Effects of Dieldrin, Diet, and Bedding on Enzyme Function and Tumor Incidence in Livers of Male CF-1 Mice

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ABSTRACT

The effects of naturally occurring microsomal enzyme inducers on hepatocellular drug-metabolizing enzyme systems and also upon the incidence of "spontaneous" liver tumors in CF-1 mice were investigated, using animals maintained on semisynthetic diet and filter-paper bedding as controls. The administration of dieldrin, a potent microsomal enzyme inducer with tumorigenic properties in livers of CF-1 mice, to some of the experimental treatment groups served as a positive control. Conventional diet and sawdust bedding caused induction of the liver monooxygenase system, although this effect was far less pronounced than that produced by dieldrin. The incidence of liver tumors in mice exposed to conventional diet and sawdust bedding was similar to that seen in the control group. The incidence of liver tumors was significantly increased in dieldrin-treated mice, including those maintained on semisynthetic diet and filter-paper bedding. Both benign and malignant tumors were found in dieldrin-treated mice, the latter type of lesion showing evidence of lung metastasis. These results, together with evidence that dieldrin and its mammalian metabolites possess neither genotoxic activity nor potential, are consistent with the concept that dieldrin exacerbates or facilitates the expression of a preexisting oncogenic factor which is genetically linked and possibly viral in origin.

INTRODUCTION

Prolonged p.o. exposures of CF-1 mice to dieldrin, phenobarbitone, DDT,[2] and β- and γ-hexachlorocyclohexane (benzenehexachloride) result in an increase in the incidence of liver tumors, some of which possess invasive properties and can be transplanted (22, 24). Such malignant tumors also occur in control CF-1 mice, and increases in the incidence of "spontaneous" liver tumors have also been reported for inbred strains of mice exposed to dieldrin or to phenobarbitone (6, 16).

It is well established that phenobarbitone and DDT can enhance the carcinogenic response of rat liver to 2-acetylaminofluorene (15, 17, 18). By analogy, it has been suggested that these compounds do not exert an intrinsically carcinogenic effect on mouse liver but function by enhancing the expression of a preexisting oncogenic factor, which may be of environmental or genetic origin. However, it has been argued that, even though the oncogenic factor may be of environmental origin, its expression must be genetically determined (26). All

of the compounds that have been shown to cause an increase in the incidence of hepatic tumors in mice are lipophilic substances that are inducers of the microsomal monooxygenase system of mammalian liver (5). This has led to the suggestion that a general property of microsomal enzyme inducers may be to cause an increase in the liver tumor incidence in certain strains of mice (26).

Several reports (1, 9, 11, 23) indicate that diets and bedding used routinely in toxicological studies with rodents may contain naturally occurring and, possibly, adventitious microsomal enzyme inducers. If apparently unrelated xenobiotic microsomal enzyme inducers can enhance the incidence of liver tumors in various strains of mice, similar effects might be expected as a consequence of exposure to naturally occurring or adventitious microsomal enzyme inducers in the environment of the animal. Accordingly, the principal objectives of the current study were to determine the capacity of diets and bedding used in this laboratory (Tunstall) to induce microsomal monooxygenases and related enzyme systems and to study the relationships between these effects and tumor incidence in livers of male CF-1 mice. The effects of a CD and of bedding material, e.g., SB, were investigated, using animals maintained on SSD and FPB as controls. Dieldrin was used as a positive control.

MATERIALS AND METHODS

Animal Experiments

The experiment entailed 8 treatment groups: (a) SSD plus FPB; (b) SSD plus SB; (c) SSD plus FPB plus dieldrin (10 mg/kg diet); (d) SSD plus SB plus dieldrin (10 mg/kg diet); (e) CD plus FPB; (f) CD plus SB; (g) CD plus FPB plus dieldrin (10 mg/kg diet); and (h) CD plus SB plus dieldrin (10 mg/kg diet).

