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THE CARCINOGENIC AND OTHER CHRONIC EFFECTS OF PERSISTENT HALOGENATED ORGANIC COMPOUNDS

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Renate D. Kimbrough

Toxicology Branch, Center for Disease Control
United States Public Health Service
Department of Health, Education, and Welfare
Atlanta, Georgia 30333

A number of persistent halogenated organic compounds have been shown to produce liver tumors in rodents, such as DDT, dieldrin, mirex, Kepone®, hexachlorobenzene, polychlorinated and polybromonated biphenyls (PCBs and PBBs) and now also 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) as well as hexachlorodibenzodioxin. TCDD also induced tumors of the lungs, hard palate or nasal turbinates in rats. It is possible that other isomers of the halogenated dibenzodioxins are also carcinogenic. Representatives of other groups of compounds in this category, the halogenated dibenzofurans, naphthalenes and terphenyls have not been tested for a carcinogenic effect. Most of the chemicals mentioned are persistent in mammals once they have been absorbed. They are also poorly degraded in the environment. For this class of compounds it is generally assumed that not the chemicals themselves but their metabolites are the proximate carcinogens. TCDD may be an exception. While most of the chemical compounds mentioned are commercial products, the halogenated dibenzodioxins and dibenzofurans are contaminants of a number of technical products. Some of these products that may theoretically be contaminated are listed in TABLE I.

The persistent halogenated organic compounds under discussion induce mixed-function oxidases and cause hepatomegaly in rodents and other mammals. The hepatomegaly is the result of the hypertrophy of individual hepatocytes. Some hyperplasia also occurs and increased mitotic activity can be noted. The hepatocytes may accumulate lipid in their cytoplasm. At the ultrastructural level the enlarged hepatocytes show an increase in smooth endoplasmic reticulum and inclusions within the cytoplasm, which appear like concentric whorls, surrounding lipid vacuoles. Morphologic changes in the mitochondria have also been described (Gaines *et al.*[1]; Kimbrough *et al.*[2]). In addition to these alterations, the PCBs, PBBs, some isomers of the halogenated dibenzodioxins as well as hexachlorobenzene induce experimental hepatic porphyria. In the rat experimental hepatic porphyria only occurs in the female, but in other species it can be induced in both sexes. On microscopic examination an increase in macrophages and prominent Kupffer cells containing brown ceroid pigment and necrobiosis of liver cells is prominent. Lipid accumulates in the cytoplasm of hepatocytes resulting at times in hepatocytes with foamy cytoplasm. Similarly, lipid accumulation, lipofuchsin (ceroid pigment) have also been reported in livers of humans suffering from porphyria cutanea tarda (Bickpica *et al.*[3]).

Adenofibrosis (cholangiofibrosis) (Kimbrough *et al.*[2]) has been observed in rats fed PCBs and technical but not pure pentachlorophenol (Kimbrough and Linder[4]). The PCBs were contaminated with trace amounts of chlorinated dibenzofurans and the pentachlorophenol with chlorinated dibenzodioxins and dibenzofurans. The significance of adenofibrosis and its relationship to the development of hepatocellular carcinomas is presently not understood. It usually occurs concomitantly with

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hepatocellular carcinoma in rodents (Kimbrough and Linder[5], Kimbrough *et al.*[6]) and has also been described in rats exposed to other carcinogens, such as *p*-dimethylaminoazobenzene (Edwards and White[8]). Although hepatocellular tumors have also been reported in rodents after exposure to DDT, dieldrin, mirex, and Kepone, these compounds do not produce the same type of liver pathology as outlined above. It is possible that PCBs, PBBs and related compounds cause lipid peroxidation while mirex, Kepone, DDT, and dieldrin do not. Whether the experimental hepatic porphyria produced by these compounds is in any way related to the induction of hepatocellular carcinoma is presently not known. A study reported by Jones and Sweeney[9] is interesting in this regard. These authors treated genetically responsive (DBA/2J) or nonresponsive (DBA/2J) mice to the induction of aryl-hydrocarbon hydroxylase with TCDD. The nonresponsive mice had normal hepatic uroporphyrinogen decarboxylase activity while it was decreased in the responsive mice, and uroporphyrin excretion was increased in this group. In the study of Kociba *et al.* presented in this volume, only the female rats showed hepatic porphyria, and they also developed hepatocellular carcinomas while the male rats had neither. It should be explored whether an effect on certain enzymes related to porphyrin metabolism is coupled with an effect on enzymes somehow related to the induction of hepatocellular

TABLE 1

EXAMPLES OF COMMERCIAL PRODUCTS THAT MAY BE CONTAMINATED WITH HALOGENATED DIBENZODIOXINS OR DIBENZOFURANS

2,4,5-Trichlorophenol	Trichlorphos (tronoch)
Pentachlorophenol	Ureon
Hexachlorobenzene	Trichloronate
2,4-Dichlorophenyl-*p*-nitrophenyl ether (TOK)	Hexachlorophene
2,4,5-Trichlorophenoxyacetic acid	2,4,4'-Trichloro-2'-hydroxydiphenyl ether (Irgasan DP300)
Lenoprop (Silvex)	Polychlorinated biphenyls

carcinomas, or whether hepatic porphyria and hepatocellular carcinoma only coexist.

