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THE PROMOTING EFFECTS OF POLYHALOGENATED BIPHENYLS
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By

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ABSTRACT

THE PROMOTING EFFECTS OF POLYHALOGENATED BIPHENYLS AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON NASAL AND TRACHEAL TUMORS

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Male Syrian golden hamsters were allotted to 4 groups of 30 each and were used for an initiation-promotion study of respiratory tract carcinogenesis. Hamsters were given a single subcutaneous dose of 0 or 80 mg N-nitrosodiethylamine (NDEA)/kg of body weight and were fed diets containing 0 or 100 mg polybrominated biphenyls (PBB)/kg of diet for 140 days. Basal diet was fed from day 140 until the end of the experiment on day 273. The number of tracheal papillomas was significantly increased in hamsters given NDEA and PBB as compared to those in hamsters given only NDEA. Tracheal papillomas were not seen in the other two groups. Nasal tumors occurred at approximately the same incidence in hamsters given NDEA as in those given NDEA and PBB.

To characterize precursor lesions in the trachea and their relationship to the tumor promoting ability of PBB after initiation with NDEA, young male hamsters were allotted to 4 groups of 36 each and were given a single subcutaneous dose of 0 or 80 mg NDEA/kg of body weight. Diets containing 0 or 100 mg PBB/kg were fed for the remainder of the experiment. Twelve hamsters from each

group were killed on days 21, 42 or 63. Precursor lesions were not observed during the time frame of this study.

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, 100 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.

Results indicate that PBB promotes the development of tracheal papillomas in the hamster and TCDD enhances formation of nasal tumors in the rat.

Dedicated With Love To

My mother

Rr. Hastari Wuryastuti, my wife

My sisters and their husbands, my brother and his wife,
and their children:
Ria, Bagus, Nila, Windri, Galih, Imok, Denta, Rizki,
Uuk and Bayu

You

And in memory of my father: R. Mohammad Ichram

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TABLE OF CONTENTS

Page

LIST OF TABLES vi

LIST OF FIGURES vii

INTRODUCTION 1

LITERATURE REVIEW 4

Experimental Nasal Carcinogenesis 4
N-nitrosamines 8
Introduction 8
Metabolism 8
Respiratory Carcinogenesis 11
Polybrominated Biphenyls 16
Introduction 16
Metabolism 18
Pathology 21
Factors Modifying Respiratory Carcinogenesis . . . 24
Initiation and Promotion in Carcinogenesis 25

CHAPTER I: THE PROMOTING EFFECT OF POLYBROMINATED BIPHENYLS ON NASAL AND TRACHEAL TUMORS IN SYRIAN GOLDEN HAMSTERS 28

Introduction 28
Materials and Methods - Experiment 1 32
Experimental Design 32
N-nitrosodiethylamine Administration 34
Diet Preparation 38
Necropsy and Histopathologic Procedures . . . 38
Statistical Evaluation 39
Results 40
Materials and Methods - Experiment 2 47
Experimental Design 47
Necropsy and Histopathologic Procedures . . . 48
Results 48
Discussion 56

CHAPTER II: THE PROMOTING EFFECT OF TETRACHLORODIBENZO-p-DIOXIN AND 2,2',4,4',5,5'-HEXACHLOROBIPHENYL ON NASAL CAVITY TUMORS IN SPRAGUE-DAWLEY RATS 65

Introduction 65
Materials and Methods 68

Page

Experimental Design 68
Necropsy and Histopathologic Procedures . . . 70
Statistical Evaluation 70
Results 71
Discussion 75

SUMMARY AND CONCLUSIONS 79

BIBLIOGRAPHY 82

VITA 105

LIST OF TABLES

Table		Page
1	Experimental design	33
2	Body weights of Syrian golden hamsters (g)	41
3	A comparison of papillomas in the larynx or trachea of Syrian golden hamsters treated either with NDEA alone or with a combination of NDEA and PBB	44
4	Tumors in the nasal cavity of Syrian golden hamsters	46
5	Experimental design	69
6	The incidence and type of nasal tumors in rats by 420 days	72

LIST OF FIGURES

Figure		Page
1	Photomicrograph of nasal cavity from a control hamster	36
2	Photomicrograph of nasal cavity from a hamster given 80 mg NDEA/kg bw	36
3	Photomicrograph of nasal cavity from a control hamster	37
4	Photomicrograph of nasal cavity from a hamster given 80 mg NDEA/kg bw	37
5	Papilloma of the trachea from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	50
6	Papilloma of the nasal cavity from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	50
7	Adenoma of the nasal cavity from a hamster given 80 mg NDEA/kg bw	51
8	Adenocarcinoma of the nasal cavity from a hamster given 80 mg NDEA/kg bw	51
9	Squamous cell carcinoma of the nasal cavity from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	52
10	Papilloma of the trachea from a hamster given 80 mg NDEA/kg bw	52
11	Papilloma of the trachea from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	53
12	Papilloma in a bronchiole from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	53
13	Adenoma of the lung from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet	54

Figure | Page

14 Adenoma of the lung from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet 54

15 Trachea of a hamster fed a commercial diet for 42 days after administration of 80 mg NDEA/kg bw 55

16 Papilloma of the trachea from a hamster fed a diet containing 100 mg of PBB/kg for 63 days after administration of 80 mg NDEA/kg bw 55

17 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 74

18 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 74

INTRODUCTION

Neoplasms of the upper respiratory tract in people and animals have been recently emphasized in a 3 volume monograph by CRC Press Inc. (Reznik-Stinson, 1983) and in a textbook edited by Barrow (1986) that specifically deal with nasal tumors and by continuing research on the pathogenesis and morphogenesis of tracheal tumors (Reznik-Schuller, 1980). The various N-nitrosamines have been studied extensively. N-nitrosodiethylamine (NDEA), for example, is considered as a mutagen and carcinogen in people and animals, is detected in food, water and air, and can be formed *in vivo* from ingested amines and nitrites (International Agency for Research on Cancer, 1978). NDEA is metabolically activated via the cytochrome P-450-dependent monooxygenase system and a reactive intermediate formed during metabolism interacts with DNA to yield alkylated products (Hecht *et al.*, 1983). NDEA is considered to act as a tumor initiator and studies have shown that it targets specific cells in the nasal cavity (Reznik-Schuller, 1982; Jensen and Sleight, 1987) and trachea (Reznik-Schuller and Hague, 1981a,b) and can cause tumors to develop in these cells (Montesano and Saffiotti, 1968; Reznik-Schuller, 1980).