CF-1 mice were bred and reared on their specific experimental treatments in order to ensure continuous exposure during both the prenatal and postnatal periods. After weaning, males from the same treatment group were housed together (maximum, 5 mice/cage), and parent females and female offspring were discarded. The animals were housed in plastic cages approximately 30 x 13 x 12 cm with a wire mesh top (stainless steel) and a layer of SB or shredded FPB on the bottom. The cages were cleaned twice weekly. The temperature of the animal room was maintained at 20 ± 1°. Both food and water (supply from local mains) were offered ad libitum. Mice were killed at intervals of 15, 52, 65, and 92 weeks for biochemical and morphological investigations of their livers.

Liver Biochemistry

The animals were killed by cervical dislocation. The livers were quickly excised, the gall bladder was removed, and the tissue was chilled in ice-cold 0.25 M (isotonic) sucrose solution, pH 7.4, for a few min. The livers were weighed, and weighed samples of tissue (approximately 1 g) were pressed into a homogenizing tube and homogenized in approximately 6 to 7 ml of ice-cold isotonic sucrose solution (pH 7.4). Fifteen passes of the pestle were used at 1452 rpm. The final

volume of the homogenate was adjusted to 10% w/v by the addition of ice-cold isotonic sucrose solution (pH 7.4). Liver homogenates were fractionated to yield microsomal and soluble fractions (25).

Enzyme Assays

Monooxygenase. p-Nitroanisole-O demethylase activity was used to monitor monooxygenase activity in accord with the method of Netter and Seidel (13).

Epoxide Hydratase (EC 4.2.1.63). This activity was measured as described by Oesch et al. (14) using tritiated styrene oxide as a substrate.

Glutathione S-epoxide Transferase (EC 4.4.1.7). This activity was measured in the soluble fraction by the method of James et al. (10) except that tritiated styrene oxide substrate was used at a concentration of 16 mM.

UDP:Glucuronyltransferase (EC 2.4.1.17). This activity was measured by recording the rate of disappearance of p-nitrophenol in the presence of uridine:diphosphoglucuronic acid and TX-100 (membrane solubilizer) at 400 nm as described by Pogell and Krisman (19).

Chemical Assays

Protein. Homogenized tissue samples were diluted with distilled water to a protein concentration of approximately 100 μg/ml, and the protein content was determined according to the method of Lowry et al. (12).

Liver DNA. Homogenized tissue samples (2 ml) were washed 3 times with 10 ml 0.2 N perchloric acid at 0°. DNA was subsequently extracted in 2 × 3 ml 0.5 N perchloric acid by heating at 70° for 15 min. The DNA extract was adjusted to 10 ml with 0.5 N perchloric acid, and the DNA concentration was measured colorimetrically after reaction with diphenylamine as described by Burton (4).

Pathological Examination of Liver

The incidence of liver tumors was assessed in all treatment groups at the age of 65 weeks. A second assessment was carried out in the 4 non-dieldrin treatment groups after 91 to 92 weeks. All other mice except those taken for liver biochemistry were allowed to live their natural life span.

The animals were killed (if not found dead) by i.p. injection of 0.5 ml pentobarbitone:sodium solution (60 mg/ml). All organs were inspected for gross pathological changes, but only liver and lungs were examined histologically. The organs were fixed in 10% neutral formalin, and histological sections (5 μm) were made of all liver lobes, including sections of any gross pathological lesions. All lobes of the lung were sectioned. The sections were stained with hematoxylin and eosin.

Liver nodules were classified according to the method of Walker et al. (24) as adenomas (nodular growths of solid cords of parenchymal cells) and carcinomas (papilliform and adenoid growth with cells proliferating in confluent sheets with necrosis, increased mitoses, and sometimes associated metastases to the lungs).

Statistics

Results obtained in the various treatment groups were analyzed using Student's t test. Mice maintained on SSD and FPB were used as controls. The incidence of liver tumors in the various treatment groups was tested against that observed in mice maintained on SSD and FPB using Fisher's exact test (3).

Materials

Diets. The standard laboratory food was Laboratory Animal Diet 2 supplied by Spillers, Ltd., Newmarket, U. K. SSD was compounded in this laboratory (Tunstall) on the basis of the 1969 recommendations of the U. K. Laboratory Animals Association (8). The composition of this diet is shown in Table 1.