Hepatic porphyria (porphyria cutanea tarda) has also been reported in humans following occupational exposure to some of these chemicals and porphyrin excretion should be screened for carefully in such cases in the future. The sex difference for the induction of hepatic porphyria found in rats does not exist in humans. The PCBs, TCDD and perhaps also the PBBs affect reproduction. The reduction of hormone-dependent tumors in the study by Kociba *et al.* may be indicative of an influence of these compounds on steroids. Other halogenated persistent organic compounds also affect endocrine glands. Kepone has been shown to cause hyperplasia of the adrenal cortex in rats (Cannon and Kimbrough[10]) and in quail (Heinz and Wilson[11]). High doses of technical hexachlorobenzene also caused hyperplasia of the adrenal cortex in rats (Kimbrough and Linder[5]).

All of the halogenated organic compounds that I have mentioned are persistent in biologic systems. Certain isomers of PBBs and PCBs seem to be retained indefinitely (Kimbrough *et al.*[12]). If a single high dose of a mixture of PBBs is given to weanling rats, hepatocellular tumors can be induced. It is therefore not necessary to conduct 2-year feeding studies with these compounds to show that they are carcinogenic. A single high dose at least of the PBBs will have the same effect. When rats were given

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1 p PBB/kg body weight by stomach tube as weanlings, neoplastic nodules began to appear at about 6 months after dosing (Kimbrough *et al.*[7]). It was also found that PBB levels in the liver did not regress. It is presently not known whether the isomers that persist are the ones that are carcinogenic. If the persistent isomers prove to be carcinogenic, then the tumor induction would be due to the continuous internal exposure of the target organ to these isomers.

Neoplastic nodules are considered to represent part of the spectrum of response elicited by hepatocarcinogens in rodents. First, areas of alteration will develop, some of these will become neoplastic nodules and some of the neoplastic nodules will in time transform into hepatocellular carcinomas (Squire and Lewit[14]). A number of carcinogens have produced this same spectrum of response and there is no difference in the response, whether the chemical producing it is a carcinogen of long standing or whether it is one of the halogenated compounds discussed here. Although it has been shown many times that the neoplastic nodules are part of the carcinogenic response they are not always included in the statistical evaluation of bioassays, which may lead to erroneously interpreted results, particularly when they are classified as hyperplastic nodules or "nodular hyperplasia" and when the number of animals studied was small (Carcinogenesis Testing Program, 1977[3]).

Since all of these compounds are also inducers of mixed function oxidases and would enhance the metabolism of other xenobiotics, they may, depending on the circumstances, prevent or enhance the tumor induction by other chemicals. Active metabolites would have to be formed for many of the halogenated compounds; tetrachlorodibenzo-*p*-dioxin perhaps being an exception. The dermal application of these compounds that have to be activated would therefore not necessarily result in tumor formation. Further studies will have to be done to elucidate these problems. The question of skin carcinogenesis is particularly important in workers that have had occupational dermal exposure to TCDD, PCBs or chlorinated naphthalenes as evidenced by chloracne.

References

1. Gaines, T. B. & R. D. Kimbrough. 1970. Oral toxicity of mirex in adult and suckling rats. Arch. Environ. Health **21**: 7–14.
2. Kimbrough, R. D., T. B. Gaines & R. E. Linder. 1971. The ultrastructure of livers of rats fed DDT and dieldrin. Arch. Environ. Health **23**: 460–467.
3. Kimbrough, R. D., R. E. Linder & T. B. Gaines. 1972. Morphological changes in liver of rats fed polychlorinated biphenyls. Arch. Environ. Health **25**: 354–363.
4. Bickers, J., N. Kosower, M. H. May & S. Goldfischer. 1974. Hepatic porphyrias, cytochemical and ultrastructural studies of liver in acute intermittent porphyria and porphyria cutanea tarda. Arch. Pathol. **98**: 336–343.
5. Kimbrough, R. D. & R. J. Linder. 1978. The effect of technical and purified pentachlorophenol on the rat liver. Toxicol. Appl. Pharmacol. **46**: 151–162.
6. Kimbrough, R. D. & R. E. Linder. 1974. Induction of adenofibrosis and hepatomas of the liver in Balb/cd mice by polychlorinated biphenyl (Aroclor 1254). J. Nat. Cancer Inst. **53**: 547–552.
7. Kimbrough, R. D., R. A. Squire, R. E. Linder, J. D. Strandberg, R. J. Montali & V. W. Burse. 1975. Induction of liver tumors in Sherman Strain female rats by polychlorinated biphenyl Aroclor 1260. J. Nat. Cancer Inst. **55**: 1453–1459.
8. Edwards, J. F. & J. Wirtz. 1941. Pathologic changes with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). J. Nat. Cancer Inst. **2**: 157–183.
9. Jones, K. G. & G. D. Swezey. 1977. Association between induction of aryl hydrocarbon

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hydroxylase and depression of uroporphyrinogen decarboxylase activity. Res. Commun. Chem. Pathol. Pharmacol. 17: 631–637.