The actual mechanisms of upper respiratory tract carcinogenesis have not been elucidated, but Prasad (1983) has proposed that the development of upper respiratory tract tumors from exposure to environmental compounds may involve a multistep process involving initiation and promotion. Polyhalogenated aromatic hydrocarbons (PHAH), such as polybrominated biphenyls (PBB), polychlorinated biphenyls (PCB) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are environmental contaminants and studies have shown that they act as tumor promoters in the liver (Gupta et al., 1973; Buchmann et al., 1986; Jensen and Sleight, 1986b). To date, however, the ability of PHAH to promote tumors at nonhepatic sites, such as in the respiratory tract, has not been demonstrated. PHAH can accumulate in the epithelial cells of the respiratory tract (Brandt, 1977; Appelgren et al., 1983). These cells are rich in cytochrome P-450-dependent monooxygenase (Hadley and Dahl, 1983; Voight et al., 1985; Dahl, 1986). PHAH can induce these enzymes in these cells (Bond, 1983; Voight et al., 1985). Therefore, in a study of respiratory tract carcinogenesis, one could postulate that NDEA can act as a tumor initiator and PHAH as tumor promoters.

This dissertation is divided into two chapters. The first chapter describes an initiation-promotion study in Syrian golden hamsters with NDEA as an initiator and PBB as a possible promoter. The first objective was to determine if PBB will promote respiratory tract tumors. The second

objective was to determine and characterize sequential development of tracheal tumors. We expected to find precursor lesions of tracheal tumors in NDEA-initiated hamsters given PBB. The second chapter describes an initiation-promotion study in Sprague-Dawley rats with NDEA as an initiator and TCDD and 2,2'4,4',5,5'-hexachlorobiphenyl (245-HCB) as promoters. The objectives were to determine if TCDD or 245-HCB acts as a promoter of nasal carcinogenesis and to determine if the interactions of TCDD and 245-HCB cause a synergistic effect on tumor promotion when compared with the effect of these compounds given separately.

LITERATURE REVIEW

Experimental Nasal Carcinogenesis

Many chemicals have been reported to induce tumors of the nasal cavity in people and animals (Reznik and Stinson, 1983; Barrow, 1986). In experimental carcinogenesis, N-nitrosamines are the most well known chemicals that are capable of inducing tumors in the respiratory tract, particularly in the nasal cavity. In addition, nasal tumors have been related to a wide variety of important industrial chemicals, such as formaldehyde. Exposure to formaldehyde in rats and mice induced squamous cell carcinomas in the nasal tissue (Swenberg et al., 1980; Kerns et al., 1983).

In chemical carcinogenesis, it is thought that compounds, such as N-nitrosamines, require metabolic activation before they can produce tumors. Formaldehyde, in contrast, requires no metabolic activation. Inhaled formaldehyde must first be deposited on the surface of the epithelial cells, particularly in the anterior part (respiratory region) of the nasal cavity. At low ambient air concentrations, formaldehyde deposited on the epithelial cells can be removed from proximity to target tissue via normal mucociliary clearance. The target tissue of formaldehyde is presumed to be basal cells. Once inside the

basal cells, formaldehyde must penetrate the nuclear membrane and react with DNA to yield adducts (Starr, 1983). Carcinogenesis in the nasal epithelium, resulting from formaldehyde toxicity, may be directly related to increase in cell proliferation. During increased cell proliferation, the likelihood of interaction of formaldehyde with DNA would increase, as would fixation of adducts before DNA repair could occur (Swenberg et al., 1983).

An early clinical sign of nasal tumors in experimental animals was reported to be unilateral or bilateral ocular discharge (Swenberg et al., 1980; Takano et al., 1982). Other signs described in animals included bloody nasal discharge (Tucker, 1975; Takano et al., 1982), dyspnea, mouth breathing and loss of weight (Hoch-Ligeti, 1970; Takano et al., 1982). Marked swelling of the nose and orbital regions has been reported as a gross evidence of nasal tumors (Montesano and Saffiotti, 1968; Albert et al., 1982), while in other instances, gross lesions of tumors were found only when multiple frontal sections were made. The gross lesions consisted of opaque, greyish-white masses within the nasal cavity and involved naso-maxilloturbinates and ethmoturbinates. The tumors sometimes had eroded bone (Herrold, 1964c; Sellakumar et al., 1983).

Results of numerous experimental studies demonstrated that tumors of the nasal cavity were of multicentric origin and of many different histologic types. Generally, however, the main types of induced neoplasms may be divided into two

categories: those that arise in the anterior part (respiratory region) of the nasal cavity (e.g. papilloma, adenoma, adenocarcinoma and squamous cell carcinoma) and those that originate in the posterior part (olfactory region) of the nasal cavity (e.g. adenoma, adenocarcinoma, squamous cell carcinoma and olfactory neuroepithelial tumor) (Reznik-Schuller, 1983; Feron et al., 1986).

Papilloma. These tumors are composed of infolded masses of squamous epithelial cells with a well developed stalk and no infiltrative growth into the subepithelial tissues (Herrold, 1964b; Mohr et al., 1977; Reznik-Schuller, 1983). Other histopathologic features of papillomas include keratin formation and microcysts containing debris of necrotic cells and inflammatory cells (Herrold, 1964b).

Adenoma. In contrast to papillomas, adenomas have a somewhat glandular and papillary growth pattern and contain mucous cells (Mohr et al., 1977; Reznik-Schuller, 1983).