Table 1
Composition of semisynthetic diet

Component	Amount/100 g
Protein	
Casein	23.6 g
Carbohydrates	
Corn starch	46.7 g
Potato starch	10.0 g
Sucrose	5.0 g
Fat	
Corn oil	10.0 g
Vitamins	
Thiamine (B₁)	0.4 mg
Riboflavin (B₂)	0.5 mg
Pyridoxine-HCl (B₆)	0.6 mg
Nicotinic acid (niacin)	1.0 mg
Calcium pantothenate	1.2 mg
Biotin (H)	0.1 mg
Menapthone (K₃)	0.2 mg
α-Tocopherol (E)	7 IU
Vitamin D₃	100 IU
Vitamin A	500 IU
Cyanocobalamin (B₁₂)	2 μg
Choline chloride	100 mg
Minerals	
Calcium citrate	47.6 mg
Potassium dihydrogen phosphate	1.36 g
Magnesium carbonate	141 mg
Magnesium sulfate:7H₂O	338 mg
Manganous sulfate:4H₂O	18.4 mg
Zinc carbonate	4.0 mg
Ammonium ferric citrate	45.0 mg
Copper sulfate	2.6 mg
Sodium fluoride	0.5 mg
Potassium iodate	0.3 mg
Calcium hydrogen orthophosphate	1.05 g
Calcium carbonate	910 mg
Sodium chloride	700 mg

Bedding Materials. SB material was obtained from W. P. Ushers Ltd., London. This SB is derived predominantly from *Pseudotsuga* spp. (Douglas fir) grown in Scandinavian countries. Control bedding material was prepared by shredding Whatman No. 1 filter paper, supplied by Scientific Furnishings, Ltd., Chichester, U. K.

Chemicals. [7-³H]Styrene oxide (50 μCi/mmol) was purchased from the Radiochemical Centre, Amersham, U. K. Radiochemical purity was demonstrated by thin-layer chromatography on silica gel GF plates with authentic styrene oxide in 4 different solvent systems: benzene: chloroform (1:1, v/v); benzene:light petroleum (1:1, v/v); benzene: ethyl acetate:chloroform (1:1:1, v/v/v); and chloroform:light petroleum (1:1, v/v). Dieldrin, purity greater than 99%, was supplied by the Agricultural Chemicals Division, Shell Biosciences Laboratory, Sittingbourne, U. K. All reagents and solvents were of analytical reagent grade.

RESULTS

Effects on Body Weight, Liver Weight, Total Liver DNA, Liver Proteins, and Hepatocellular Enzymes Related to the Metabolism of Foreign Compounds. Exposure of CF-1 mice to dieldrin p.o. had no effect on body weight (Table 2). At the age of 15 weeks, mice fed on CD showed slightly higher body weights than those maintained on SSD, but this tendency disappeared with increasing duration of treatment (data not shown). Results obtained with 15-week-old CF-1 mice showed that the administration of 10 mg dieldrin per kg diet resulted in pronounced generalized liver enlargement, ranging from 37.5% in mice maintained on CD and FPB to 56.5% in mice maintained on SSD and SB (Table 2).

Total liver DNA was increased in all 4 dieldrin groups. These increases ranged from 25.8% in mice maintained on SSD and FPB to 36.8% in mice fed CD and maintained on SB. In the

Table 2

Body weight, relative liver weight, total liver DNA, liver protein:liver DNA quotients, and the activities of liver p-nitroanisole O-demethylase, epoxide hydratase, glutathione S-epoxide transferase, and UDP:glucuronyltransferase in 15-week-old male CF-1 mice (4 mice/treatment group).