10 CASSEDY, S. B. & R. D. KIMBROUGH. Short-term Kepone toxicity in rats, including reproduction, organ pathology and reversibility. Toxicol. Appl. Pharmacol. In press.

11 EROSHENKO, V. P. & W. O. WILSON. 1975. Cellular changes in the gonads, livers, and adrenal glands of Japanese quail as affected by the insecticide Kepone. Toxicol. Appl. Pharmacol. 31: 491–504.

12 KIMBROUGH, R. D. & R. E. LINDER. 1974. The toxicity of technical hexachlorobenzene in the Sherman strain rat. A preliminary study. Res. Commun. Chem. Pathol. Pharmacol. 8: 653–664.

13 KIMBROUGH, R. D., V. W. BURSE & J. A. LIDDLE. 1978. Persistent liver lesions in rats after a single oral dose of polybrominated biphenyls (Fire Master FF-1) and concomitant PBB tissue levels. Environ. Health Perspect. 23: 265–273.

14 SQUIRE, R. A. & M. H. LEVITT. 1975. Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Res. 35: 3214–3223.

15 Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institute of Health, Bethesda, Md. 20014. October 20, 1977. Bioassay of Aroclor 1254 for possible carcinogenicity.

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STUDIES WITH CHLORINATED DIBENZO-*p*-DIOXINS, POLYBROMINATED BIPHENYLS, AND POLYCHLORINATED BIPHENYLS IN A TWO-STAGE SYSTEM OF MOUSE SKIN TUMORIGENESIS. POTENT ANTICARCINOGENIC EFFECTS*

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David L. Berry, Thomas J. Slaga, John DiGiovanni,† and Mont R. Juchau†

Cancer and Toxicology Program
Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

†Department of Pharmacology, School of Medicine
University of Washington
Seattle, Washington 98195

The chlorinated dibenzo-*p*-dioxins and chlorinated dibenzo-furans are among the most potent teratogens and toxins that are found as environmental contaminants.[1] The polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) are not nearly as toxic as the dioxins but they are environmentally more prevalent than the dioxins.[2,3] Both classes of compounds are inducers of the cytochrome P_{450}-mediated microsomal monooxygenases[4-8] and of δ-aminolevulinic acid synthetase.[7,9] The dioxins induce aryl hydrocarbon hydroxylase (AHH, E.C. 1.14.14.2), the enzyme system responsible for converting polycyclic aromatic hydrocarbons (PAH) to active and inactive metabolites, much the same as 3-methylcholanthrene (3-MC) but for periods up to 35 days after a single i.p. injection and at much lower doses.[7,10] PCBs and PBBs are mixtures of various isomers and induce the monooxygenase system with properties similar to both phenobarbital and 3-MC but the duration of induction is much shorter than with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).[7,9,11] The toxicologic response is similar for both classes of compounds in that they are hepatotoxic,[6,12-14] cause a decreased growth rate[13,15] and produce chloracne in test animals and man.[11,16]

The effects of PCBs on carcinogenicity of various chemicals have been investigated by numerous groups. Kanechlor-500 in combination with several hepatocarcinogens (3'methyl-4-dimethylaminoazobenzene, *N*-2-fluorenylacetamide and diethylnitrosamine) in the diets of rats markedly decreased the formation of hepatocarcinomas.[17] Kimura *et al.*[18] demonstrated that pretreatment with Kanechlor-400 in diets of rats 4 months prior to and 2 months during treatment with 3'methyl-4-dimethylaminoazobenzene protected the rats against the formation of hepatocarcinomas induced by this carcinogen. Treatment of animals with the organochlorine insecticide 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane (DDT) has been shown to inhibit 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors.[19] These findings as well as other reports[20,21] suggest that in terms of effects on carcinogenesis, timing is important in the application of PCB to the diets of laboratory animals. Treatment with the PCBs prior to the administration of carcinogen results in

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reduction of the tumor response whereas treatment subsequent to application of the carcinogen results in enhancement of the tumorigenic response. The inhibitory effects of the PCBs in these studies were attributed to the induction of hepatic microsomal enzymes while the enhancement was ascribed to the promoting properties of the PCBs.

The two-stage system of mouse skin tumorigenesis allows one to evaluate critically the initiation and promotion phases of carcinogenesis individually.[4-7] The initiation phase requires a single subthreshold dose of a polycyclic aromatic hydrocarbon and is irreversible, while the promotion stage requires *multiple applications of a promoter* and is reversible up to a given time point.[6,7] This system allows one to study the *effects of modifiers on initiation and promotion* separately in a skin carcinogenesis assay. The results presented here demonstrate that certain environmental chemicals (PCBs, PBBs, and TCDD) possess the capacity to modify tumor initiation in a mouse skin assay. At the doses utilized, these chemicals exhibited little or no initiating or promoting properties.

