SAFETY EVALUATION OF TOOTHPASTE CONTAINING CHLOROFORM III. LONG-TERM STUDY IN BEAGLE DOGS

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Beagle dogs were given chloroform in a toothpaste base orally in gelatin capsules on 6 d/wk for 7½ yr, followed by a 20-24 wk recovery period. Groups of 16 males and females received 0.5 ml/kg/d of the vehicle (toothpaste without chloroform) and 8 dogs of each sex remained untreated. Treated groups comprised 8 dogs of each sex, receiving doses equivalent to 15 and 30 mg $CHCl_3$/kg/d in the toothpaste vehicle; another group of the same size received an alternative non-chloroform toothpaste (0.5 ml/kg/d). Eleven of the 96 dogs died during the study, only two of these being in the $CHCl_3$-treated groups. The only significant toxic response during treatment was a moderate rise in serum enzyme levels (e.g. SGPT), reaching a peak in the sixth year of the study and probably corresponding to minimal liver damage. Few palpable growths were noted while the dogs were alive. "Fatty cysts" were seen in the liver of several dogs at post mortem possibly associated with the chloroform treatment but the distribution of a nodular change in the liver was not obviously dose related. A small number of macroscopic and microscopic neoplasms were seen; one dog in each chloroform-treated group had a malignant tumour but there were no tumours in the liver or kidney of any dog. Overall, exposure to chloroform in a toothpaste base was not associated with any effect on the incidence of any kind of neoplasm.

From this and related studies in mice and rats, it is concluded that repeated exposure to chloroform (3.5 percent) in toothpaste is unlikely to result in any hazard to human health.

INTRODUCTION

Strain-related differences in renal tumour incidence in mice given toothpaste containing chloroform for 80 wk (Roe, *et al.*, 1978) suggested the need for a carcinogenicity study using a non-rodent species. At the time concerned (1967), guidance was given by the U.S. Food Protection

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Committee (1959) recommendation for a 4-yr dog study. Interest in the dog stemmed from the well-known finding of Hueper, *et al.*, (1938) that bladder tumours in dogs given 2-naphthylamine required a latent period of only about 2 yr; such tumours could not be demonstrated in rats or mice though they did occur in man. Several later reviews discussed the use of dogs for carcinogenicity screening, e.g. Berenblum (1969), Bonser (1969) and Lalonde *et al.* (1973).

The present report deals with preliminary studies for the selection of dose levels and the main study, which was planned to continue for 7 yr.

MATERIALS AND METHODS

Animal Management and Treatment

For the main study, 48 male and 48 female pure bred beagles, initially 18-24 wk old, were housed singly in kennels. Another 8 males and 8 females were used for preliminary studies. All the dogs were clinically examined and inoculated against distemper, canine hepatitis and leptospirosis as well as being dosed with piperazine adipate as an anthelmintic. Inoculation and anthelmintic treatment were repeated annually.

The dogs were fed weighed amounts (200 g) of a dry diet at fixed times twice daily. When new food was offered, any residue from the previous meal was removed for weighing and calculation of food consumption. From week 300, the daily food ration of dogs considered to be obese was reduced from 400 to 300 g. Water was freely available at all times and fresh milk (200 ml) offered to each dog on weekday mornings for the first 6 mon.

The test material given to the dogs was a toothpaste complying with the formula given by Roe *et al.*, (1978) except that the carragheen gum and glycerol concentrations were reduced for ease of transfer from a syringe into gelatin capsules. Transfer was effected immediately before dosing, to minimize volatilization of chloroform or softening of the gelatin. Doses, calculated weekly after weighing the individual dogs, were given at a fixed dose-volume of 0.5 ml/kg on 6 d/wk.

For humane reasons, treatment e.g. with antibiotics was given to a few dogs with obvious illness in the late stages of the main study. A vehicle control bitch developed a large mammary growth which was surgically removed during the final year and another in the same treatment group was operated upon for the repair of an inguinal hernia during the 5th year of the study.

Dose-Levels (Preliminary Study)

The schedule of treatment given 7 d/wk is shown in Table 1. At the time of commencing these studies, reference data on untreated beagles were not readily available through Brunk (1969), Noel (1970), Deavers *et al.*, (1972) and Lutzen *et al.*, (1976) have since given details. Data were,

however, available to us from another 64 beagles of similar age from the same supplier.