Adenocarcinoma. Tumors classified as adenocarcinomas are composed, for the most part, of anaplastic or poorly differentiated cells and may have a gland-like growth pattern (e.g. formation of acini and secretion of mucus) (Huang and Ho, 1978; Reznik-Schuller, 1983). A papillary growth pattern and a few small solid areas of tumor cells are sometimes present (Huang and Ho, 1978). Occasionally, these tumors may have focal areas of squamous metaplasia indicating they might differentiate into squamous cell carcinomas at a later stage (Reznik-Schuller, 1983). The

tumors may invade the periorbital tissue, lacrimal gland, maxillary sinus and facial muscle, and extend posteriorly through the cribriform plate to involve the frontal lobes of the brain (Feron and Kroes, 1979).

Squamous cell carcinoma. These tumors are composed of anaplastic or poorly differentiated cells, but pronounced keratinization and epithelial pearl formation are usually present (Lijinsky and Taylor, 1975a; Reznik-Schuller, 1983). The neoplastic squamous cells are usually arranged in clusters. These tumors tend to invade the bony skeleton of the nasal cavity, and may then eventually lead to ulceration. In some cases, the tumor may grow into the posterior part of the ethmoturbinates and, after penetrating the cribriform plate, invade the brain (Reznik-Schuller, 1983).

Olfactory neuroepithelial tumor. Tumors from the olfactory epithelial cells are classified as (esthesio)neuroepitheliomas (Althoff et al., 1973; Haas et al., 1973; Rivenson et al., 1983). This tumor is sometimes referred to as an (esthesio)neuroblastoma (Herrold, 1964c; Mirvish et al., 1980). Histologically, these tumors are characterized by the formation of rosettes and pseudorosettes and by fairly uniform cuboidal to columnar cells (Herrold, 1964c; Rivenson et al., 1983; Sellakumar et al., 1983). The neurogenic origin of such tumors can best be defined by the presence of neurogenic elements such as neurosecretory granules and sustentacular cells, by electron

microscopy (Reznik-Schuller, 1983). Olfactory neuroepithelial tumors occasionally invade the nasal bones and the tumors may extend caudally to invade the brain (Herrold, 1964c; Sellakumar et al., 1983).

N-nitrosamines

Introduction

N-nitrosamines are a group of environmental carcinogens that are carcinogenic in many organs of various animal species. The carcinogenic property is dependent on species and strain of the animals and type of N-nitrosamine used, and may vary depending on the route of administration, dose and interval between individual doses. For example, the respiratory tract (nasal cavity, larynx, trachea and lung) is the main target area for N-nitrosodiethylamine (NDEA) carcinogenicity in Syrian golden hamsters. In contrast, the liver is the main target organ for NDEA carcinogenicity in rats. The mechanism responsible for this marked specificity is not clearly understood. However, N-nitrosamines must first be metabolically activated before their carcinogenic effect is seen.

Metabolism

Like many other N-nitrosamines, NDEA requires metabolic activation by cytochrome P-450 into an ultimate carcinogen (active intermediate or alkylating agent) in order to exert its carcinogenic effects (Vainio and Hietanen, 1980;

Schuller and McMahon, 1985). In Syrian golden hamsters, the possibility exists that NDEA is metabolically activated by cytochrome P-450 in the liver and the active intermediate is then transported by the circulation to target tissues, particularly in the respiratory tract. Conversely, it is more likely that enzymatic activation occurs in the respiratory tract itself as has been shown by several autoradiographic studies with NDEA. Reznik-Schuller and Hague (1981a,b) found that (^{3}H)-NDEA and its metabolites were selectively bound in specific cell types of the trachea and that NDEA-induced tumors developed from these cells. In the nasal cavity, Reznik-Schuller (1982) reported that 1 hour after administration by gavage of a single dose of (^{3}H)-NDEA to Syrian golden hamsters, most bound radioactivity was concentrated in the mucous cells of the respiratory epithelium and the secretory cells of submucous glands. These cells are rich in endoplasmic reticulum, the major source of cytochrome P-450 enzymes which are involved in the metabolic activation of N-nitrosamines (Reznik-Schuller and Hague, 1981b; Schuller and McMahon, 1985).

The metabolism of NDEA is typical of the structurally simple dialkylnitrosamines. NDEA is metabolized by α-C-hydroxylation involving cytochrome P-450. This oxidative monodealkylation yields the unstable α-hydroxy-N-nitrosamine which decomposes to acetaldehyde and ethyldiazonium hydroxide (Montesano and Bartsch, 1976; Hecht et al., 1983). The aldehyde generated is further oxidized to yield CO_2,

which is the major product of NDEA metabolism *in vivo* (Heath, 1962; Mundt and Hadjiolov, 1974). The ethyldiazonium hydroxide is unstable and may sufficiently react with H_2O to decompose and be excreted from the body (Hecht *et al.*, 1983). Blattmann and Preussman (1973) found N-nitrosoethyl-N-(2-hydroxyethyl)amine and N-nitrosoethyl-N-(carboxymethyl)amine in the urine of rats after they were given NDEA. The ethyldiazonium hydroxide is also thought to be capable of covalently modifying nucleophilic groups in cellular macromolecules to generate alkylating intermediates, such as ethylated derivatives of DNA (DNA ethylation or DNA adducts) (Hecht *et al.*, 1983).

In NDEA-treated rats and hamsters, several DNA adducts were produced in target organs (Becker *et al.*, 1985). In the lungs (nontarget organ) of rats, DNA adducts were not detected, while in the lungs (target organ) of hamsters, both O^7-ethylguanine (O^7-etG) and O^6-ethylguanine (O^6-etG) were detected following NDEA administration. In NDEA-treated rats, both O^7-etG and O^6-etG were detected in the livers (target organ). In rats, NDEA induces mainly liver tumors (Reid *et al.*, 1963; Lijinsky *et al.*, 1981), whereas only respiratory tract tumors develop in similarly treated Syrian golden hamsters (Herrold, 1964a; Montesano and Saffiotti, 1968; Montesano and Saffiotti, 1970). Therefore, the capability of electrophilic reactants to covalently modify DNA suggests that tissue differences in the

metabolism of NDEA by rats and hamsters are related to NDEA organotropism in these species.