Materials and Methods

Animals

Female CD-1 mice were purchased from the Charles River Mouse Farms (North Wilmington, MA). Mice 6 to 8 weeks old were carefully shaved with surgical clippers 2 days *prior* to treatment, and only those mice in the resting phase of the hair cycle were used for the biochemical and tumor experiments. Animals were housed in plastic cages, received water and food *ad libitum*, and were weighed weekly.

Chemicals

DMBA was purchased from Sigma Chemical Company (St. Louis, MO). Benzo(a)pyrene (BaP) was supplied by the NCI Carcinogenesis Research Program (Bethesda, MD). The phorbol diester promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) was purchased from Dr. P. Borchert, University of Minnesota, Minneapolis, MN. PCB (Aroclor 1254) was purchased from Analabs, Inc. (North Haven, CT). PBB was supplied by A. C. Kolbye, Jr. of the FDA. TCDD (Lot #851-144-2) was generously supplied by the Dow Chemical Company (Midland, MI). A Poland generously supplied samples of 2,7-dichlorodibenzo *p* dioxin (DCDD) and 3,4,3,4-tetrachloroazobenzene (TCAB).

Tumor Induction Experiments

Each experimental group consisted of 30 pre-shaved mice. DMBA (200 nmol) was dissolved in acetone immediately before use, and 0.2 ml of the acetone solution was applied topically to the shaved area of the back. One week after DMBA initiation, a control group received application twice weekly of TPA (10 μg) in 0.2 ml acetone. The following test compounds: PBB (100 μg); PCB (100 μg) and TCDD (0.1 μg) were applied twice weekly topically in acetone to the shaved dorsal skin area of the DMBA-initiated mice. Promotion was continued for 30 weeks, when the experiments were terminated. In another set of animal experiments, animals were pretreated with PCB (100 μg and 625 μg) 18 hours prior to initiation; TCDD (1.0 μg) at 1, 3 and 5

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days prior to initiation, DCDD (100 μg) 3 days prior to initiation; and TCAB (10 μg) 3 days prior to initiation. The animals were initiated with 10 nmol DMBA and promoted with TPA. Incidences of both papillomas and carcinomas were observed weekly and papillomas and carcinomas were removed at random for histopathologic verification. Initial histologic experiments were performed with PCBs, PBBs, and TCDD to assess toxicity and changes in intrafollicular epidermis prior to dose selection for the experiments described.

Enzyme Induction by PCBs and TCDD

In order to induce epidermal monooxygenase systems functional in the oxidative biotransformation of DMBA, mice were pretreated with single doses of the following inducing agents: 100 μg of PCB 18 hours prior to sacrifice, 200 nmol of DMBA, dibenz[a,c]anthracene or 3-methylcholanthrene 18 hours prior to sacrifice and 1 μg of TCDD 72 hours prior to sacrifice. High-pressure liquid chromatography was utilized to assess the profiles of metabolites of ^{14}C-DMBA. Details of the methodology have been published by DiGiovanni *et al.*[7] To further assess the bioactivation process, binding to epidermal protein, RNA and DNA by ^{3}H-DMBA was measured using the method of Huberman and Sachs.[9]

Results

Visual and histopathologic examination of mouse skin treated with TPA at 2 μg per mouse indicated marked inflammation and an increase in the number of layers in the intrafollicular epidermis from 1 to between 4 and 5 layers. PCBs and PBBs at either 100 μg or 625 μg per mouse did not affect the intrafollicular epidermis. TCDD at 1 μg per mouse increased the numbers of cells in the intrafollicular epidermis but the effect was not pronounced. DCDD or TCAB did not affect the thickening of the epidermis as compared to the strong hyperplastic response of TPA.

The chlorinated dioxins and biphenyls were tested for promoting activity in the mouse skin with a high (200 nmol) initiating dose of DMBA. In a 30-week treatment period, the normal DMBA-initiated, TPA-promoted controls yielded approximately 8 papillomas per mouse (Table 1). The two biphenyls (at doses of 100 μg/mouse given twice weekly) did not promote the development of skin tumors nor did TCDD at a dose of 0.1 μg twice weekly. When tested without DMBA initiation, neither the dioxin nor the biphenyls demonstrated any carcinogenic activity. By the end of 30 weeks of treatment, the dioxin-treated mice showed a reddening of the skin as well as ear lesions resembling acne. The biphenyls, however, did not produce any observable skin lesions.