Dose-Levels (Main Study)

From the observations made in the preliminary study and to ensure that the upper dose level would reach the threshold for early toxic changes but with as many dogs as possible remaining alive and well for at least 7 yr, daily dose-levels of 0, 15, and 30 mg $CHCl_3$/kg were selected for the main study. The treatments are detailed in Table 2.

Observations

A full range of data was collected during the preliminary study and terminal histopathology was carried out. In the main study, clinical signs were recorded daily, food consumption twice daily, water consumption intermittently and bodyweight once per week. Ophthalmoscopy was

TABLE 1. Treatments given in Preliminary Study

No. of dogs ♂	No. of dogs ♀	Treatment[1] (mg $CHCl_3$/kg/d)	Duration (weeks)
1	1	120	12
1	1	90	12
2	2	60	18
2	2	45	13
2	2	30	13

[1]In toothpaste base (including peppermint oil and eucalyptol) given orally by gelatin capsule.

performed on each dog once during the pre-treatment period and then at 3-month intervals after the start of treatment. Each dog had a thorough clinical examination at least twice yearly. A comprehensive range of laboratory investigations was performed on all dogs once during the pre-treatment period and then, after treatment started, at 6 and 13 wk, 6,9 and 12 mon and thereafter at 6-mon intervals. Additional serum enzyme studies were undertaken in the later stages of the investigation to monitor liver status.

Post-Treatment Recovery Phase and Terminal Studies

As 84 of the original 96 dogs were still alive and in good condition after 7 yr, the intention to end the study at this stage was reconsidered. The only sign of toxicity was a moderate elevation of certain serum enzyme levels, mainly at the higher chloroform dose-level; there were no palpable tumours but a few subcutaneous nodules thought to be of mammary gland origin were detectable.

Continuation of the experiment would have allowed further time for possible treatment-related lesions to develop, but it was thought better to sacrifice all the animals at one time so that strictly valid comparisons could be made between different groups. The dogs were, however, kept under observation for several months after the cessation of treatment at week 376 to see whether the elevated serum enzyme levels reverted to normal.

Between weeks 395 and 399, each dog still alive received an intravenous injection of sodium pentobarbitone. Immediately after killing or as soon as possible, in the case of animals found dead, full macroscopic examination was made of all tissues and any abnormalities noted. The principal organs were then removed and weighed; small portions of these and a wide range of other tissues along with portions of any lump or tumour were placed in fixative. Sections were routinely stained with haematoxylin and eosin; in addition, liver and kidney sections were stained with Oil Red O for fat. Further examination, by electron microscopy, was conducted terminally on liver and kidney sections from two untreated control dogs and three dogs from the 30 mg $CHCl_3$/kg/d group.

TABLE 2. Treatments given in Main Study

No. of dogs ♂	No. of dogs ♀	Treatment (mg $CHCl_3$/kg/d)
8	8	30[a]
8	8	15[a]
16	16	0[a,b]
8	8	Untreated
8	8	Alternative non-chloroform toothpaste[c]

[a]In toothpaste base (including peppermint oil and eucalyptol) given orally by gelatin capsule

[b]Vehicle Control Group

[c]The same alternative non-chloroform toothpaste referred to by Roe *et al.* (1978)

RESULTS

Preliminary Study

No dogs died during the preliminary study. Vomiting occurred at times in dogs given 120, 60 or 30 mg $CHCl_3$/kg/d and one dog that re-

ceived 120 mg $CHCl_3$/kg/d became jaundiced after treatment for 4-5 weeks. Loss of general condition was noted in both dogs at 120 mg $CHCl_3$/kg/d, in the male at 90 mg $CHCl_3$/kg/d and in one dog of each sex at 60 mg $CHCl_3$/kg/d. Marked bodyweight losses occurred in the dogs given 60 mg $CHCl_3$/kg/d or above and some others had reduced weight gain. Appetite was apparently suppressed at first in all dogs and not regained in dogs which showed effects on bodyweight.