DNA alkylation products are repaired by two distinct DNA repair processes. O^6-alkylguanine is repaired by the O^6-alkylguanine-DNA alkyltransferase which transfers the miscoding alkyl group (either methyl or ethyl) from the O^6-position of guanine to a sulfhydryl group of a cysteine in the repair protein, thereby restoring the fidelity of the DNA and inactivating the receptor protein. The 7-alkylguanine is lost from DNA by a combination of spontaneous and enzyme catalyzed depurination (Lindahl, 1982). The failure to correctly repair these premutagenic DNA adducts suggests that mutations occurring during DNA replication are critical for the initiation of carcinogenesis by N-nitrosamines (Cayama et al., 1978).

Respiratory Carcinogenesis

Hamsters. Of the three different hamster species (European, Chinese and Syrian golden) used in research, the Syrian golden hamster is utilized most frequently as a model in N-nitrosamine-induced respiratory tract carcinogenesis. The upper respiratory tract (nasal cavity and trachea) of this species appears to be highly sensitive to the carcinogenic effects of NDEA. Mohr et al. (1966) reported multiple papillomas in the tracheas in the offspring of Syrian golden hamster dams that had been given daily subcutaneous doses of 2 mg NDEA for 1-7 days during the second half of the gestation period. The tracheas of the

NDEA-treated mothers had similar tumors 25 weeks after the first administration. In another study, a single subcutaneous dose of 55, 33 or 5.5 mg NDEA/kg of body weight given to newborn Syrian golden hamsters induced tumors in the tracheas, larynges, nasal cavities, bronchi and lungs (Montesano and Saffiotti, 1970). The tumors observed in the tracheas were papillomas with histopathologic features similar to those observed in adult NDEA-treated Syrian golden hamsters (Herrold and Dunham, 1963; Herrold, 1964a; Schuller and McMahon, 1985). In a sequential study of NDEA-induced tracheal tumors in Syrian golden hamsters, there were initial ultrastructural changes in the epithelial cells of tracheas including an increase in the amount of rough endoplasmic reticulum and a change in the orientation of the nuclei from perpendicular to parallel to the basement membrane. However, the tracheal tumors, including papillomas and squamous cell carcinomas, arose from the basal cells, although these cells apparently were not affected during the initial treatment (Reznik-Schuller, 1980).

Neoplasms described in the nasal cavities of NDEA-treated hamsters include papillomas, adenocarcinomas, anaplastic carcinomas and neuroepithelial tumors (Montesano and Saffiotti, 1970). These neoplastic changes were similar to those reported by Herrold (1964b), Montesano and Saffiotti (1968) and Stenback (1973). In addition,

papillomas of the bronchi and adenomas and carcinomas of the lungs were also seen (Montesano and Saffiotti, 1970).

Many other N-nitrosamines have been investigated for their ability to cause tumors of the respiratory tract. N-nitrosodimethylamine (Herrold, 1967), N-nitroso-β-hydroxypropyl-n-propylamine (Pour et al., 1974), N-nitrosopiperidine (Haas et al., 1973), N-nitrosopyrrolidine (McCoy et al., 1980) and N-nitrosonornicotine (Hilfrich et al., 1977) have been reported to be weak carcinogens for the respiratory tract, whereas N-nitrosodiethanolamine (Hilfrich et al., 1978), N-nitroso-di-n-propylamine and N-nitroso-β-oxopropyl-n-propylamine (Pour et al., 1974), N-nitrosomethyl-n-propylamine (Pour et al., 1974; Pour et al., 1979), N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (Pour et al., 1979), N-nitrosomorpholine (Haas et al., 1973) and N-nitrosohexamethyleneimine (Althoff et al., 1973) have been reported to be moderate carcinogens for the respiratory tract. N-nitroso-2,6-dimethylmorpholine (Althoff et al., 1978; Reznik et al., 1978) and N-nitrosodiallylamine (Althoff et al., 1973) are similar to NDEA in that they also are strong inducers of nasal cavity tumors in Syrian golden hamsters.

Rats. In rats, NDEA induced mainly tumors of the liver (Reid et al., 1963; Lijinsky et al., 1981). Available information indicates that NDEA is not a potent carcinogen for the nasal cavity in this species (Lijinsky and Taylor, 1978; Beer et al., 1986). However, in rats,

N-nitrosodimethylamine and N-nitroso-hydroxypropyl-n-propylamine (Reznik et al., 1975), N-nitroso-di-isopropanolamine and N-nitroso-3,4-dichloropyrrolidine (Mohr et al., 1977), N-nitrosomorpholine, N-nitroso-2,6-dimethylmorpholine and N-nitrosoheptamethyleneimine (Lijinsky and Taylor, 1975a), N-nitroso-3-piperidinol, N-nitroso-4-piperidinol and N-nitroso-4-piperidinone (Lijinsky and Taylor, 1975b), N-nitrosopiperidine (Lijinsky and Taylor, 1975b; Taylor and Lijinsky, 1975) and N-N'-dinitroso-2,6-dimethylpiperazine (Hecht et al., 1980) strongly induced nasal cavity tumors. N-nitrosamine-induced tumors arising in the nasal cavity included papillomas, adenomas, adenocarcinomas, squamous cell carcinomas, esthesioneuroepitheliomas and rhabdomyosarcomas.

A procedure has been standardized for the histopathologic examination of the nasal cavity in rats (Young, 1981). This procedure results in four nasal tissue slices from standard comparable regions and permits a thorough gross examination without destroying anatomic relationships. After embedding and sectioning, histopathologic evaluation of four transverse sections of the nasal cavity results in a uniform assessment and interpretation of histologic changes. Serial sections can also be made if necessary.