A series of biphenyl- and dioxin-related inducers of aryl hydrocarbon hydroxylase were tested for their ability to act as tumor initiators or for their capacity to modify the initiating activity of DMBA (see Table 2). Aroclor 1254 and TCDD were very weak initiators as was shown previously by DiGiovanni *et al.*[7] Aroclor 1254 exhibited no effect on tumorigenesis when given 5 minutes prior to an initiating dose of DMBA. Aroclor 1254 given at 18 hours and 72 hours prior to administration of DMBA inhibited initiation by as much as 45% in a time-dependent fashion. The much stronger monooxygenase inducer, TCDD (at 1 μg) decreased DMBA initiation by 93% when applied 3 days prior to initiation. The less active DCDD inhibited DMBA initiation only slightly. A close structural analogue of the dioxins, 3,4,5,4'-tetrachloroazobenzene,[7] which is much less potent than TCDD in terms of AHH

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TABLE 1*

LACK OF PROMOTING ABILITY OF PCB, PBB, AND TCDD ON SKIN-TUMOR INITIATION WITH DMBA†

Initiator‡	Promoter	Dose§	Papillomas per Mouse	Percent with Papillomas
DMBA	None		0	0
None	TPA	10 μg¶	0.03	3
DMBA	TPA	2 μg	8.1	92
DMBA	PCB	100 μg	0	0
None	PCB	100 μg	0	0
DMBA	PBB	100 μg	0	0
None	PBB	100 μg	0	0
DMBA	TCDD	0.1 μg	0	0
None	TCDD	0.1 μg	0	0

*Data taken from Res. Commun. Chem. Pathol. Pharmacol. 20: 101–107 (1978).
†30 female mice per group; promoted for 30 weeks.
‡DMBA was applied at a dose of 200 nmol per mouse.
§Promoter applied twice weekly in acetone.
¶TPA applied weekly in acetone.

induction in the mouse, slightly enhanced DMBA initiation at a dose of 10 μg given 3 days prior to initiation.

To further study the inhibitory effects of the dioxins on PAH initiation, a series of tumor experiments were conducted using DBMA or BaP as initiators. As shown in TABLE 3, pretreatment times of 1, 3 and 5 days (at a dose of 1 μg per mouse) resulted in a 94% inhibition of the tumor response to DMBA. To test the generality of the inhibition, the same experimental design was used with BaP as the initiator. A similar inhibitory action of TCDD on BaP tumor-initiation was also observed (unpublished observations). With BaP as the initiator, the dependence on the time of pretreatment with TCDD was similar to that observed with DMBA as the initiator. The maximum

TABLE 2

EFFECTS OF VARIOUS ORGANOHALIDES ON SKIN-TUMOR INITIATION BY DMBA
(Pretreated mice were initiated with 2.56 μg of DMBA and promoted with twice weekly applications of 10 μg TPA*)

Experiment	Pretreatment (μg/mouse)	Pretreatment Time	Papillomas per Mouse†	Percent of Control‡
1	Acetone	5 min	3.80	100
2	Aroclor 1254 (100)	5 min	3.60	95
3	Aroclor 1254 (100)	18 hr	2.10	55
4	Aroclor 1254 (100)	3 days	3.30	80
5	Aroclor 1254 (625)	18 hr	2.70	71
6	DCDD (100)	3 days	3.44	91
7	TCAB (10)	3 days	4.73	125
8	TCDD (1)	3 days	0.34	7

*30 mice were used per experimental group. Promotion was continued for 20 weeks.
†Average number of papillomas per mouse after 20 weeks of promotion.
‡The average number of papillomas per mouse expressed as a percentage of the DMBA-initiated, TPA-promoted group (experiment 1).

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inhibition of tumor formation occurred with pretreatment at 5 days prior to initiation, but the inhibitory response of TCDD pretreatment on BaP initiation was less than that observed with DMBA as the initiator. In all cases where the animals received 1 μg of TCDD, the weight gain, morphologic and histologic characteristics of the skin were normal. At topical doses of 2 μg, toxicity was observed and approximately one-third of the mice died at the end of the 20-week period.

To further show that the inhibition of PAH initiation by TCDD was related to the induction of the epidermal monooxygenase system, a dose-response study was designed. As illustrated above, maximum inhibition was observed at 3 to 5 days prior to DMBA initiation. As shown in TABLE 3, TCDD, in a dose-dependent fashion, inhibited skin-tumor initiation by DMBA. At doses of 0.01 μg, the inhibition of DMBA-initiation was approximately 80%, whereas 0.1 μg of TCDD was almost as effective as 1.0 μg. At this writing, these experiments are only in the 15th week and the

TABLE 3

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON SKIN-TUMOR INITIATION BY DMBA

(Pretreated mice were initiated with 2.56 μg of DMBA and promoted with twice weekly applications of 10 μg of TPA *)

Experiment	Pretreatment (μg/mouse)	Pretreatment Time	Papillomas per Mouse†	Percent of Control‡
1	Acetone	5 min	3.80	100
2	TCDD (5)	5 min	3.70	97
3	TCDD (1)	1 day	0.53	14
4	TCDD (1)	3 days	0.34	9
5	TCDD (1)	5 days	0.23	6
6	Acetone	5 min	2.43	100
7	TCDD (0.01)	3 days	0.27	11
8	TCDD (0.10)	3 days	0.10	4
9	TCDD (2.0)	3 days	0.07	3

*30 mice were used per experimental group. Promotion was continued for 20 weeks for experiments 1, 5, and 15 weeks for experiments 6–9. Promotion is still in progress for both groups of experiments.

†Average number of papillomas per mouse after 15 or 20 weeks of promotion.