Diet	Bedding	Dieldrin (mg/kg diet)	Body wt (g)	Relative liver wt (g liver/100 g body wt)	Total liver DNA (mg/100 g body wt)	mg liver protein/mg liver DNA	p-Nitroanisole O-demethylase (nmol p-nitrophenol/mg microsomal protein/min)	Epoxide hydratase (nmol styrene glycol/mg microsomal protein/min)	Glutathione transferase (nmol styrene oxide conjugated/mg soluble protein/min)	UDP:glucuronyltransferase (nmol p-nitrophenol conjugated/mg microsomal protein/min)
SSD	FPB	0	34.2 ± 0.8^a	4.2 ± 0.2	12.8 ± 0.5	75.5 ± 3.4	0.8 ± 0.1	1.5 ± 0.1	281 ± 12	1.1 ± 0.1
		10	35.4 ± 1.9	6.4 ± 0.2^b	16.1 ± 0.9^b	90.4 ± 6.2^c	6.2 ± 0.6^b	2.8 ± 0.8^d	617 ± 32^b	1.6 ± 0.2^d
SSD	SB	0	35.1 ± 0.6	4.6 ± 0.1^d	12.6 ± 0.6	82.3 ± 2.5^d	3.0 ± 0.4^c	1.8 ± 0.5	387 ± 66	1.2 ± 0.3
		10	33.8 ± 1.9	7.2 ± 0.4^b	16.4 ± 0.7^b	93.4 ± 2.8^b	6.9 ± 0.8^b	1.9 ± 0.3	660 ± 77^b	1.6 ± 0.04^c
CD	FPB	0	39.1 ± 1.7^c	4.8 ± 0.2^c	13.0 ± 0.5	83.9 ± 3.0^d	1.6 ± 0.4^d	1.5 ± 0.2	333 ± 8^b	1.4 ± 0.2
		10	36.4 ± 1.8	6.6 ± 0.6^b	17.1 ± 1.0^b	85.4 ± 8.9	4.9 ± 0.5^b	2.8 ± 0.3^c	634 ± 166^c	1.6 ± 0.02^c
CD	SB	0	35.8 ± 4.1	4.8 ± 0.2^c	12.5 ± 1.0	80.8 ± 3.8	1.9 ± 0.5^d	1.7 ± 0.3	360 ± 70	1.2 ± 0.4
		10	37.8 ± 1.7^c	7.0 ± 0.9^b	17.1 ± 2.5^d	92.2 ± 6.9^c	4.9 ± 1.0^b	3.0 ± 0.5^c	638 ± 98^b	1.7 ± 0.03^c

^{a} Mean ± S.D.
^{b} Significant difference from the control mean (SSD plus FPB plus 0 ppm dieldrin), $p < 0.001$.
^{c} Significant difference from the control mean (SSD plus FPB plus 0 ppm dieldrin), $p < 0.01$.
^{d} Significant difference from the control mean (SSD plus FPB plus 0 ppm dieldrin), $p < 0.05$.

case of animals maintained on CD, the increases in liver DNA were nearly proportional to the increases in liver weight. Small increases in liver protein:liver DNA quotients were also observed in dieldrin-treated animals (Table 2).

Liver enlargement induced by dieldrin was accompanied by the induction of drug-metabolizing enzymes, e.g., p-nitroanisole-O-demethylase, epoxide hydratase, glutathione-S-epoxide transferase, and UDP:glucuronyltransferase activity (Table 2).

In the case of non-dieldrin-treated mice and using data obtained in mice maintained on SSD and FPB as the baseline, the administration of CD to mice and exposure to SB, both singly and in combination, caused only marginal enlargement of the liver. No increases in total hepatic DNA were observed in these mice (Table 2). Induction of the liver monooxygenase system was noted. However, other drug-metabolizing enzymes appeared to be only very slightly increased by CD and SB (Table 2).

Biochemical investigations were also conducted in 52-, 65-, and 92-week-old mice (data not shown). The results of these experiments showed that the effects of dieldrin and other environmental factors (diet, bedding) on hepatocellular enzyme activities were very similar to those measured in 15-week-old CF-1 mice.