Mice. Relatively few N-nitrosamines have been reported to induce respiratory tract tumors in mice. NDEA (Clapp and

Craig, 1967; Ward et al., 1984), N-nitroso-N-bis(2-hydroxypropyl)amine and N-nitroso-N-bis(2-acetoxypropyl)-amine (Green et al., 1980) have been used for carcinogenicity studies in mice, and, so far, all of these N-nitrosamines mainly induced liver tumors, and their carcinogenic effect in the respiratory tract (nasal cavity and lung) was not great.

Other animals. Carcinogenicity studies with N-nitrosamines, particularly NDEA, have also been done in other animal species. Guinea pigs (Argus and Hoch-Ligeti, 1963), rabbits (Rapp et al., 1965), cats (Schmahl et al., 1978), dogs (Schmahl et al., 1964; Hirao et al., 1974), pigs (Schmahl et al., 1967), monkeys (Kelly et al., 1966), parakeets (Schmahl et al., 1966), chickens (Schmahl et al., 1978), fish (Stanton, 1965), frogs (Khudoley, 1977) and snakes (Schmahl and Scherf, 1983) when administered NDEA developed mainly liver tumors.

Human beings. Strong evidence that N-nitrosamines cause cancer in people is lacking. However, epidemiological studies suggest that N-nitrosamines contribute to human carcinogenesis. Consumption of large quantities of Cantonese-style salted fish (Ho, 1972; Yu et al., 1986) or exposure to cigarette smoke (Lin et al., 1973; Mabuchi et al., 1985) has been implicated in the development of nasopharyngeal carcinomas in people. Cantonese-style salted fish and tobacco smoke contained a high level of N-nitrosodimethylamine and NDEA (Fong and Walsh, 1971;

McCormick et al., 1973; Iyengar et al., 1976). Tobacco-specific N-nitrosamines, including N-nitrosonornicotine, 4-(N'-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, N'-nitrosoanabasine and N'-nitrosoanatabine were found at high concentrations in tobacco smoke, snuff and chewing tobacco (Boyland et al., 1964; Hecht et al., 1978; Hoffmann et al., 1979). Therefore, N-nitrosamines are likely to be involved in the development of tobacco-related tumors of the larynx, lung, oral cavity, esophagus, pancreas and urinary bladder (Hirayama, 1981; Hoffmann and Adams, 1981; Winn et al., 1981).

Polybrominated Biphenyls

Introduction

Commercial mixtures of polybrominated biphenyls (PBB), marketed as Firemaster (FM) (Michigan Chemical Co/US), Octa and Deca (White Chemical Co/US), Bromkal 80-9D (Chemische Fabrik Kalk/West Germany), Flammex B-10 (Berk/Great Britain), Adine 0102 (Ugine Kuhlmann/France) and HFO 101 (Hexcel/UK) have been widely used as a flame retardant additive for numerous polymeric resins (Brinkman and Dekok, 1980; Safe, 1984). Firemaster was produced by Michigan Chemical Company which also manufactured Nutrimaster, a magnesium oxide-containing feed supplement. In 1973, a major contamination of the food chain by PBB occurred in Michigan. This was due to inadvertently substituting FM for Nutrimaster in feed formulation. This error subsequently

resulted in widespread contamination of meat, poultry and milk products as a result of PBB-contaminated feed being fed to dairy cattle and other livestock. There was major concern as to the implications on human health (Jackson and Halbert, 1974; Carter, 1976; Kay, 1977).

The mixture of PBB consists of approximately 30 different congeners, and 13 of these are major congeners (Moore et al., 1980; Aust et al., 1982). The mixture causes a mixed-type induction of liver microsomal drug-metabolizing enzymes since it induces phenobarbital (PB)-type and 3-methylcholanthrene (MC)-type microsomal enzymes (Dent et al., 1976). The PB-type of microsomal enzyme induction results in increased activity of cytochrome P-450, whereas the MC-type of microsomal enzyme induction results in increased activity of P-448 (P_1-450) (Alvares et al., 1967; Lu and West, 1978; Wilkinson, 1980). Apparently, there is a structure-activity correlation between the toxicity of an individual congener and its ability to induce specific microsomal enzymes. For example, a minor congener in FM BP-6, 3,3',4,4'-tetrabromobiphenyl (TBB) (Robertson et al., 1982; Robertson et al., 1983; Millis et al., 1985b) was reported to be an MC-type inducer and was considered toxic. Other MC-type inducers, 3,4,4'-tribromobiphenyl, 3,4,4',5-tetrabromobiphenyl, 3,3',4,4'-5-pentabromobiphenyl are also toxic (Robertson et al., 1982). Robertson et al. (1982) suggested that the presence of bromine atoms at both para positions and at one, two, three or four meta positions on

biphenyl rings contributes to the properties of PBB as a 3-MC type inducer. Mono ortho-substituted congeners in FM which possess only lateral positions of bromine atoms may also be MC-type inducers. The PBB congeners in FM that have two ortho-substitutions, such as 2,2',3,4,4',5,5'-heptabromobiphenyl (Moore et al., 1979) and 2,2',4,4',5,5'-hexabromobiphenyl (HBB) (Moore et al., 1978; Akoso et al., 1982a) are PB-type inducers and are relatively nontoxic. Of the congeners in the commercial PBB mixture, 2,4,5,3',4',5'-hexabromobiphenyl (HBB) (Dannan et al., 1978b), 2,4,5,3',4'-pentabromobiphenyl (Dannan et al., 1982b) and 2,3,4,5,3',4'-HBB (Dannan et al., 1982a) are each classified as mixed-type microsomal enzyme inducers and are toxic.

At room temperature, the commercial mixtures of PBB are white, odorless solids, insoluble in water but highly soluble in fat and organic solvents, such as toluene, benzene and chloroform. These compounds begin to melt at 72^{o} C and decompose at 300 to 400^{o} C (Kay, 1977). Most congeners of PBB are slowly metabolized and highly lipophilic (Brinkman and Dekok, 1980; Tuey and Matthews, 1980). Ultraviolet radiation will readily degrade PBB to lesser brominated biphenyls (Ruzo and Zabik, 1975; Millis et al., 1985a).