‡The average number of papillomas per mouse expressed as a percentage of the DMBA-initiated, TPA-promoted groups for each period of promotion (i.e., experiments 1 and 6).

animals have not yet reached plateau tumor levels. The trend is, however, readily apparent.

FIGURE 1 shows high-pressure liquid chromatographic profiles of metabolites formed upon incubation of ^{14}C-DMBA with epidermal homogenates from inducer-pretreated CD-1 mice. The doses of TCDD and Aroclor 1254 utilized and the pretreatment times were identical to the conditions employed in the tumor experiments in which maximal inhibition of tumor initiation was observed. These high-pressure liquid chromatographic profiles demonstrate that chlorinated hydrocarbons such as Aroclor 1254 and TCDD are capable of inducing epidermal monooxygenases that are responsible for converting DMBA to a variety of hydroxylated products. Furthermore, a good correlation between the increased rates of oxidative metabolism induced by Aroclor 1254 and TCDD and the inhibition of DMBA tumor-initiation was observed.

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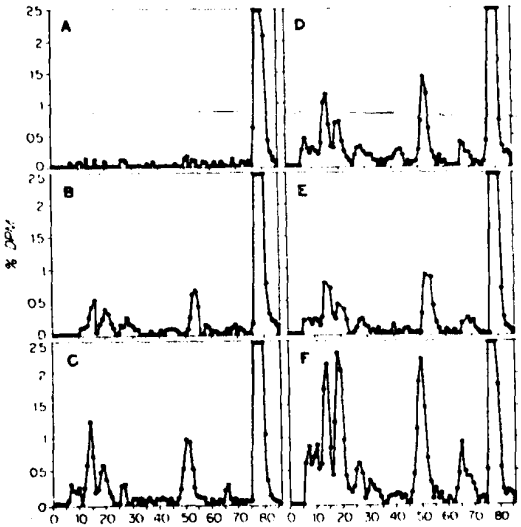

Figure 1. High pressure liquid chromatographic profiles of [^{3}H]-DMBA *metabolites generated in vitro using epidermal homogenates from various pretreated, female CD-1 mice: Panel A: heat inactivated control; panel B,* DMBA-pretreatment (200 nmol 18 hr prior to sacrifice); *panel C,* 3-methylcholanthrene pretreatment (200 nmol 18 hr prior to sacrifice); *panel D* dibenz(a,c)anthracene pretreatment (200 nmol 18 hr prior to sacrifice); *panel E* Aroclor 1254-pretreatment (100 μg 18 hr prior to sacrifice); and *panel F,* TCDD pretreatment (1 μg, 72 hr prior to sacrifice). All profiles have been normalized with respect to protein concentration. The peak fractions in which various synthetic standard compounds elute in this system are as follows: DMBA, 78; 5-hydroxy-DMBA, 72; 4-hydroxy-DMBA, 70; 2-hydroxy- and 3-hydroxy-DMBA, 61; 12-hydroxymethyl-7-methylbenz(a)anthracene, 57; 7-hydroxymethyl-12-methylbenz(a)anthracene, 52; 5,6-dihydro-5,6-dihydroxy-DMBA, 22; 7,12-dihydroxymethylbenz(a)anthracene 19; 8,9-dihydro-8,9-dihydroxy-DMBA, 13. Further details of the methodology can be found in reference 27.

To further test the possibility that the effects of TCDD on DMBA-mediated tumor initiation were due to changes in metabolism of the procarcinogen, a series of experiments were designed to monitor covalent binding of ^{3}H-DMBA to epidermal DNA, RNA and protein (TABLE 4). TCDD was applied topically to the shaved backs of CD-1 mice (1 μg per mouse) 3 days prior to application of 2.56 μg ^{3}H-DMBA (10

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μCi). Control animals received acetone alone. At 3 and 24 hours after treatment, epidermal DNA, RNA and protein were extracted using the method of Huberman and Sachs.[30] TCDD pretreatment reduced the binding of ^{3}H-DMBA to DNA at 3 and 24 hours respectively by 63% and 72% (TABLE 4). Similar results were observed with respect to covalent binding of ^{3}H-DMBA to RNA although the magnitude was less. However, the binding of ^{3}H-DMBA to epidermal protein was not affected by TCDD pretreatment.

DISCUSSION

TCDD and Aroclor 1254 previously were shown to possess little or no tumor-initiating properties[31] or tumor-promoting properties[32] in mouse skin using the two-stage, initiation-promotion system of carcinogenesis. Previous studies by Kociba[1] and Van Miller[2] indicated that TCDD was carcinogenic in the rat; however, this carcinogenic effect appeared to be related mainly to the promotion of spontaneously occurring tumors. Furthermore, with a hepatic system utilizing an initiation-promotion regimen,[33,34] PCBs appeared to act as promoters.

