The Promoting Effects of 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin on Nasal Tumors in Sprague-Dawley Rats

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ABSTRACT

It was hypothesized that exposure to environmentally relevant dietary levels of 2, 3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 2,2',4,4',5,5'-hexachlorobiphenyl (245-HCB) could cause the development of nasal tumors and a combined exposure to these compounds could have a potentiating effect on tumor development. Thirty days after partial hepatectomy and NDEA administration, groups of 12 or 24 female Sprague-Dawley rats were fed a basal diet or diets containing 10 ppt TCDD, 10 ppt TCDD, 5 ppm 245-HCB, 10 ppt TCDD and 245-HCB or 100 ppt TCDD and 245-HCB for 140 days. Basal diets were fed from day 140 until rats were killed on day 210 or day 420. Results indicated that TCDD or a combination of TCDD and 245-HCB significantly increased the incidence of nasal tumors by day 420. However, a combined exposure to TCDD and 245-HCB had no potentiating effect on the incidence of nasal tumors.

INTRODUCTION

The cells of the nasal cavity in experimental animals have not been widely recognized as an important target site for carcinogenic environmental compounds. However, the growing interset in and importance of neoplasms in the nasal cavity of man and animals have been emphasized by the publication of a 3 volume monograph by CRC Press, Inc. (Reznik and stinson, 1983) and by a textbook edited by Barrow (1986) in which major emphases are placed on the pathology of those tumors and upon nasal tumors experimentally induced by environmental compounds.

Naturally occurring nasal cancer is extremely rare in the rat (Goodman *et al.*, 1979), but many chemicals, such as N-nitrosamines, can induce these tumors (Reznik-Schuller, 1983). Although the mechanism of nasal carcinogenesis is poorly understood, it is known that metabolism of N-nitrosamines occurs in nasal epithelial cells (Reznik-Schuller, 1982; Brittebo and Tjalve, 1983), and DNA adducts can be formed. Little is known about promotion of nasal carcinogenesis, but a multistep process in which environmental factors are important has been proposed (Prasad, 1983).

Polyhalogenated aromatic hydrocarbons (PHAH), such as polybrominated biphenyls (PBB), polychlorinated biphenyls (PCB) and tetrachlorodibenzo-p-dioxin (TCDD), are a class of widespread environmental pollutants which are known to act as promoters of hepatocarcinogenesis in rodents (Gupta *et al.*, 1973; Buchman *et al.*, 1986). An experimental study demonstrated that dietary exposure of rats to a diet containing 2200 ppt TCDD for 2 years increased the incidence of squamous cell carcinomas in the hard palate and nasal cavity, whereas the tumors were not evident in rats fed diets containing either 22 ppt or 210 ppt TCDD for 2 years (Kociba *et al.*, 1978). Jensen and Sleight (1986a), in an experiment designed to assesss hepatic tumor promotion, demontrated that PBB enhanced the development of nasal tumors in rats initiated with a subcarcinogenic dose of NDEA. PBB decreased the latency time, but did not alter the incidence of nasal carcinomas. However, the number of nasal adenomas was apparently increased by a diet containing PBB.

Until now, the possibility that PHAH could act as tumor promoters at nonhepatic sites, such as the nasal cavity or trachea, has not been addressed. Certain PHAH, such as PCB (Brandt, 1977) and

TCDD (Appelgren *et al.*, 1983), when given to rodents, accumulate in nasal epithelial cells. These cells have relatively high levels of cytochrome P-450 enzymes (Hadley and Dahl, 1983; Voight *et al.*, 1985; Dahl, 1986), and there is evidence that PHAH can induce enzymes in these cells (Bond, 1983; Voight *et al.*, 1985). If PHAH are present in nasal epithelial cells and can cause physiologic responses in these cells, it is logical that promotion of NDEA-initiated cells could occur. Therefore, the major hypothesis underlying this study is that exposure to environmental chemicals, such as PHAH, can enhance the development of nasal tumors in rats initiated with a subcarcinogenic dose of NDEA.

A major objective of the following study was to determine if interactions of 2,2′,4,4′,5,5′-hexachlorobiphenyl (245-HCB) and tetrachlorodibenzo-p-dioxin (TCDD) in a long term sequential study caused a synergistic effect on nasal tumor promotion in Sprague-Dawley rats given a single low dose of NDEA when compared to the effect of these compounds given separately. There was an apparent synergistic effect on the development of γ-glutamyl transpeptidase-positive altered hepatic foci and the development of hepatic nodules caused by the simultaneous exposure to 2,2′,4,4′,5,5′-hexabromobiphenyl and 3,3′,4,4′,5,5′-hexabromobiphenyl (Jensen and Sleight, 1986b). The low concentrations of TCDD and PCB used in this study have been reported in food products, such as fish (Zabik *et al.*, 1982; Cardle, 1983) Therefore, this study may have important public health implicatios if simultaneous exposure to environmentally relevant concentrations of these chemicals can be shown to have an additive or synergistic effect on carcinogenic response.