The development of liver tumors in some of the animals fed 10 mg dieldrin per kg diet necessitated the separation of normal (host) and liver tumor tissue for biochemical measurements. The results of these experiments (data not shown) demonstrated that the activities of drug-metabolizing enzymes in host liver tissues were similar to those measured in animals from the same treatment group but which had no apparent hepatic tumors. In contrast, hepatic tumor tissue exhibited slightly higher drug-metabolizing enzyme activities than did host liver tissue. Marked depressions of glucose-6-phosphatase activity and glutathione concentration were observed in hepatic tumor tissue.

Survival. Survival in each treatment group is shown in Chart 1. The data were adjusted for interim kills. The results demonstrate a significant reduction in the survival time of mice exposed to dieldrin.
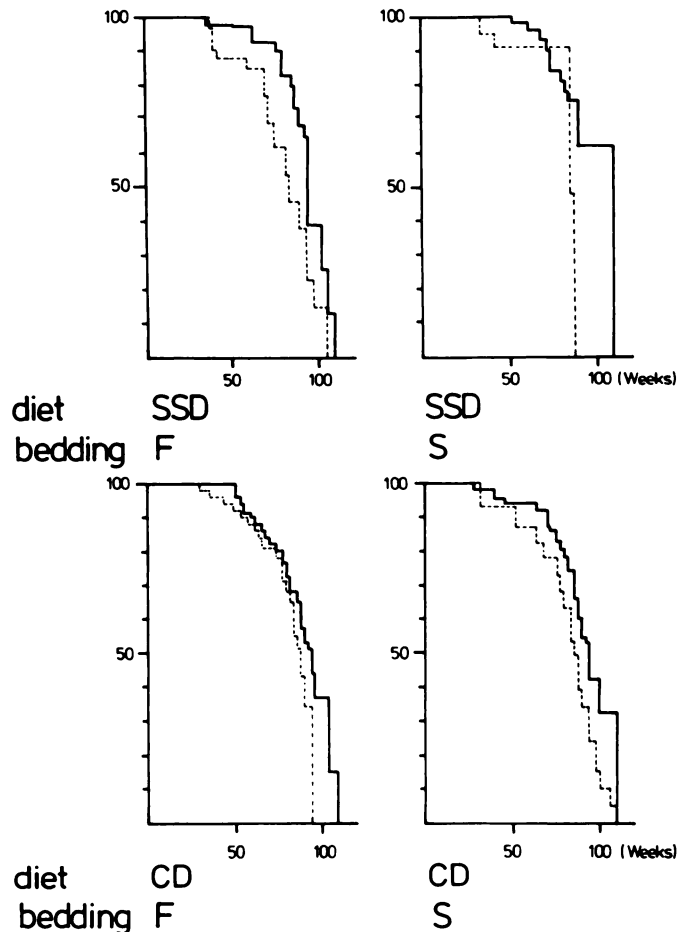
% SURVIVAL

Chart 1. Survival (adjusted for scheduled kills) against time. The experiment was terminated after 110 weeks. ——, 0 mg dieldrin per kg diet; - - - -, 10 mg dieldrin per kg diet. F, FPB; S, SB.

One-half of the animals receiving 10 mg dieldrin per kg diet survived at Week 86 (approximately 20 months). The corresponding interval, i.e., 50% survival, in non-dieldrin-treated

mice was 94 weeks (approximately 22 months). On average, 10% of dieldrin-treated mice survived to 100 weeks (approximately 23 months) compared with 40% of mice not exposed to dieldrin.

Liver Tumor Incidence. The administration of dieldrin to mice resulted in the relatively early appearance of nodular hepatic lesions, the first being observed in a mouse aged approximately 43 weeks. High incidences of liver tumors were

