors was not mentioned.

Chloroform treatment did cause a highly significant increase in the incidence of chronic renal disease in CBA ($p = <0.01$) and CFl ($p = <0.001$) male mice. Relatively few CFl mice survived beyond the 80th week when renal neoplasia was most likely to be seen. By the end of the first year 51% of the treated and control mice had died, and only six mice were alive at the termination of the study. Renal disease may have caused the high mortality.

The renal disease was "chronic nephritis" and varied in degree from mild and moderate to severe. The severe disease was described as: "The most severely affected kidneys were usually visibly enlarged and had surfaces that were irregular because of fibrotic scarring or cyst formation. In a few cases, widespread necrosis and fibrosis had led to kidneys appearing shrunken. Microscopically, severely diseased kidneys showed both tubular and glomerular changes. Some glomeruli were greatly enlarged and were infiltrated by lymphocytes. Some showed thickening of Bowman's capsule. Extensive tubular changes with areas of degeneration and hyperplasia, numerous tubular casts, fibrosis, and chronic inflammatory infiltration were also usually prominent."

In summary, chloroform in toothpaste base was carcinogenic for the kidney of male ICI-Swiss mice, thus confirming the findings in the previous study using mice of this strain. Male CBA and CFl mice given chloroform developed significant chronic renal disease, whereas male C57BL did not.

Chloroform Administered With Arachis Oil

The carcinogenicity of chloroform (4%) with arachis oil as a vehicle was examined for ICI-Swiss male mice in the previous study. The dose of chloroform was 60 mg/kg-day. The chemical was given by oral gavage six days a week for a total of 80 weeks, followed by a 24-week period without the chemical. A group of 100 untreated mice was also used for controls.

Table 24. Incidence of hepatic and renal tumors in male ICI-Swiss, C57BL, CBA, and CFl mice given chloroform (60 mg/kg).[a]

Strain	Treatment[b]	Hepatic tumors	Renal tumors
ICI-Swiss	(−)	7/49 (14%)	1/49 (2%)
ICI-Swiss	(+)	10/47 (21%)	5/47 (11%)
CBA	(−)	36/51 (71%)	0/51 (0%)
CBA	(+)	29/51 (57%)	0/51 (0%)
CFl	(−)	4/45 (4%)	2/45 (4%)
CFl	(+)	5/48 (14%)	1/48 (2%)
C57BL	(−)	2/46 (4%)	0/46 (0%)
C57BL	(+)	0/51 (0%)	0/51 (0%)

[a]The numbers of mice have not been corrected for survival time, i.e., time of appearance of first renal or hepatic tumor. The earliest renal tumor was observed in a chloroform-treated mouse dying during the 71st week.
[b]Control mice received the toothpaste base without chloroform.

Table 25. Incidence of hepatic and renal tumors in male ICI-Swiss male rats given chloroform in arachis oil[a]

Dose, mg/kg	Hepatic tumors	Renal tumors
0	8/83 (10%) 9/133 (7%)	0/83 (0%)
Vehicle	1/50 (2%)	9/50 (18%)
60	8/48 (17%), $p = 0.04699$[b] $p = 0.01305$	12/48 (25%), $p = 0.00156$[c]

[a]Numbers of mice have not been corrected for survival time, i.e., time of appearance of first renal or hepatic tumor. The earliest renal tumor was found in a dead mouse at 71 weeks.
[b]$p = 0.01305$ using vehicle controls only.
[c]Statistics based on combined controls.

Twelve of 48 mice (25%) developed tumors of the kidney, whereas 1 of 50 (2%) receiving the vehicle and none of the untreated mice had renal tumors (Table 25). Eight of 48 (17%) treated mice had hepatic tumors, compared to 9 of 133 (7%) controls ($p = 0.04699$).

In summary, chloroform in arachis oil was carcinogenic for the kidney of male ICI-Swiss mice. These results represent the third time that chloroform was carcinogenic for the kidney of male ICI-Swiss mice.

Sprague-Dawley Rats

Sprague-Dawley male and female rats, 50 per group, were given 60 mg/kg/day of chloroform for 95 weeks. The chemical was given to six-week-old rats in toothpaste base by gavage six times a week. There were 50 rats in each group.

Roe concluded that there was no "effect on tumor incidence in either sex," and there was no increase in hepatic or renal tumors in chloroform-treated rats. One female rat had a "cystadenoma of the liver." Benign and malignant tumors were not evaluated separately. Plasma cholinesterase levels were decreased in male and female rats given 60 mg/kg of chloroform, as well as female rats on 15 mg/kg. Control rats had a high incidence of tumors.