MATERIALS AND METHODS

Experimental Design

Two hundred and sixteen female Sprague-Dawley rats initially weighing 180-200 g at 5-6 weeks old were used. Rats were acclimated for 7 days and were fed a basal diet and tap water *ad libitum*.

Rats were 70% partially hepatectomized (PH) 24 hr prior to intraperitoneal administration of 10 mg NDEA/kg body weight. Rats used as a controls were not PH or given NDEA. Thirty days after PH, rats wererandomly allotted into 12 groups of 24 or 12 each. The experimental design is illustrated in Table 1.

Diets were prepared by adding appropiate amounts of tetrachlorodibenzo-p-dioxin (TCDD) or 2,2′,4,4′,5,5′-hexachlorobiphenyl (245-HCB) dissolved in corn oil to a basal diet. Rats were fed the diets for 140 days. Rats continued on experiment were main-

Table 1. **Experimental design**

Group	Treatment	Diets (day)		Termination (day)		
		0 — 140	140 — 420	140	210	420
A	PH + NDEA	Basal diet	Basal diet	6[a]	6	12
B	None	Basal diet	Basal diet	3	3	6
C	PH + NDEA	10 ppt TCDD	Basal diet	6	6	12
D	None	10 ppt TCDD	Basal diet	3	3	6
E	PH + NDEA	100 ppt TCDD	Basal diet	6	6	12
F	None	100 ppt TCDD	Basal diet	3	3	6
G	PH + NDEA	5 ppm 245-HCB	Basal diet	6	6	12
H	None	5 ppm 245-HCB	Basal diet	3	3	6
I	PH + NDEA	10 ppt TCDD + 5 ppm 245-HCB	Basal diet	6	6	12
J	None	10 ppt TCDD + 5 ppm 245-HCB	Basal diet	3	3	6
K	PH + NDEA	100 ppt TCDD + 5 ppm 245-HCB	Basal diet	6	6	12
L	None	100 ppt TCDD 5 ppm 245-HCB	Basal diet	3	3	6

[a]Rats in each group were killed on day indicated.

tained on basal diets from that point on until the experiment was terminated on day 420.

The rats were housed according to groups in stainless wire-top, plastic cages, 2 or 6 rats per cage, and the bedding was changed twice a week. Cages containing TCDD or 245-HCB-treated rats were placed in filtered laminar flow units. The room was maintained at 22°C with a 12 hr light/dark cycle. Rats were observed daily for clinical signs.

Necropsy and Histopathologic Procedures

Six rats from each treated group and 3 from each control group were killed with CO_2 at 140 and 210 days. The remaining rats were killed at 420 days. At necropsy, nasal cavities were infused with approximately 3 ml of 10% neutral buffered formalin through the posterior opening of the nasal pharynx and tissues were fixed for 7 days. Methods for preparation of nasal cavities for histopathologic examination were according to procedures of Young (1981).

Formalin-fixed specimens were processed in an automatic tissue processor, embedded in paraffin, cut with a microtome at 5 μm and stained with hematoxylin and eosin.

Statistical Evaluation

Incidence of tumors of the nasal cavity was calculated on the basis of results of histopathologic examination. Differences in the tumor incidence among groups were analyzed by chi-square. The differences were considered significant at the level of $p < 0.05$ (Gill, 1981).

RESULTS AND DISCUSSION

Gross Lesions

There were no noticeable swellings of the nasal or orbital regions observed in any of the rats during this experiment. Gross lesions in the nasal cavity were observed only when multiple frontal sections of the nasal cavity were made. Typical lesions were firm, white-yellow masses within the nasal cavities.

