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# Tumor-Initiating Ability of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Arochlor 1254 in the Two-Stage System of Mouse Skin Carcinogenesis

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Many chemicals in the environment are responsible for outbreaks of toxicity either among industrial workers or inhabitants of a particular community. Two classes of compounds that have received considerable attention in recent months are the dioxins and the polychlorinated biphenyls (PCB's). This report deals with the highly potent dioxin TCDD and Arochlor 1254, a widely studied PCB. TCDD is found as a contaminant in the herbicide and defoliant 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), whereas PCB's have received wide usage in industry as plasticizers, heat exchangers and industrial fluids. The agents are prevalent in the environment and are considered significant environmental pollutants.

The toxicity of TCDD (ARGUS et al., 1973; GREIG et al., 1973; GUPTA et al., 1973; HARRIS et al., 1973; JONES and BUTLER, 1974; KOCIBA, et al., 1976; and SCHWETZ et al., 1973) and PCB's (FISHBEIN, 1974; KIMBROUGH and LINDER, 1974; KIMBROUGH et al., 1975; and NELSON, 1972) has been extensively studied. TCDD is an extremely potent inducer of hepatic aryl hydrocarbon hydroxylase (E.C. 1.14.14.2) in the rat (POLAND and GLOVER, 1974a). Arochlor 1254 also induces hepatic drug metabolizing systems in rats, although it is not as potent as TCDD (ALVARES et al., 1973). It is interesting to note that both TCDD and Arochlor 1254 share enzyme inducing properties with 3-methylcholanthrene, a prototypic polycyclic hydrocarbon inducing agent. TCDD and Arochlor 1254 both are known to produce hepatic tumors in rats (ARGUS et al., 1973; KIMBROUGH et al., 1975). Therefore, it seemed important to study the tumor-initiating ability of these two substances using the two-stage, initiation-promotion system of carcinogenesis. By testing potential carcinogens as tumor initiators in this system, one can eliminate the promoting phase as a factor in the carcinogenicity of the compounds. We have examined and report here on the tumor-initiating capacity of these compounds in mouse skin. We have also studied the effects of simultaneous administration of either TCDD or Arochlor 1254 with 7,12-dimethylbenz[a]anthracene (DMBA), an established, highly potent initiator in this system.

MATERIALS AND METHODS. TCDD was a gift from the Dow Chemical Co., Midland, Mich. (98.6% pure by glc, Lot #851-144-II). Arochlor 1254 was purchased from Analabs, Inc., No. Haven, Conn. DMBA was



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MONS 001161

obtained from the Sigma Chemical Co., St. Louis, Mo. 12-O-Tetradecanoylphorbol-13-acetate (TPA) was prepared as previously described (BAIRD and BOUTWELL, 1971) and purified by preparative thin-layer chromatography.

Female Charles River CD-1 mice were purchased from Charles River Mouse Farms, North Wilmington, Mass. Mice 7-9 weeks old were carefully shaved with surgical clippers 2 days prior to initial treatment and only those mice in the resting phase of the hair cycle were used in the tumor experiments. Each experimental group contained 30 preshaved mice. TCDD ( 2 µg/mouse) and Arochlor 1254 (100 µg/mouse) in 0.2 ml of acetone were applied alone as initiators or 5 min prior to initiation with DMBA (2.56 µg/mouse). The dose of TCDD was based on the $ED_{50}$ and on the ability of this compound to induce aryl hydrocarbon hydroxylase after topical administration to mice (2 µg/mouse/day) (POLAND et al., 1974b). Both "responsive" and "nonresponsive" mice exhibited significant induction with this dose of TCDD. The dose of Arochlor 1254 was based on the ability of this PCB to induce drug-metabolizing enzymes in skin after a single topical application to rats (25 mg/kg) (BICKERS et al., 1974). Preliminary results in this laboratory have shown that Arochlor 1254, when applied topically to mice (100 µg/mouse), increases rates of oxidative DMBA metabolism in the skin within 18 hrs after pretreatment. In addition, TCDD, at the dose described above, produced lethality in approximately one-third of the animals at 32 weeks. A higher dose would have produced higher lethality and was therefore impractical for the tumor experiments. One week after initiation, mice received applications of 5 µg of TPA in 0.2 ml acetone twice weekly and promotion was continued for 32 weeks. The incidences of both papillomas and carcinomas were observed and recorded weekly. Papillomas and carcinomas were removed at random for histologic verification.

RESULTS AND DISCUSSION. The tumor-initiating capacity of TCDD is illustrated in figure 1. Shown also is the effect of simultaneous administration of DMBA and TCDD on tumor initiation. TCDD, when administered alone at a dose of 2 µg per mouse, showed only weak initiating ability after promotion for 32 weeks (0.1 papillomas/mouse, 14% of survivors with papillomas). When TCDD was given concurrently with DMBA, the number of tumors observed increased slightly (2.2 papillomas/mouse, 63% of survivors with papillomas) when compared with the initiating ability of DMBA alone; i.e., when TCDD and DMBA were given together, an approximately additive effect was observed. This is in contrast to recent data showing that concomitant administration of the weak initiator dibenz(a,c)anthracene [DB(a,c)A] caused a reduction in the tumorigenic effect of DMBA (SLAGA and BOUTWELL, 1976). It was speculated that DMBA and DB(a,c)A may compete for bioactivation resulting in the observed decrease in effect. TCDD, on the other hand, has an extremely long half-life and no metabolites have been detected to date (POLAND and KENDE, 1976). Lack of biotransformation of TCDD possibly could explain the difference seen with TCDD vs. DB(a,c)A on tumor initiation by DMBA.