Beagle Seven and One-Half Year Study

Male and female beagle dogs were given 15 mg/kg or 30 mg/kg/day of chloroform in toothpaste base for 376 weeks, and were killed after 396-400 weeks. The chemical was given in capsules six days a week.

Treatment with chloroform did not affect the survival of dogs. The SGPT values in dogs given 30 mg/kg of chloroform were two times those in controls.

Table 26. Incidence of neoplasms in dogs given chloroform.

Dose, mg/kg	Males	Females	Total
0	0/8 (0%)	0/8 (0%)	0/16 (0%)
Vehicle	0/16 (0%)	2/16 (13%)	2/32 (6%)
15	1/8 (13%)	3/8 (38%)	4/16 (25%)
30	2/8 (25%)	0/8 (0%)	2/16 (13%)
15-30	3/16 (19%)	3/16 (19%)	6/16 (37%)
		$p = 0.0378$[a]	

[a]Departure from trend.

Neoplasms were seen in 2 of the high dose, 4 of the low dose of chloroform, and 2 of the dogs receiving the vehicle (Table 26). Tumors were not present in untreated dogs. Of the chloroform-treated male dogs 19% (3/16) developed tumors, compared to 0 of 16 dogs in the vehicle and 0 of 8 untreated dogs. Three of 16 female dogs (19%) given chloroform had tumors, whereas 2 of 16 (13%) dogs on the vehicle and 0 of 16 untreated dogs had tumors. "Trichoepithelioma of the skin and hemangiosarcoma of the spleen" were seen in the two male dogs on the high dose.

In addition to tumors: "A variety of liver abnormalities was seen in both control and treated dogs, including slight bile-duct hyperplasia, nodules of enlarged pale-staining, vacuolated or dark-staining hepatocytes, and a type of lesion that has been described in the literature as 'fatty cyst'."

Roe concluded that "no dog in the experiment developed a neoplasm of the liver." However, further data are required in order to accurately assess the hepatic nodules in the dogs.

In summary, there was an increase in tumors in male and female dogs given chloroform. Hepatic nodules were observed in some dogs.

Summary

Male ICI-Swiss mice given chloroform develop renal tumors, as well as "hepatic degeneration." Significant chronic renal disease was seen in male CFl and CBA mice receicing chloroform. Chloroform also induced tumors in beagle dogs.

Other Chloroform Studies

Eschenbrenner Study

Strain A male and female mice were given chloroform in doses ranging from 37.5 to 600 mg/kg/day by gavage once every four days for four months (*8*). They were killed one month later. Survival times are given in Table 27.

"Hepatomas" and cirrhosis occurred in female strain A mice given the 150 or 300 mg/kg doses of chloroform (Table 28). Within one day after single exposures to chloroform, hepatic necrosis was observed in both sexes at the three highest doses, but not at the two lowest doses. Kidney necrosis was observed at all doses in males, but not in female mice.

This study did demonstrate that tumors of the liver were observed in female strain A mice under the conditions of the study. Numbers of mice were small, duration was limited, and mortality of male mice was too high in order for tumors to develop.

Rudali Study

Rudali studied the carcinogenic effects of halogenated hydrocarbons (*9*). Chloroform was administered to NLC mice of unspecified sex via gav-

Table 27. Survival of male and female strain A mice given chloroform.

	Survival					
	0 mg/kg	37.5 mg/kg-day	75 mg/kg-day	150 mg/kg-day	300 mg/kg-day	600 mg/kg-day
Males	5/5	5/5	3/5	0/5	0/5	0/5
Females	5/5	5/5	5/5	3/5	4/5	0/5

Table 28. Incidences of "hepatomas" in male and female strain A mice given chloroform.

	Hepatoma incidence					
	0 mg/kg	37.5 mg/kg-day	75 mg/kg-day	150 mg/kg-day	300 mg/kg-day	600 mg/kg-day
Males	0/5	0/5	0/3	—	—	—
Females	0/5	0/5	0/5	3/3, $p = 0.01786$	4/4, $p = 0.00794$ $p = 5.864 \times 10^{-6}$ [a]	—

age in oil solution in two doses of 60 mg (2800 mg/kg) each and the animals observed for 10 months. Out of an initial group of 24 animals, five survived and three had "hepatomas." No control group was reported.

Under the conditions of the study, mice given a limited exposure to chloroform and observed for a relatively short duration developed tumors of the liver.