Increase in SAP, SGOT, bilirubin and ICD was seen in the male at 120 mg $CHCl_3$/kg/d. An indication of the SGPT values found is given in Table 3. The female given 120 mg $CHCl_3$/kg/d and both dogs given 90 mg $CHCl_3$/kg/d showed increased SGPT on several occasions and bili-

TABLE 3. SGPT Values in Preliminary Study[1]

Treatment (mg $CHCl_3$/kg/d):	Untreated		30		45		60		90		120	
Sex:	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mean SGPT[2] (mU/ml):	28	29	68	45	50	69	92	58	84	114	146	47

[1]At 12 wk for treated dogs, 5 wk for untreated controls.

[2]32 males and 32 females in Untreated group; 2 males and 2 females in 30, 45 and 60 mg $CHCl_3$/kg/d groups, other data are for single dogs.

rubin once but no other definite abnormality. There was an increase in SGPT at the dose level of 60 mg $CHCl_3$/kg/d and, in the two dogs showing marked loss of condition, an effect on SAP and SGOT. Only occasional increases in SGPT were seen at 45 and 30 mg $CHCl_3$/kg/d. At post-mortem there was discolouration of the liver and increased liver weight at 45 mg $CHCl_3$/kg/d and above. Nothing abnormal was seen at 30 mg $CHCl_3$/kg/d. At 60 mg $CHCl_3$/kg/d and above, hepatocyte enlargement and vacuolation together with deposition of fat within the hepatocytes was seen in all dogs. Variation in hepatocyte size with slight fat deposition was noted at 45 mg $CHCl_3$/kg/d.

Main Study

Shortly after the study began, frequently observed changes were soft and pale faeces along with occasional vomiting in all treated groups and in the vehicle controls. Regular veterinary examination revealed no evidence of disease apart from transient minor abnormalities and one case of inguinal hernia in a vehicle control bitch, none of which were treatment related.

About 20 percent of the dogs were hyperexcitable, especially during the first 2-3 yr. These dogs were prone to convulsive episodes and altogether 10 dogs died after one or more fits. A vehicle control bitch in renal failure was killed in week 299 for humane reasons; this dog was apparently in good health for the first 5½ years but then deteriorated rapidly, showing various degenerative tissue changes at post mortem without obvious cause. Deaths are listed in Table 4. During the final year, a bitch from the group receiving the alternative non-chloroform toothpaste was missing after kennel cleaning and not subsequently found.

Bodyweight changes are summarized in Figs. 1 and 2 for males and females respectively still alive at the time of weekly weighing. The findings were complicated by obvious obesity; dogs considered obese, whose diet was reduced at the beginning of week 300 are listed in Table 5. Neither the development of obesity in individual dogs nor the group mean bodyweight changes were treatment related. Food consumption was not influenced by the treatments given. Males consistently left not more than 5 percent of their food but females regularly left up to 15 percent. Daily water consumption was in the region of 1000-1500 ml for all dogs.

TABLE 4. Deaths during Main Study

Treatment (mg $CHCl_3$/kg/d)	No. of Deaths ♂	No. of Deaths ♀	Wk. of Death
30	1	0	87
15	1	0	328
Vehicle Control	1	4	96, 125, 151, 173, 299[a]
Untreated	1	3	134, 147, 153, 192
Alternative non-chloroform toothpaste	0	0	

[a]Killed for humane reasons.

Haematological findings during the study and at the end of treatment (week 374) showed no discernible differences between groups. The most obvious deviation found in biochemical analyses was a dose-related elevation in SGPT values, later accompanied by less marked elevation of SAP and SGOT. No other parameters originally studied showed any abnormality in the treated dogs. Since moderate liver damage was suggested by the analytical findings, bromsulphthalein retention tests were performed during the sixth year of the study but no influence due to treatment was detected. Later the biochemical analyses were extended to include further indices for presumptive liver damage, namely LAP, γGT

and GDH. Tables 6 and 7 show indications of hepatic dysfunction while treatment continued; however, all but one of the dogs at the lower $CHCl_3$-dose level and several of the higher dose-level dogs reverted to normal SGPT values during the post-treatment recovery phase, whereas several dogs in other groups had increased SGPT values at this stage. Changes noted in ophthalmoscopic examination included peripheral capsular opacities associated with the ends of posterior lens sutures, a degree of nuclear sclerosis and some degenerative change involving the tapetum