Histopathology

At 140 days, nasal tissues of rats in all groups were histologically normal. Adenomas in the nasal cavities were first observed at 210 days in 1 of 6 rats given a diet containing 100 ppt TCDD (group E) or 100 ppt TCDD + 5 ppm 245-HCB (group K). Adenocarcinomas as well as adenomas were observed at 420 days (Table 2). In 1 rat each from groups given a diet containing 10 ppt TCDD (group C), 100 ppt TCDD (group E), 10 ppt TCDD + 5 ppm 245-HCB (group I) or 100 ppt TCDD + 5 ppm 245-HCB (group K), multiple types of adenomas and adenocarcinomas were also noted. Five rats died as a result of adenocarcinomas. Of these rats, 2 from group K died at days 288 and 323, respectively, and 1 each from groups A, E and G diet at days 401, 417 and 387, respectively. Adenomas were composed of well-differentiated cells with a well-defined glandular structure and mostly with a papillary growth pattern (Figure 1). Adenocarcinomas were composed of poorly differentiated cells with mostly solid areas and with prominent nuclear features of malignancy, such as pleomorphism, hyperchromatism and abnormal mitotic figures. Evidence of a glandural pattern was occasionally present (Figure 2). Tumors were mainly observed in the lining epithelium of posterior regions of the nasal cavities.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is present as a trace contaminant in several industrial organic chemicals. Polychlorinated biphenyls (PCB) have been used in a variety of industrial processes since the 1930's. The production of PCB ceased during the 1970's. Both TCDD and PCB have been identified as environmental contaminants (Rappe and Buser, 1980). People may be exposed to TCDD or PCB from many sources, such as watwer, soil and food products, such as fish. Concentration of TCDD as high as 30 ppt and with an average value of 25 ppt were found in edible portions of salmonid fish, such salmon and trout. Lower levels were in the edible portions of species, such as bullhead, perch, catfish and sucker (Cordle, 1983). PCB have been detected in fish at approximately 2 ppm on an edible tissue basis, and on a fat basis the value was approximately 23 ppm (Zabik *et al.*, 1982).

TCDD and PCB are known to be toxic and carcinogenic (Poland and Knutson, 1982). At present, some researchers have examined the effect of TCDD or PCB exposure following administration of N-nitrosodiethylamine (NDEA), a known tumor initiator, and results appear to indicate that TCDD or PCB function as a promoter of hepatocarcinogenesis in laboratory animals (Pitot, *et al.*, 1980; Buchman *et al.*, 1986). It is important to understand, however, their carcinogenic potential at nonhepatic sites since

it is becoming apparetnt that TCDD or related compounds my enhance the development of cancer in the nasal cavity (Kociba *et al.*, 1978; Jensen and Sleight, 1986a). It is possible that when these compounds (NDEA, TCDD and PCB) exist together in the environment, there is an increased likelihood of finding respiratory tract tumors, particularly in the nasal cavity. In order to assess this risk, an attemp was made to simulate natural exposure of animals to chemicals, such as NDEA, TCDD and PCB. This was done by using an initiation-promotion assay for nasal carcinogenesis with the low dose of NDEA, as an initiator, and the low doses of TCDD and PCB, as promoters.

Table 2. **The incidence and type of nasal tumors in rats by 420 days**

Group	No. of rats	Initiation[a]	Chemicals in diets	Nasal tumors		Total no. of rats with nasal tumors
				Adenomas	Adenocarcinomas	
A	12	PH + NDEA	Basal diet	3	1	4[b]
B	6	None	Basal diet	0	0	0
C	12	PH + NDEA	10 ppt TCDD	6	3	9
D	6	None	10 ppt TCDD	0	0	0
E	12	PH + NDEA	100 ppt TCDD	7	2	9
F	6	None	100 ppt TCDD	0	0	0
G	12	PH + NDEA	5 ppm 245-HCB	6	1	7
H	6	None	5 ppm 245-HCB	0	0	0
I	12	PH + NDEA	10 ppt TCDD + 5 ppm 245-HCB	8	1	9
J	6	None	10 ppt TCDD + 5 ppm 245-HCB	0	0	0
K	12	PH + NDEA	100 ppt TCDD + 5 ppm 245-HCB	7	2	9
L	6	None	100 ppt TCDD + 5 ppm 245-HCB	0	0	0

[a]Initiation consisted of a 70% partial hepatectomy (PH) and N-nitrosodiethylamine (NDEA) administration 30 days prior to dietary treatment.
[b]Significantly different ($P < 0.05$) from groups C, E, I and K.