Metabolism

In vitro metabolism of congeners of PBB by liver microsomal drug metabolizing enzymes has been demonstrated.

Among the major congeners in FM, 2,2',4,5,5'-pentabromobiphenyl and 2,2',3,4',5',6-hexabromobiphenyl (HBB) are most rapidly metabolized, and similar findings have been reported for other PBB congeners, such as 2,2'-dibromobiphenyl (DBB), 2,4,2',5'-tetrabromobiphenyl (TBB) and 3,3',4,4'-TBB (Dannan et al., 1978a; Millis et al., 1985b; Mills et al., 1985). The major congener in FM, identified as 2,2',4,4',5,5'-HBB, is slowly metabolized (Dannan et al., 1978a). The metabolism of PBB congeners appears to be correlated with the number and position of bromines on the biphenyl rings. According to Moore et al. (1980), the metabolism of PBB is facilitated when the number of para substitutions decreases, the number of ortho substitutions increases and the total number of substitutions decrease.

FM BP-6, as already mentioned, is characterized as a mixed (PB and MC)-type inducer of liver microsomal drug metabolizing enzymes. The microsomal enzymes induced by PB- and MC-type inducers are located mainly in the liver. These enzymes also are present to some degree in extrahepatic tissues, such as in the respiratory tract (Azzopardi and Thurlbeck, 1968; Schuller and McMahon, 1985; Roberts et al., 1986). The microsomal enzyme system is a nonspecific metabolizing system which possesses catalytic activity towards many substrates, such as drugs and xenobiotics, via conjugation and excretion as well as generation of dangerous reactive intermediates (Kappas and Alvares, 1975; Vainio and Hietanen, 1980; Gelboin, 1983). The system consists of

complex enzymes including flavoproteins (cytochrome P-450 reductases) (Yasukochi and Masters, 1976; Guengerich, 1977), hemoproteins (cytochrome P-450) (Cooper et al., 1965; Lu and West, 1980) and a phospholipid, phosphatidylcholine (Strobel et al., 1970). The microsomal enzyme system is an NADPH-dependent transport chain that inserts one atom of atmospheric oxygen (O_2) into their substrates (monooxygenase) (Mason, 1957; Conney, 1967). This electron transport pathway transfers electrons via cytochrome P-450 reductases (flavoproteins) from NADPH to the terminal oxidases, cytochrome P-450 (Lu et al., 1969; Nebert et al., 1982). Since one atom of O_2 is incorporated into the substrate at the P-450 enzyme active site and the other atom of O_2 is ultimately reduced to water, this system is also called the mixed function oxidase (MFO) system.

It has been postulated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can stereospecifically bind to a cytosolic polypeptide receptor called the TCDD receptor (Ah receptor) that mediates a 3-MC type microsomal enzyme induction with aryl hydrocarbon hydroxylase (AHH) activity (Poland et al., 1976; Jones et al., 1985). Compounds, such as 3,3',4,4'-tetrabromobiphenyl, 3,3',4,4',5,5'-hexabromobiphenyl, 2,3,4,5,3',4'-hexabromobiphenyl and 3,3',4,4'-tetrachlorobiphenyl that are closely analogous in chemical and toxicological properties to the TCDD, have the ability to bind to this receptor (Dannan et al., 1982a; Millis et al., 1985b; Buchmann et al., 1986). The complex of receptor

and compound translocates to the nucleus in a temperature-dependent step (Okey et al., 1980). Within the nucleus, this complex appears to associate with chromatin (Eisen et al., 1983; Roberts et al., 1985), thereby, inducing synthesis of specific messenger ribonucleic acid (mRNA) (Negishi and Nebert, 1981; Tukey et al., 1982). The mRNA leaves the nucleus, moves to the rough endoplasmic reticulum (RER) which subsequently is translated into new protein (enzymes), including P-448 and associated AHH (Poland et al., 1979; Eisen et al., 1983).

Pathology

Gross lesions and clinical signs. According to Collins (1982), guinea pigs are much more sensitive to the lethal effects of PBB than Syrian golden hamsters. Hamsters treated with up to 3200 mg PBB/kg body weight had reduced weight gain, but no deaths occurred. However, when single doses of 400 or 800 mg PBB/kg body weight were given to guinea pigs, severe body weight loss and mortality were observed. In all guinea pigs that died, clinical signs including anorexia, rough hair coat and hypersalivation were observed. In both of these species, there were hepatomegaly and thymic involution. Studies have shown that TCDD is more highly toxic than PBB in laboratory rodents. The guinea pig is the most sensitive to the lethal effects of TCDD with an LD_{50} of 2 µg TCDD/kg (Gupta et al., 1973) whereas the hamster is the least sensitive with an LD_{50} of 1157 µg TCDD/kg (Olson et al., 1980). Loss of weight, hepatomegaly

and thymic involution have also been reported in rats (Sleight and Sanger, 1976), mice (Gupta et al., 1983a,b), chickens (Ringer, 1978; Dharma, 1980), pigs (Ku et al., 1978; Howard et al., 1980), cattle (Jackson and Halbert, 1974), dogs (Farber et al., 1978) and nonhuman primates (Allen et al., 1978) exposed to PBB.

Massive enlargement of the common bile duct was observed in rats after prolonged feeding of a vitamin A-deficient diet containing 100 ppm PBB (Darjono et al., 1983). However, Wasito (1984) failed to observe any gross lesions in the common bile duct in rats fed a vitamin A-deficient diet containing 100 ppm PBB for 28 days.

A variety of clinical signs and gross lesions in people were reported to be associated with an acute or chronic exposure to PBB. These include porphyria, immunologic defects, headaches, fatigue, bronchitis, persistent coughing and reproductive failures (Bekesi et al., 1978; Valciukas et al., 1978; Stross et al., 1981).