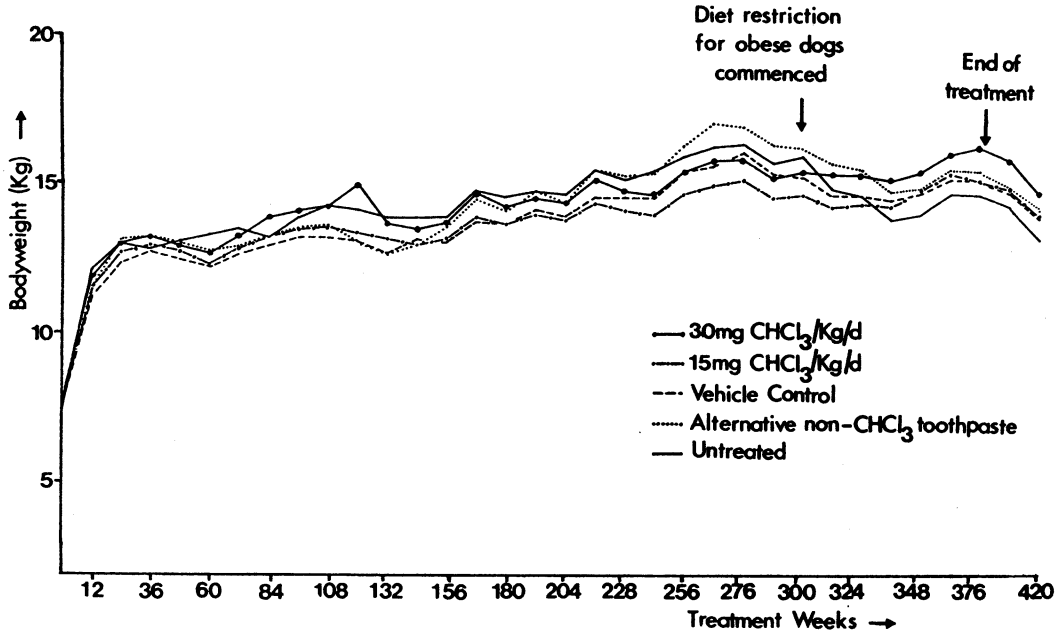

FIGURE 1. Group Mean Bodyweight (Males)

lucidum; none of these age-related changes was considered to be treatment-related. There were few anomalies in urinalysis at the end of treatment or at any intermediate stage.

Autopsies on the dogs that died during the treatment phase of the study showed no obvious changes in the brain, other parts of the central nervous system, liver, kidney or other organs and there were no tumours.

At the end of the study there were no treatment-related differences in either absolute or relative weights of various organs (Table 8); considerable individual variations were noted but group means showed no significant deviations when compared with the untreated or vehicle controls.

Histopathological Findings Other Than Neoplasms

Liver and Gall Bladder. Generalised disruption of lobular architecture was seen in two of the upper dose-level males and one male vehicle control. Varying incidence of bile duct hyperplasia, either focal or

generalised, was seen in all groups without any clear relationship to treatment; similarly, the frequency of minimal hepatic fibrosis was not dose-dependent. Aggregations of vacuolated histiocytes forming so-called "fatty cysts" (Jubb and Kennedy, 1970) were seen in all groups, although the cysts were larger and more numerous in the treated dogs (Table 9).

Haemosiderin, almost entirely within macrophages, was found in the livers of all dogs but most prominently in the group treated with the alternative non-chloroform toothpaste (40 percent of group with haemosiderin in numerous macrophages; highest in other groups was 11 percent of dogs in the vehicle controls). Twelve dogs had small areas of