This report demonstrates that TCDD possesses remarkable inhibitory actions on skin tumor initiation by polycyclic aromatic hydrocarbons (PAH). Almost complete inhibition of DMBA tumor initiation is achieved with a single nontoxic topical dose of 0.1 *μg* and with a dose of 0.01 *μg* approximately 80% inhibition was achieved. This potent anticarcinogenic effect may be related to the ability of TCDD to induce epidermal enzyme pathways responsible for detoxifying PAH carcinogens in the skin. Induction of microsomal enzymes in several tissues has been postulated as a mechanism for the anticarcinogenic effects of a wide variety of compounds such as PAH, flavones, coumarins, phenothiazines, and phenobarbital as reviewed by Wattenberg.[35] Induction of microsomal enzyme systems also has been implicated in the anticarcinogenic activity of DDT[36] and various polychlorinated biphenyl mixtures.[37] However, investigations such as these have not demonstrated conclusively that the inhibitory actions are a result of induction within the target tissue for a particular carcinogen or that the effect is on tumor-initiation *per se*.

The unusually potent inhibitory action of TCDD on skin tumor-initiation by PAH described in this report stands in contrast to a report by Komri[38] who showed an enhancing effect of TCDD on the formation of 3-MC-induced sarcomas at the site of subcutaneous injection. Both compounds were administered simultaneously and the

TABLE 4

COVALENT BINDING OF ^{3}H-DMBA TO MOUSE EPIDERMAL MACROMOLECULES AS MODIFIED BY PRETREATMENT WITH TCDD

Treatment	Time (Hr)*	Hydrocarbon bound to macromolecule (fmoles, mpi)†		
		DNA	RNA	Protein
DMBA	3	48	29	698
DMBA + TCDD‡	3	18	16	638
DMBA	24	176	72	888
DMBA + TCDD‡	24	48	32	894

*Time of sacrifice after application of ^{3}H-DMBA; 40 mice were used for each experimental group.

†Mice in this group received 1 μg of TCDD, applied topically, 72 hours prior to application of ^{3}H-DMBA.

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increased tumor formation was attributed to the ability of TCDD to enhance the metabolism of 3-MC *in vivo*. In view of the time of administration, this explanation seems somewhat unlikely. Furthermore, as shown in TABLE 3, when TCDD was applied 5 minutes prior to initiation with DMBA, little or no effect on the tumor response was observed.

The anticarcinogenic effects of TCDD and Aroclor 1254 appear to correlate well with their ability to induce monooxygenase enzymes of the skin. Furthermore, the quantity of ^{3}H-DMBA bound to DNA and RNA (but not protein) in the presence and absence of TCDD-pretreatment correlated well with the tumor response under similar conditions. When taken together, these experiments suggest that pretreatment with TCDD gives rise to an increased rate of inactivation of the DMBA molecule relative to the rate of activation in mouse skin. Although these data point to induction of oxidative biotransformation as a possible mechanism for the inhibitory effects exhibited by TCDD, other mechanisms could be operating. Possibilities include induction in epidermal tissues of nonoxidative metabolic pathways such as epoxide hydratase, glutathione-S-transferase, UDP-glucuronosyltransferase and others. Other possibilities include effects on DNA-repair systems and on the distribution of the carcinogen to the critical target site(s).

It is deemed possible that future research will enable the development of compounds that evoke minimal toxicity but retain the capacity to inhibit tumor initiation by virtue of their enzyme-inducing properties. Of particular advantage would be the production of chemicals capable of selectively inducing various inactivating enzymes in particular target tissues. Such chemicals could feasibly serve as important chemopreventive agents in cases in which contact with tumor initiators is necessary or unavoidable.

REFERENCES

1. POLAND, A. & A. KENDE. 1976. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: environmental contaminant and molecular probe. Fed. Proc. 35: 2401–2411.
2. POLAND, A. & E. GLOVER. 1977. Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: a study of the structure-activity relationship. Mol. Pharmacol. 13: 924–938.
3. KOUBA, R. J., D. G. KEYES, J. E. BEYER, R. M. CARLSON, C. F. WADE, D. A. DITTENBER, R. P. KENNAN, L. F. HANSON, C. N. PARKE, S. D. BARNARD, R. A. HUMISTON & C. G. HUMISTON. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicol. Appl. Pharmacol. In press.
4. MCKINNEY, J. D., K. CHAE, B. N. GUPTA, J. A. MOORE & J. A. GOLDSTEIN. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. Toxicol. Appl. Pharmacol. 36: 65–80.
5. GOLDSTEIN, J. A., J. D. MCKINNEY, G. W. LUCIER, P. HICKMAN, H. BERGMAN & J. A. MOORE. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. II. Effects on drug metabolism and porphyrin accumulation. Toxicol. Appl. Pharmacol. 36: 81–92.
6. BICKERS, D. R., J. C. HARBER, A. KAPPAS & A. R. ALVARES. 1972. Polychlorinated biphenyls. Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. Res. Commun. Chem. Pathol. Pharmacol. 3: 505–512.
7. BICKERS, D. R., A. KAPPAS & A. P. ALVARES. 1974. Differences in inducibility of cutaneous and hepatic drug metabolizing enzymes and cytochrome P-450 by polychlorinated biphenyl and 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane (DDT). J. Pharmacol. Exp. Therap. 188: 301–309.
8. MARUMO, T., M. NOMAKAMI, H. VAINIO & K. J. W. HARTIALA. 1977. Stimulation of hepatic ch... H. Kuplen... 9. VAINIO, H. ... liver by ... ... 10. POPLACK, ... poison and ... Pharmacol. 11. GOLDSTEIN, ... Separation ... Res. Commun. 12. GUPTA, B. N. ... effects of ... Perspect. 13. ... 14. ... 15. ... 16. ... 17. ... 18. ... 19. ... 20. ... 21. ... 22. ... 23. ... 24. ... 25. ... 26. ... 27. ... 28. ... 29. ...