Histopathology. The histopathologic lesions associated with PBB toxicosis consisted mainly of enlargement and intracytoplasmic vacuolation of hepatocytes and hepatic necrosis in the livers of rats (Sleight and Sanger, 1976), hamsters (Collins, 1982), guinea pigs (Sleight and Sanger, 1976; Collins, 1982) or sows and their pigs (Werner and Sleight, 1981). The ultrastructural hepatic lesions in rats have been characterized by an increase in size of hepatic mitochondria, an increase in the amount of smooth

endoplasmic reticulum (SER) and an increase in cytoplasmic vacuolation (Sleight and Sanger, 1976; Mangkoewidjojo, 1979; Render et al., 1982).

Hyperplasia of extraparenchymal bile ducts was reported in rats after prolonged feeding of a vitamin A-deficient diet containing 10 or 100 ppm PBB (Darjono et al., 1983). Similar but less pronounced lesions were observed when rats were fed a vitamin A-deficient diet containing PBB for 28 days (Wasito, 1984).

In the thymus, the lesions were characterized by loss of demarcation between the cortical and medullary regions as well as depletion of cortical lymphocytes (Howard et al., 1980; Collins, 1982). In the thyroid, hypertrophy and hyperplasia of the follicular cells and vacuolation and depletion of colloid were reported in rats fed the mixture of PBB (Sleight et al., 1978; Mangkoewidjojo, 1979; Akoso et al., 1982b).

Carcinogenicity. Results of experimental studies suggest that PBB and its congeners induce neoplastic nodules and hepatocellular carcinomas in the livers. For example, hepatocarcinogenic effects were observed in the livers of rats (Kimbrough et al., 1981; Gupta et al., 1983b) and in mice (Gupta et al., 1983b) given high doses of PBB. Results of in vitro studies by Williams et al. (1984) and Kavanagh et al. (1985) indicated that Firemaster BP-6 (FM) and 2,2',4,4',5,5'-hexabromobiphenyl (245-HBB) are not genotoxic or mutagenic. FM and 245-HBB were reported to inhibit

metabolic cooperation in Chinese hamster V-79 cells (Tsushimoto et al., 1982) and WB-F344 (rat epithelial) cells (Evans, 1987) in culture, a property of known tumor promoters (Yotti et al., 1979; Trosko et al., 1981). Jensen et al. (1982) and Jensen et al. (1984) concluded that PBB act as a hepatic tumor promoter. In these studies, PBB consistently enhanced the formation of γ-glutamyl transpeptidase (GGT) positive enzyme-altered foci (EAF) and neoplastic nodules in the livers of rats previously initiated with NDEA. A few hepatocellular carcinomas were also observed. In addition, Jensen and Sleight (1986b) demonstrated that simultaneous exposure to 2,2',4,4',5,5'-hexabromobiphenyl and 3,3',4,4',5,5'-hexabromobiphenyl caused a synergistic effect on hepatic tumor promotion.

In the skin, tumor promoting activity of PBB was reported by Poland et al. (1982) who found skin papillomas in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-initiated HRS/J hairless mice.

Factors Modifying Respiratory Carcinogenesis

The respiratory tract has been reported to be the main target organ of NDEA carcinogenicity in Syrian golden hamsters. There is relatively little information concerning the factors that may modify respiratory carcinogenesis and give either an increased or decreased tumor yield. For example, cigarette smoke was reported to be able to potentiate tumor development in the nasal cavities, larynges

and tracheas in Syrian golden hamsters given 12 weekly subcutaneous injections of NDEA at a total dose of 10 mg/animal during lifetime observation. When 1% vitamin C in the diets was fed to the Syrian golden hamsters treated with NDEA and exposed to cigarette smoke, incidence of the nasal cavity tumors was decreased, while incidence of the laryngotracheal tumors was increased (Harada et al., 1985).

Schuller and McMahon (1985) reported that piperonylbutoxide (PIP) possesses respiratory (lung and trachea) anticarcinogenic activity in Syrian golden hamsters initiated with NDEA. They concluded that PIP causes a decrease in carcinogenic effects of NDEA because this compound inhibits microsomal enzyme activity in the respiratory tract (Boyd and Burka, 1978; Boyd et al., 1978) that may lead to inhibition of metabolic activation of NDEA.

A combined treatment of Syrian golden hamsters with two different respiratory carcinogens resulting in a synergistic respiratory carcinogenic response was reported by Montesano et al. (1974). They found an increase in incidence of malignant respiratory tract tumors in Syrian golden hamsters given a combined treatment of benzo(a)pyrene (BP) plus ferric oxide (Fe_2O_3) (BP-Fe_2O_3) and NDEA as compared with Syrian golden hamsters given BP-Fe_2O_3 or NDEA alone.

Initiation and Promotion in Carcinogenesis

Carcinogenesis is thought to be a multistep process consisting of two major phases: initiation and promotion

(Berenblum, 1941; Pitot and Sirica, 1980; Miller and Miller, 1986). Initiation is defined as an irreversible event that occurs rapidly after treatment with an agent, either chemical, physical or biological, that is capable of directly or indirectly altering the native molecular structure of the genetic component (DNA) of cells (genotoxic) (Cairns, 1975; Farber, 1981; Pitot et al., 1981). Such alteration may be the result of a covalent binding (mutation) of an initiating agent (initiator), or one of its metabolites, to DNA molecules or the result of damaged DNA-repair enzyme systems (nonmutation). The initiator may therefore cause either one or more complete scissions of the DNA chain, an elimination of purine or pyrimidine sequences of DNA, or an error in repair of DNA (Boutwell, 1974; Farber, 1981; Pitot et al., 1981). Another characteristic of the initiator is that it can induce tumors without a promoter when a high enough dose is used (Berenblum, 1941; Pitot et al., 1981).