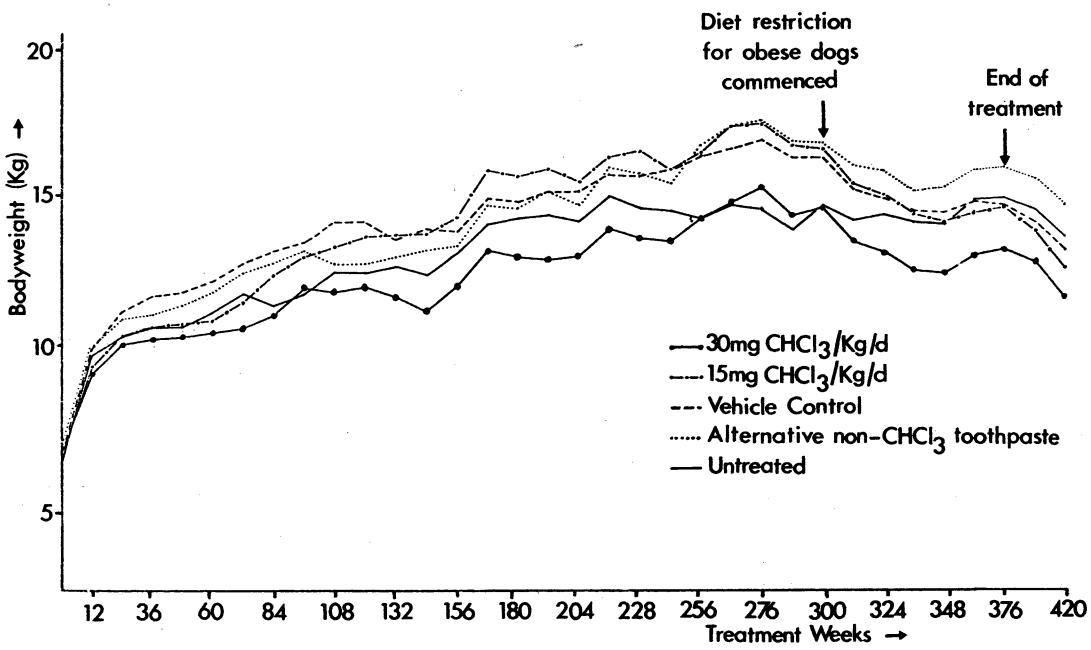

FIGURE 2. Group Mean Bodyweight (Females)

well-defined nodules of altered hepatocytes, in seven of which only microscopic nodules were detectable. The nuclear morphology of the altered hepatocytes was normal; no mitoses were seen and the areas appeared to be early or small hyperplastic nodules without evident neoplasia. Dogs in all groups showed these changes, which had no obvious relationship to treatment. Numerous minor hepatic changes and minor abnormalities of the gall bladder were seen, apparently unrelated to the treatment given.

Cardiovascular System. Small focal areas of arteriosclerosis were seen in the aorta of about one-half of the dogs in each group and endocardiosis of the mitral and/or tricuspid valves in a small proportion of dogs from all groups. These and other less marked changes did not appear to be treatment-related.

Reproductive System. Ectopic testes with inhibition of spermatogenesis were noted in two dogs at the upper chloroform dose level, one at the lower dose level and one untreated control. Other abnormalities in males occurred in various groups without any treatment relationship. Most of the changes in the ovaries and uterus of the females were obviously consistent with normal oestrus activity. Nodular hyperplasia of the mammary gland was noted in three dogs at 15 mg $CHCl_3$/kg/d, five of the vehicle controls and one untreated control.

Urinary System. Prominent changes, usually chronic interstitial nephritis, were seen in the kidneys in all groups but most often in the untreated controls (4/12). Fat deposition accounted mainly for the differences between groups in glomerular abnormalities. Many glomeruli were affected in 6/15 dogs of the 30 mg $CHCl_3$/kg/d with fewer glomeruli affected in all other groups.

Other Systems. Numerous changes were recorded in individual dogs, without evident treatment relationships.

TABLE 5. Dogs Considered Obese at Wk 300

Treatment (mg $CHCl_3$/kg/d)	No. of Obese Dogs ♂	♀
30	2	6
15	2	8
Vehicle Control	7	9
Untreated	5	4
Alternative non-chloroform toothpaste	3	5

Histopathological Findings: Neoplasms

No neoplasms were seen in dogs which died during the study. The details for neoplasms found in dogs killed at the end of the study are given in Table 10. There was no evidence of invasion of other tissues by any of the tumours, although two were classified as histologically malignant.