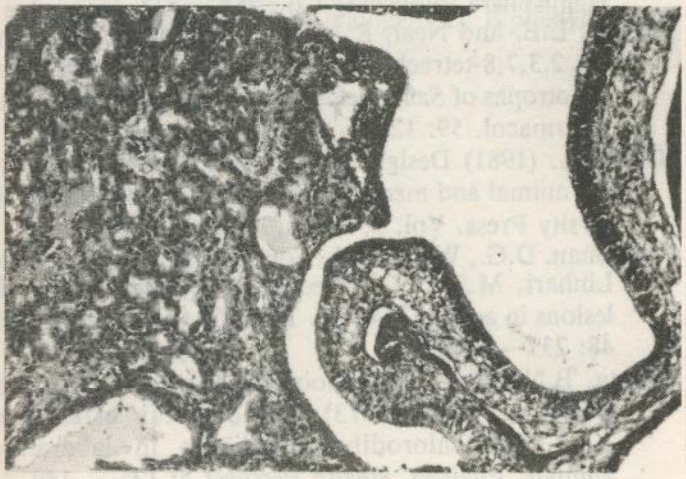

Figure 1. **Adenoma of the nasal cavity from a NDEA-initiated rat fed a diet containing 100 ppt TCDD plus 245-HCB for 140 days and killed on day 420. Notice well differentiated cells have formed glandular structures with a papillary growth pattern. (H & E stain, 218x.).**

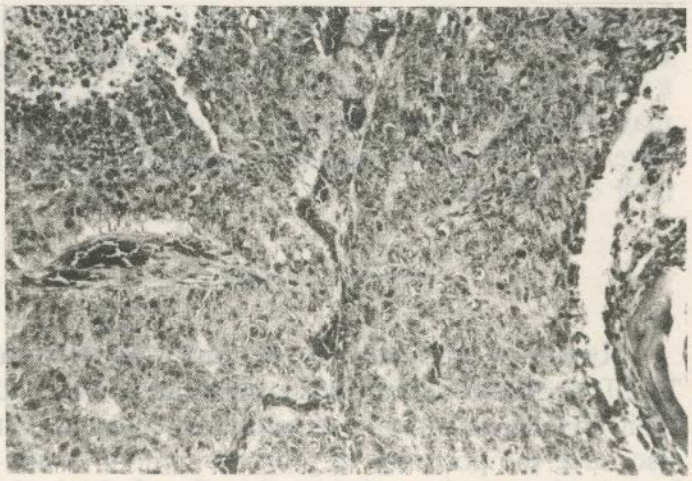

Figure 2. **Adenocarcinoma of the nasal cavity from a NDEA-initiated rat fed a diet containing 100 ppt TCDD plus 245-HCB for 140 days. Rat died on day 323. Notice solid areas of poorly differentiated cells with few glandular structures. (H & E stain, 360x.).**

Results of this experiment indicated that dietary exposure of rats to diets containing both TCDD and 2,4,5-HCB did not have a potentiating effect on the incidence of nasal tumors in NDEA-initiated rats. However, the incidence of nasal tumors was apparently increased by exposure to diets containing either TCDD or a combination of TCDD and 245-HCB. Therefore, it is apparent that dietary exposure to TCDD may have acted as a promoter in nasal carcinogenesis. Little previous work has been done to determine whether TCDD or PCB are carcinogenic in the nasal cavity. In one study, squamous cell carcinomas of the hard palate or nasal cavities could be detected in rats not previously initiated and chronically administered TCDD at 2200 ppt in the diet (Kociba *et al.*, 1978). Naturally occurring nasal cancer is extremely rare in the rat (Goodman *et al.*, 1979). Chemicals that can induce tumors without a promoter when given at a high enough dose are generally considered tumor initiator (Berenblum, 1941; Pitot *et al.*, 1981). Since TCDD has no properties of know tumor initiators (e.q. not mutagenic or genotoxic) (Wassom *et al.*, 1978; Poland and Glover, 1979; Geiger and Neal, 1981; Roberts *et al.*, 1985), it is possible that these tumors resulted from promotion of spontaneously initiated cells. Alternatively, this chemical could act as both an initiator and promoter and thus behave as a complete carcinogen.

In this study, there was deaths from nasal carcinomas among animals exposed to NDEA, TCDD or TCDD and 245-HCB. The earliest deaths occurred in 2 of 12 rats given a combination of 100 ppt TCDD and 245-HCB. The highest incidence of hepatocellular carcinomas also occurred in rats from this group (Sleight *et al.*, 1987). Thus, these results suggest that a combined exposure to 100 ppt TCDD and 2,4,5-HCB may decrease the latency time for nasal carcinomas to develop as well as increase the incidence of hepatocellular carcinomas.

CONCLUSIONS

Results of this study are very important because TCDD or related compounds accumulate in the food chain and can act not only as hepatic carcinogens, but also have the potential to promote tumors in non-hepatic sites, such as the nasal cavity. Indeed, the results indicate that risks to animals or people from environmental chemicals found in food may be enhanced by interaction between such chemicals.

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