Table 3
Liver tumor incidence

| | Time period (wk) | | | | | | | | | | | |
| | 0–65 wk | | | | 65 (interim kill) wk | | | | 66–90 wk | | | |
	Total no. of mice	No. of mice with liver adenomas[a]	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis	Total no. of mice	No. of mice with liver adenomas	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis	Total no. of mice	No. of mice with liver adenomas	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis
SSD												
FPB												
0 mg dieldrin/kg diet	3	0	0	0	15	2	0	0	10	0	0	0
10 mg dieldrin/ kg diet	5	0	0	0	15	7	4	0	6	1	4[b]	1
SB												
0 mg dieldrin/kg diet	2	0	0	0	15	1	0	0	18	6	0	0
10 mg dieldrin/ kg diet	5	1	1	0	12	10[d]	2	0	2	2[b]	0	0
CD												
FPB												
0 mg dieldrin/kg diet	8	0	0	0	15	0	0	0	17	1	0	0
10 mg dieldrin/ kg diet	10	2	4	0	16	9[c]	4	0	21	10[c]	11[b]	8
SB												
0 mg dieldrin/kg diet	7	0	0	0	15	0	0	0	25	0	1	0
10 mg dieldrin/ kg diet	3	0	1	0	16	7	5[c]	1	13	4	9[b]	7

| | Time period (wk) | | | | | | | | | | | | |
| | 90–92 (interim kill) wk | | | | 92–110 wk | | | | 0–110 wk | | | | |
	Total no. of mice	No. of mice with liver adenomas	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis	Total no. of mice	No. of mice with liver adenomas	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis	Total no. of mice	No. of mice with liver adenomas	No. of mice with liver carcinomas	No. of mice with liver carcinomas showing lung metastasis	Liver tumor incidence (%)
SSD													
FPB													
0 mg dieldrin/kg diet	21	0	1	0	6	0	0	0	55	2	1	0	5.5
10 mg dieldrin/ kg diet					5	2	3	0	31	10	11	1	67.7
SB													
0 mg dieldrin/kg diet	11	4[c]	0	0	1	1	0	0	47	12	0	0	25.5
10 mg dieldrin/ kg diet									19	13	3	0	84.2
CD													
FPB													
0 mg dieldrin/kg diet	21	2	0	0	7	1	0	0	68	4	0	0	5.9
10 mg dieldrin/ kg diet					4	2	2	1	51	23	21	9	86.3
SB													
0 mg dieldrin/kg diet	29	5	0	0	6	0	0	0	82	5	1	0	7.3
10 mg dieldrin/ kg diet					6	2	4	1	38	13	19	9	84.2

[a] Mice with liver carcinomas frequently displayed liver adenomas, as well but have *not* been included in the columns summarizing liver adenoma incidence.
[b] Significance of the difference between control (SSD plus FPB plus 0 mg dieldrin per kg) and treatments, $p < 0.01$.
[c] Significance of the difference between control (SSD plus FPB plus 0 mg dieldrin per kg) and treatments, $p < 0.05$.
[d] Significance of the difference between control (SSD plus FPB plus 0 mg dieldrin per kg) and treatments, $p < 0.001$.

recorded in the dieldrin-treated mice at 65 weeks, including those maintained on SSD and FPB (Table 3). At this stage, the incidence of liver carcinomas in dieldrin-treated mice ranged from 16.7% (2 of 12) in animals maintained on SSD and SB to 31.3% (5 of 16) in mice on CD and SB. The low overall incidence of hepatocellular carcinomas in dieldrin-treated mice on SSD and SB is probably due to a shorter survival time, (all mice had died by 88 weeks). Morphological changes characteristic of hepatocellular carcinomas could be seen developing in the large adenomas. These observations indicate that benign tumors may progress towards a malignant state. More than 50% (18 of 33) of dieldrin-treated mice with hepatocellular carcinomas examined after the 65-week necropsy showed lung metastases (Table 3).

Liver tumors also occurred in mice from the nondieldrin groups. However, these tumors occurred much less frequently and were smaller than those observed in the dieldrin groups, being usually less than 5 mm in diameter. Most of these tumors (23 of 25) were classified as liver adenomas. Two tumors showed morphological characteristics of hepatocellular carcinomas (Table 3). Lung metastases were not observed in non-dieldrin-treated mice bearing liver carcinomas (Table 3). Towards the end of the study, non-dieldrin-treated mice on SSD and SB showed a significantly increased incidence of liver adenomas. No such contrast was observed between non-dieldrin-treated mice maintained on CD plus FPB and those on CD plus SB. The overall incidence of liver tumors in these latter treatment groups was very similar to that observed in control mice maintained on SSD plus FPB.