DISCUSSION

The prevalence of a type of idiopathic epilepsy (Koestner and Rehfeld, 1968; Martinek and Dahme, 1977) in the early stages of our main study raised doubts about the feasibility of achieving the projected 7-yr term but fortunately, after the population of most excitable animals had died as a

result of fits, there were no more deaths from this cause. However, the optimal duration for a carcinogenicity study using dogs is still debatable. Compared to many dog studies terminated after only 2 to 4 yr, the work reported here provides more comprehensive long-term findings, and it compares favourably also with earlier studies lasting 9 or more years with only two or three dogs per treatment. Recently, Weikel and Nelson (1977) reported a 7-yr study of contraceptive steroids given to substantial numbers of female beagles.

TABLE 6. Group Mean Values in Biochemical Analyses (Main Study) (males and females combined)

Treatment (mg $CHCl_3$/kg/d)	30		15		Vehicle Control		Un-treated		Alternative non-chloroform toothpaste	
No. of dogs	15		15		27		12		15	
Wk.	374[a]	395[b]	374	395	374	395	374	395	374	395
Urea (mg %)	30	28	32	26	31	31	33	27	34	29
Glucose (mg %)	99	99	100	100	101	98	99	95	102	98
Total serum proteins (g%)	5.9	6.1	6.1	6.2	6.3	6.0	6.3	6.0	6.4	6.1
Albumin	2.9	2.8	3.0	2.8	3.1	2.8	3.2	2.9	3.2	2.9
α_1 globulin	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3
α_2 globulin	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.8
β globulin	1.6	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.7	1.6
γ globulin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.6	0.4
A/G ratio	0.98	0.86	0.98	0.87	0.99	0.91	1.04	0.97	1.00	0.94
SAP (KA units)	30	26	20	17	14	16	12	14	10	13
SGPT mU/ml	102	111	66	48	51	128	50	56	57	87
SGOT mU/ml	33	33	27	29	25	32	22	32	25	31
LAP (GR units)	90	72	82	62	73	54	81	56	82	60
Bilirubin (mg %)	<0.2	<0.2	<0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Erythrocyte cholinesterase (Δ pH/h)	0.73	0.73	0.78	0.81	0.77	0.81	0.77	0.82	0.65	0.71
Plasma (Δ pH/h)	1.08	0.91	1.00	0.88	1.00	0.87	1.02	0.86	1.05	0.91
γ GT (mU/ml)	3.6	3.3	2.9	2.5	2.9	2.1	2.5	1.7	2.8	1.8
GDH (mU/ml)	7.2	5.3	6.5	3.9	4.9	4.0	3.9	3.0	4.9	3.0
ICD (B & B units)	11.8	11.4	11.2	10.7	9.9	12.9	9.2	11.7	10.6	12.9

[a]End of Treatment.

[b]End of post-treatment recovery period.

TABLE 7. SGPT Changes Throughout Main Study

Treatment (mg $CHCl_3$/kg/d)	Group Mean SGPT (mU/ml)																				
	Pre-treatment	Treatment Stage (weeks)																		Post-Treatment (weeks)	
		6	13	26	39	52	78	104	130	156	182	208	234	260	286	312	338	364	372	14	19
30mg $CHCl_3$/kg/d	24	34[a]	37[b]	58[c]	63[c]	52[c]	73[c]	64[c]	51[c]	76[c]	108[c]	91[c]	80[c]	147[c]	138[c]	128[c]	134[c]	104[c]	102[c]	105[b]	111
15mg $CHCl_3$/kg/d	22	29	30	33	39	32	43	45	34[b]	46[b]	61[b]	55[b]	55[a]	95[c]	93[b]	89[c]	73[a]	79[a]	66	53	48
Vehicle Control	22	29	29	30	39	29	35	40	22	30	33	40	34	33	65	47	49	50	51	56	128
Alternative Non-chloroform Toothpaste	28	31	31	33	40	28	37	36	21	30	34	36	35	50	47	52	47	59	57	48	87
Untreated	24	30	30	30	38	27	37	37	21	29	33	30	33	32	48	50	44	50	50	53	56

[a]Comparison with untreated group; $p < 0.05$.
[b]Comparison with untreated group; $p < 0.01$.
[c]Comparison with untreated group; $p < 0.001$.