Roe Study

An experiment was carried out by Roe et al., using groups of approximately 50 (C57BL X DBA/2)F_1 mice including equal numbers of males and females (*10*). Chloroform was given as a single dose of 200 μg in 0.02 ml arachis oil when the mice were less than 24 hr old or as eight daily doses of 200 μg starting before the mice were 24 hr old. All mice were injected subcutaneously in the intrascapular region. Survivors (over 36 mice in each group) were killed between the 77th and 80th week of the experiment and examined for tumors of all sites. The authors concluded that a small number of tumors of the lung, liver, and mammary gland and a few cases of generalized lymphoma were encountered in all groups, but the incidence was no higher in the treated groups than in the control groups. No other lesions attributable to treatment were observed. This is not unexpected because of the low dose which was given subcutaneously.

Guinea Pig Study

Guinea pigs were given doses of 35 mg/kg and some of them died during the course of the experiment (*11*). Five of the guinea pigs lived longer than two months, but only two of these lived longer than three months. The guinea pigs which died had fatty change, necrosis, and cirrhosis of the liver.

Summary

Strain A female mice given large doses of chloroform by gavage for a few months developed cirrhosis of the liver and "hepatomas." "Hepatomas" also were reported in NLC strain mice given chloroform.

Guinea pigs given chloroform had cirrhosis of the liver. It is likely that guinea pigs given a dose of chloroform over a long period of time would also develop tumors of the liver.

Comments

The formation and occurrence of tumors in man and other mammals, such as rats and mice, is quite similar (*12*). In tests undertaken to date, it has been demonstrated that virtually every chemical which has been found to be carcinogenic in man is also carcinogenic in one or more mammalian test animals. It has been shown that if these compounds will produce neoplasms in one species, they will very likely produce neoplasms in more than one; thus adding weight to any finding of carcinogenicity in tests using any mammalian species.

Sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that a finding of carcinogenicity in one mammalian species should be deemed to have relevance for other mammalian species, including man. This threat may not be manifested for up to 30 or 40 years, given the long latent period of many known human carcinogens.

Statistical analyses were done by C. W. Riggs, Information Systems Department, Frederick Cancer Research Center.

REFERENCES

1. Page, N. P., Cueto, C., and Saffiotti, U. Report on carcinogenesis bioassay on chloroform. National Cancer Institute, 1976.
2. Thomas, D. G., Breslow, N., and Gart, J. J. Trend and homogeneity analyses of proportions and life table data. Computers Biomed. Res. 10: 373 (1977).
3. Reuber, M. D. Development of preneoplastic and neoplastic lesions of the liver in male rats ingesting .025 percent N,2-fluorenyldiacetamide. J. Natl. Cancer Inst. 34: 697 (1965).
4. Reuber, M. D. Morphologic and biologic correlation of hyperplastic and neoplastic renal lesions occurring in Buffalo and Fischer strain rats ingesting *N*-4-(4′-fluorobiphenyl)-acetamide. Tumori 63: 493 (1977).
5. Roe, F. J. C. Preliminary report of long-term tests of chloroform in rats, mice, and dogs. Huntington Research Centre, England. Paper, presented at the 15th Annual Meeting of the Society of Toxicology, Atlanta, Georgia, March 14-18, 1976.
6. Palmer, A. K., Halliday, J., Hague, P. H., Mawdesley-Thomas, L. E., and Roe, F. J. C. Effects of prolonged administration of emulsions containing peppermint oil, eucalyptol, or chloroform on mice. Unpublished manuscript (January 4, 1971).
7. Palmer, A. K., Hunter, B., Mawdesley-Thomas, L. E., and Roe, F. J. C. Effect of prolonged administration of an emulsion containing chloroform on male mice of C57BL, CBA, CFl and ICI strains. Unpublished manuscript (January 28, 1969).
8. Eschenbrenner, A. B. Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. J. Natl. Cancer Inst. 5: 251 (1945).
9. Rudali, G. A propos de l'activité oncogène de quelques hydrocarbures halogènés utilisés en therapeutique. (U.I.C.C. Monographs 7) 1967, pp. 138-143.
10. Roe, F. J. C., Carter, R. L., and Mitchley, B. C. V. Tests of chloroform and 8-hydroxyquinoline for carcinogenicity using newborn mice. A. R. Brit. Inst. Cancer Campaign. 46: 13 (1968).
11. Miklashevski, V. E., Tugarinova, V. N., Rakhamanina, N. L., and Yagovleva, G. P. Toxicity of chloroform administered perorally. Gig. Sanitar. 31: 320 (1966).
12. Reuber, M. D.: Carcinogenicity testing of chemicals with particular reference to organochlorine pesticides. Sci. Total Environ. 10: 105 (1978).