MONS 001162

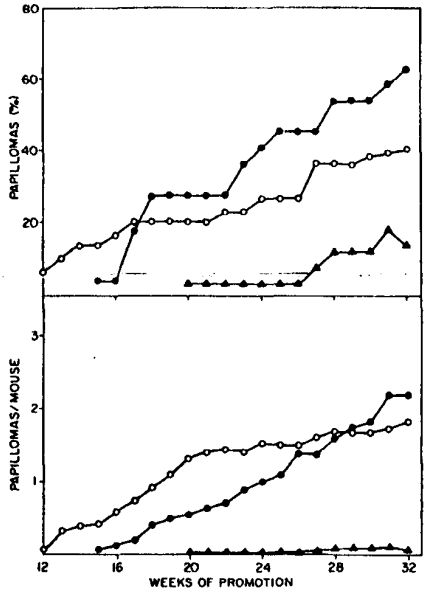


Figure 1 Tumor-initiating ability of TCDD and the effect of simultaneous administration of TCDD and DMBA on skin tumorigenesis. The animals in group 1 (O) were treated with a single application of 2.56 µg of DMBA and promoted for 32 weeks with 5 µg of TPA, group 2 (●); treated with 2.56 µg DMBA + 2 µg TCDD and promoted with 5 µg TPA for 32 weeks and group 3 (▲); treated with 2 µg TCDD and promoted with 5 µg TPA for 32 weeks. Figure 1A plots the data as % of surviving mice with papillomas. Figure 1B illustrates the data as papillomas per mouse. The number of mice surviving in each group at 32 weeks was: Group 1, 29/30; Group 2, 22/30; and Group 3, 21/30.



MONS 001163

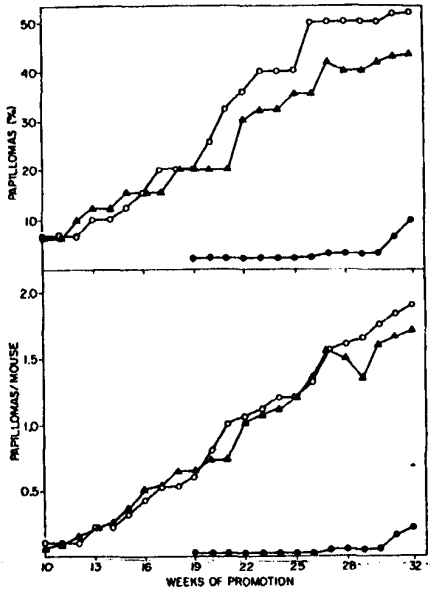


Figure 2 Tumor-initiating ability of Arochlor 1254 and the effect of simulatneous administration of Arochlor 1254 and DMBA on skin tumorigenesis. The animals in group 1 (O) were treated with 2.56 µg DMBA and promoted with 5 µg TPA for 32 weeks, group 2 (●); treated with 100 µg Arochlor 1254 and promoted with 5 µg TPA for 32 weeks, group 3 (▲); treated with 2.56 µg DMBA and 100 µg Arochlor 1254 and promoted for 32 weeks with 5 µg TPA. Figure 2A plots the data as % of surviving mice with papillomas. Figure 2B illustrates papillomas per mouse. The number of mice surviving in each group at 32 weeks was 30/30.



MONS 001164

In comparison, Arochlor 1254, at a dose of 100 µg/mouse, also displayed a weak tumor-initiating capacity (figure 2) after promotion with TPA for 32 weeks (0.2 papillomas/mouse, 10% of survivors with papillomas). Concurrent administration of Arochlor 1254 with DMBA produced a slight decrease in the number of tumors observed at 32 weeks (1.7 papillomas/mouse, 42% of survivors with papillomas) when compared with the effects of DMBA given alone.

Oral feeding of either Arochlor 1254 (JONES and BUTLER, 1974) or TCDD (ARGUS et al., 1973) appears to cause hepatocarcinomas in rats. Technical PCB's have been shown to possess promoting properties for hepatic tumors initiated with benzene hexachloride in mice (ITO et al., 1973). However, tumor initiating properties have not been tested. An earlier report (KING et al., 1973) indicated that a series of relatively non-toxic chlorinated dibenzodioxins possessed neither tumor promoting activity nor complete carcinogenic properties in mouse skin. Studies regarding the tumor-initiating ability of both PCB's and TCDD are lacking. The data presented in this communication suggests that, at the doses utilized, Arochlor 1254 and TCDD are weak tumor initiators in the two-stage system of mouse skin tumorigenesis; and when given concurrently with a known carcinogen in this system, are capable of modifying the response only slightly.

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MONS 001165

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MONS 001166