TABLE 8. Organ Weights in Main Study
(Upper Figure = Absolute wt. in g.; Lower Figure = Organ wt. as % body wt.)

Treatment mg $CHCl_3$/kg/d	30	15	Vehicle Control	Untreated	Alternative non-chloroform toothpaste
Brain	79 0.66	76 0.62	81 0.65	78 0.64	77 0.59
Pituitary (mg)	81 0.00068	77 0.00063	79 0.00063	73 0.00059	79 0.00060
Spinal Cord	20.4 0.17	19.2 0.16	20.3 0.16	19.8 0.16	20.2 0.15
Heart	114 0.94	118 0.95	121 0.96	117 0.95	120 0.90
Lungs	107 0.89	108 0.87	112 0.90	105 0.85	116 0.88
Liver	473 3.86	470 3.82	433 3.47	462 3.74	451 3.42
Spleen	88 0.71	75 0.60	71 0.56	63 0.50	65 0.49
Pancreas	34 0.28	40 0.32	35 0.28	37 0.31	39 0.29
Thymus	2.4 0.02	2.5 0.02	2.8 0.02	3.7 0.03	2.9 0.02
Prostate	22.5 0.16	21.8 0.17	23.8 0.18	20.1 0.16	21.0 0.16
Uterus	11.7 0.11	8.5 0.07	10.0 0.08	13.0 0.10	11.3 0.09
Kidneys	78 0.64	79 0.64	78 0.62	84 0.68	78 0.59
Thyroids	0.96 0.0080	0.86 0.0071	0.97 0.0077	0.91 0.0073	0.81 0.0061
Adrenals	2.23 0.0186	2.23 0.0183	2.06 0.0164	2.05 0.0170	2.09 0.0158
Testes	34.0 0.236	30.6 0.232	31.0 0.243	22.6 0.209	30.2 0.233
Ovaries	1.32 0.0120	1.57 0.0133	1.45 0.0119	1.82 0.0143	1.67 0.0124
Body-weight	12350	12420	12730	12510	13340

The upper dose level selected for our main study was based on the relatively small changes in SGPT levels during the preliminary study, since hepatotoxicity was expected to be a determining factor. This parameter evidently served the purpose well in this instance, and we were also able to include a lower dose level representing approximately one hundredfold exaggeration compared to normal human exposure.

We were reluctant to undertake any test procedure during the main study that might have prejudiced its long-term outcome. Thus we only performed limited BSP studies to assess liver function. As in the preliminary study, the most useful index of relatively low-level hepatic dysfunction

TABLE 9. Liver Changes (Nodules of Altered Hepatocytes and "Fatty Cysts")

Treatment (mg $CHCl_3$/kg/d)	Sex	No. of dogs Examined histologically at end of expt.	No. with nodules	No. with "fatty cysts"	
				Occasional or minimal	Moderate or marked
30	♂	7	0	1	6
	♀	8	4	0	7
15	♂	7	1	0	6
	♀	8	1	2	3
Vehicle Control	♂	15	0	7	1
	♀	12	3	3	0
Untreated	♂	7	1	2	0
	♀	5	1	1	0
Alternative non-chloroform toothpaste	♂	8	0	2	0
	♀	7	1	0	0

during the full 7-yr term was the modest elevation of SGPT levels, which rose steadily during the first 5 yr and later gradually declined. The most prominent histopathological change in the liver was the occurrence of *fatty cysts*. However, whereas SGPT tended to revert to normal levels during the post-treatment recovery phase, the *fatty cysts* persisted and may, therefore, have been unrelated to the enzyme changes. In man, the onset of liver damage is often accompanied by changes in ICD levels (Tietz, 1976), but no significant deviations from normal values were recorded in our study.