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hepatic drug hydroxylation and conjugation by a cutaneously applied PCB mixture (Clophen A50). Chem. Biol. Interactions 18: 247–251.

9. Vaino, H. 1974. Enhancement of microsomal drug oxidation and glucuronidation in rat liver by an environmental chemical, polychlorinated biphenyl. Chem. Biol. Interact. 9: 379–387.
10. Poland, A. & E. Glover. 1974. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. Mol. Pharmacol. 10: 349–359.
11. Goldstein, J. A., P. Hickman, H. Bergman, J. D. McKinney & M. P. Walker. 1977. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem. Biol. Interact. 17: 69–87.
12. Gupta, B. N., J. G. Vos, J. A. Moore, J. G. Zinke & B. C. Bafflock. 1973. Pathologic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. 5: 125–140.
13. Vos, J. G., J. A. Moore & J. G. Zinke. 1974. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57Bl/6 mice. Toxicol. Appl. Pharmacol. 29: 229–241.
14. Lee, K. P., R. R. Hebert, H. Sherman, J. G. Aftomis & R. S. Warren. 1975. Octabromodiphenyl-induced ultrastructural changes in rat liver. Arch. Environ. Health 36: 465–471.
15. Kociba, R. J., P. A. Keeler, C. N. Park & P. J. Gehring. 1975. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). Results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35: 553–574.
16. Kay, K. 1977. Polybrominated biphenyls (PBB) environmental contamination in Michigan, 1973–1976. Environ. Res. 13: 74–93.
17. Makiura, S., H. Aoe, S. Sugihara, K. Hirao, M. Arai & N. Ito. 1974. Inhibitory effect of polychlorinated biphenyl on liver tumorigenesis in rats treated with 3-methyl-4-dimethylaminoazobenzene, N-2-fluorenyl-acetamide and diethylnitrosamine. J. Nat. Cancer Inst. 53: 1253–1257.
18. Kimura, N. T., T. Kanematsu & I. Baba. 1976. Polychlorinated biphenyl(s) as a promoter in experimental hepatocarcinogenesis in rats. Z. Krebsforsch. 87: 257–266.
19. Okey, A. B. 1972. Dimethylbenzanthracene-induced mammary tumors in rats. I. Inhibition by DDT. Life Sci. 11: 833–843.
20. Kimbrough, R. D., R. A. Squire, R. E. Linder, J. D. Strandberg, R. J. Montali & M. W. Burse. 1975. Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. J. Nat. Cancer Inst. 55: 1453–1459.
21. Shuett-Herman, R. 1977. Two-stage control of cell proliferation induced in rat liver by α-hexachlorocyclohexane. Cancer Res. 37: 166–171.
22. Peraino, C., R. J. M. Fry, E. Staffeldt & J. P. Christopher. 1975. Comparative enhancing effects of phenobarbital, amobarbital, diphenylhydantoin and dichlorodiphenyltrichloroethane on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. Cancer Res. 35: 2884–2892.
23. Uchiyama, M., T. Chiba & K. Noda. 1974. Co-carcinogenic effect of DDT and PCB feedings on methylcholanthrene-induced chemical carcinogenesis. Bull. Environ. Contam. Toxicol. 12: 687–693.
24. Berenbaum, I. & P. Shubik. 1974. A new quantitative approach to the study of the stages of chemical carcinogenesis in mouse's skin. Br. J. Cancer 1: 383–391.
25. Boutwell, R. K. 1964. Some biological aspects of skin carcinogenesis. Prog. Exp. Tumor Res. 4: 207–250.
26. Scribner, J. D. 1973. Tumor initiation by apparently noncarcinogenic polycyclic aromatic hydrocarbons. J. Nat. Cancer Inst. 50: 1717–1719.
27. VanDuuren, B. L., A. Sivak & B. M. Goldschmidt. 1970. Initiating activity of aromatic hydrocarbons in two stage carcinogenesis. J. Nat. Cancer Inst. 44: 1167–1173.
28. Baird, W. M. & R. K. Boutwell. 1971. Tumor-promoting activity of phorbol and four diesters of phorbol in mouse skin. Cancer Res. 31: 1074–1079.
29. DiGiovanni, J., T. J. Slaga, D. L. Berry & M. R. Juchau. 1977. Metabolism of 7,12-dimenthylbenz(a)anthracene in mouse skin homogenates analyzed by high pressure liquid chromatography. Drug Metab. Dispos. 5: 295–301.

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