DISCUSSION

The main objective of this study was to ascertain whether microsomal enzyme inducers were present in CD for rodents and SB and to establish what effects such naturally occurring or adventitious agents may have on the incidence of liver tumors in CF-1 mice. The administration of 10 mg dieldrin per kg diet to some of the experimental groups served as a positive control and an aid to perspective. Using data from mice maintained on SSD and FPB as the baseline, the administration of CD and exposure to SB caused induction of the liver monooxygenase system. This effect was, however, far less pronounced than that observed in dieldrin-treated mice. Other drug-metabolizing enzyme activities were not or only very slightly increased by CD and SB.

The results of the current study indicate that spontaneous liver tumors may also occur in CF-1 mice bred, reared, and maintained on SSD and FPB. It would therefore appear unlikely that such spontaneous tumors are caused by exposure to environmental carcinogen(s) and it is postulated that these tumors are the expression of a preexisting oncogenic factor which is genetically linked and possibly viral in origin. Exposure of CF-1 mice to CD and SB did not result in a significant increase in the incidence of spontaneous liver tumors. This result suggests that naturally occurring or adventitious microsomal enzyme inducers have no intrinsic tumor-promoting activity or are present at concentrations below the threshold for overt tumor-promoting action. Interestingly, mice maintained on SSD and SB showed a significant increase in the incidence of liver adenomas toward the end of the study. However, relative risk analyses failed to demonstrate a significant in-

crease in risk of liver tumor development in mice exposed to SB (data not shown). Enhancing effects of SB on the incidence of spontaneous liver tumors in susceptible strains of mice have been reported previously (20, 21). However, in these experiments, this effect was associated with the use of cedar shavings (*Juniperus virginiana*), and control mice had been exposed to a type of bedding (Douglas fir) very similar to that used in the current study.

The incidence of liver tumors in the dieldrin treatment groups was very high, even in mice maintained on SSD and FPB. Longer-term exposure to dieldrin resulted in the development of hepatocellular carcinomas and lung metastases.

Two principal mechanisms can be envisaged by which microsomal enzyme inducers such as dieldrin could exert tumorigenic effects in livers of CF-1 mice. One possible mechanism of action could be that dieldrin may cause transformation of liver cells through somatic mutations or specific epigenetic effects. However, dieldrin and its metabolites gave entirely negative results when evaluated for mutagenic activity in a variety of test systems (2, 7). Studies have also been conducted on the binding of radioactivity to the liver cell DNA of rats and mice after exposure to [^{14}C]dieldrin *in vivo* (26). Although very small amounts of an unidentified biotransformation product were associated with DNA, there was no correlation between the extent of binding and susceptibility to tumor formation. It has also been demonstrated that acute exposure to very high doses of dieldrin does not cause DNA strand breakage in the livers of rats and mice (26).

An alternative mechanism could be that compounds, such as dieldrin, exert their tumorigenic action in mouse liver by facilitating the action of a potent environmental carcinogen. In this context, it is interesting to note that dietary phenobarbital and DDT can enhance 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat (15, 17, 18). The results of the current study, however, demonstrate that the tumorigenic effects of dieldrin are still manifest in mice fed on a SSD and maintained on a FPB, and it would therefore appear unlikely that dieldrin exerts its tumorigenic action by exacerbating the action of a potent environmental carcinogen.

The present data favor the concept that dieldrin facilitates or exacerbates the expression of an endogenous oncogenic factor in CF-1 mice. Such effects might possibly be brought about by the induction of liver enlargement and concomitant liver cell proliferation, *i.e.*, increases in total liver DNA, as seen in dieldrin-treated mice in this experiment.

Future studies should aim to substantiate and unravel a possible relationship between liver cell proliferation and tumor promotion in livers of susceptible animal species and the relevance of these effects to humans.

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