TABLE 10. Neoplasms in Dogs at End of Experiment

Treatment ($mgCHCl_3$/kg/d)	Sex	No. of dogs examined	No. of Dogs with macroscopically visible Neoplasms	Type of Neoplasm	Site	No. of Dogs with Neoplasms discovered by Microscopy	Type of Neoplasm	Site	Total No. of Dogs with Neoplasms
30	♂	7	1	Trichoepithelioma	Skin	0	—	—	2
			1	Haemangiosarcoma[a]	Spleen				
	♀	8	0	—	—	0	—	—	0
15	♂	7	1	Anaplastic Carcinoma[a]	Lung	3	Follicular adenoma	Thyroid	4
				Leydig cell tumour	Testis		Intratubular seminoma	Testis	
				Sertoli cell tumour	Testis[b]		Sertoli cell tumour	Testis	
	♀	8	3	Fibroma durum	Skin	2	Tubular adenoma	Ovary	4
				Pericanalicular Fibroadenoma	Mammary gland		Intracanalicular fibroadenoma	Mammary gland	
				Papillary cystadenoma	Mammary gland				

TABLE 10. (Continued). Neoplasms in Dogs at End of Experiment

Treatment ($mgCHCl_3$/kg/d)	Sex	No. of dogs examined	No. of Dogs with macroscopically visible Neoplasms	Type of Neoplasm	Site	No. of Dogs with Neoplasms discovered by Microscopy	Type of Neoplasm	Site	Total No. with with Neoplasms
Vehicle Control	♂	15	0	—	—	0	—	—	0
	♀	12	2	Follicular adenoma	Thyroid	2	Tubular adenoma	Mammary gland	4
				Cortical adenoma	Adrenal		Complex adenoma	Mammary gland	
Untreated	♂	7	0	—	—	1	Leydig cell tumour	Testis[b]	1
							Sertoli cell tumour	Testis[b]	
	♀	5	0	—	—	0	—	—	0
Alternative non-chloroform toothpaste	♂	8	1	Leydig cell tumour	Testis[c]	1	Follicular adenoma (ectopic thyroid)	Thymus	2
	♀	7	0	—	—	1	Chondroma	Dermis	1

[a]Histologically malignant.
[b]Ectopic organ.
[c]Bilateral.

Taking microscopically-discernible tumours into account as well as macroscopic lesions, the duration of our study was clearly sufficient to reach the time at which spontaneous tumours begin to appear in the beagle dog. Since there was no dose-dependent increase in tumours at this stage related to the treatment given, the treatment clearly did not advance their onset. Of the tumours recorded in our study, a few were found on microscopic examination alone. At the 7½-yr stage in the lifespan of the beagle, tumours of the testis and mammary gland were evidently starting to devlop rather commonly but we saw no reason to attribute malignancies to the treatment given. Above all, there was a complete absence of neoplasia affecting the liver or the kidney, even though the upper dose-level was high enough to exert some degree of hepatotoxic action and some of the dogs in all groups developed hyperplastic liver nodules.

To summarise the position with regard to the safety evaluation of toothpaste containing up to 3.5 percent of chloroform, data now exist from long-term studies in mice, rats and dogs. In mice, though only at the higher dose-level given, there was a sex- and strain-related tendency to develop renal tumours predominantly benign in character. By contrast, at dose-levels up to 400 times the estimated human exposure to chloroform from twice daily toothbrushing, we did not observe any overall increase in neoplastic changes in male mice of three of four strains, in female mice or in rats; at dose levels up to 200 times estimated human exposure, no treatment-related excess of tumours was observed in beagle dogs after nearly 7½ yr. Eschenbrenner and Miller (1945) showed that chloroform given in vegetable oil solution was carcinogenic to the liver of female mice but only when given in doses large enough to produce liver necrosis and to kill all the treated male mice. The NCI report (1976) also dealt with the effects of high dose-level administration of chloroform in vegetable oil solution, noting an excess of liver tumours in mice and renal tumours in rats. We have shown that the findings in chloroform studies may be markedly influenced by administration in vegetable oil solution rather than toothpaste. Recently Butterworth (1978) confirmed the tendency for [^{14}C] chloroform to concentrate radioactivity in the kidneys of male mice when given as a solution in a vegetable oil, but he found no significant radioactivity except in the urinary bladder 1 hr after giving the same chloroform dose in a toothpaste vehicle to male MF1 mice. Moreover Brown *et al.* (1974) demonstrated marked differences between the metabolism of chloroform given to rodents and a non-rodent (the squirrel monkey).

The range of experimental evidence now available appears to be fully consistent with our view that repeated exposure to chloroform at a level of 3.5 percent in toothpaste is unlikely to result in any hazard to human health.

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