Promotion is a reversible event that occurs after treatment with an agent, such as a hormone, drug or plant product, that alters the phenotypic expression of genetic information of the cell. The agent does not directly react with the DNA, but rather affects its phenotypic expression by a variety of mechanisms involving its interaction with cell surface receptors or with cytoplasmic and nuclear components and functions (Boutwell, 1974; Pitot and Sirica, 1980; Pitot et al., 1981). The promoting agent (promoter)

must be capable of eliciting tumors when given to an animal repeatedly after administration of a subcarcinogenic dose of an initiator. Tumors are not seen if the sequence is reversed (e.g. treatment of the animal with the promoter first followed by treatment with the initiator) (Berenblum, 1941; Boutwell, 1974; Williams, 1981). Although tumor promotion is generally considered to be a relatively long-term phenomenon, requiring weeks or months of administration of a promoting agent, short-term exposure to PBB (Jensen et al., 1983; Rezabek et al., 1987) or PCB (Pereira et al., 1982) is as effective as long-term exposure in promoting the development of enzyme-altered foci in an initiation-promotion bioassay of hepatocarcinogenesis. These chemicals are highly persistent in animal tissues and are therefore present in target tissues throughout the promotion phase.

At the present time, the concept of initiation and promotion of carcinogenesis as first developed in the skin of rabbits (Rous and Kidd, 1941) and mice (Berenblum and Shubik, 1947) has been used for several organ systems, including liver (Ward et al., 1984; Diwan et al., 1985; Buchmann et al., 1986), urinary bladder (Miyata et al., 1985), kidney (Diwan et al., 1985), thyroid (Diwan et al., 1985), lung (Pereira et al., 1985), colon and pancreas (Pitot, 1979; Farber and Cameron, 1980).

CHAPTER I

THE PROMOTING EFFECT OF POLYBROMINATED BIPHENYLS ON NASAL AND TRACHEAL TUMORS IN SYRIAN GOLDEN HAMSTERS

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Introduction

A wide variety of environmental chemicals has been found to induce tumors of the respiratory tract in various animal species, not only by the inhalation route of exposure, but also when administered in feed, drinking water (enteral route of exposure) or by injection (parenteral route of exposure). In past studies involving respiratory neoplasia induced by environmental chemicals, major emphasis was placed on the lower respiratory tract, such as the bronchi and lungs. Many investigators did not adequately examine the upper respiratory tract, especially the nasal cavities and tracheas. Current emphasis on tumors in the upper respiratory tract of man and animals is evidenced by the publication by CRC Press, Inc. of a 3 volume monograph that specifically deals with nasal cavity tumors (Reznik and Stinson, 1983) and by continuing research on the pathogenesis and morphogenesis of tracheal tumors (Reznik-Schuller, 1980; Reznik-Schuller, 1983). N-nitrosamines appear to be one of the most important groups of chemical carcinogens known to induce the development of upper

respiratory tract (nasal cavity and trachea) tumors in people and animals. More importantly, however, combined exposure to N-nitrosamines and other environmental contaminants may have the potential to increase the incidence of specific cancers, especially in various segments of the respiratory system.

N-nitrosodiethylamine (NDEA, diethylnitrosamine, DEN, N-N-diethylnitrosamine, N-ethyl-N-nitrosoethanamine) has been studied extensively. NDEA serves as a prototype for a group of environmental chemicals, the N-nitrosamines. NDEA is considered a potent carcinogen (Preussmann and Stewart, 1984) and is commonly found in the air (Fine et al., 1976), water (Fiddler et al., 1977) and food (Panalaks et al., 1974; Iyengar et al., 1976) and can be formed in vivo from ingested amines and nitrites (Sen et al., 1969). NDEA is considered to act as an initiator (genotoxin). It is metabolically activated via cytochrome P-450, and reactive electrophilic reactants formed during metabolism react with nucleophilic groups in cellular macromolecules (DNA) to yield alkylating intermediates (DNA adducts). Certain cell types in the nasal cavity (Reznik-Schuller, 1982), trachea (Reznik-Schuller and Hague, 1981a,b) and lung (Becker et al., 1985) can metabolize NDEA, and tumors develop in these cells (Herrold, 1964b; Montesano and Saffiotti, 1970; Reznik-Schuller, 1980). Chemical carcinogens are considered to be related to the process of carcinogenesis through a multistep process involving initiation and promotion.

However, most studies of carcinogenicity by NDEA for the respiratory tract have used large doses and/or repeated exposures of NDEA over long periods of time and have ignored the possibility that promotion by other chemicals may play a role in the natural development of these tumors.

Polybrominated biphenyls (PBB) belong to the class of toxic polyhalogenated aromatic hydrocarbons (PHAH) which include tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCB). These chemicals are environmental contaminants and are known to be toxic and hepatocarcinogenic in rodents (Kimbrough et al., 1978; Kimbrough et al., 1981; Gupta et al., 1983b). Several reports indicated that PBB have tumor promoting (epigenetic) activity (Jensen et al., 1982; Jensen et al., 1984; Jensen and Sleight, 1986b). In a recent study by Jensen and Sleight (1986a) designed to assess hepatic tumor promotion, PBB apparently enhanced the development of nasal tumors in rats. PBB decreased the latency time, but did not alter the incidence of nasal carcinomas. PBB increased the incidence of nasal adenomas in NDEA-initiated rats. However, little is known about organ or tissue specificities or tumor promoting effect of PBB. Certain PHAH accumulate in the epithelial cells of the respiratory tract (Brandt, 1977; Appelgren et al., 1983). These cells are rich in cytochrome P-450 (Hadley and Dahl, 1983; Voight et al., 1985; Dahl, 1986) and PHAH can induce these enzymes in these cells (Bond, 1983; Voight et al., 1985). This therefore led to

the hypothesis that PBB could act as a tumor promoter at nonhepatic sites, especially in the upper respiratory tract.

The first objective of this study was to determine the tumor promoting effect of PBB on the respiratory tract of Syrian golden hamsters initiated with NDEA. The second objective was to determine and characterize early responses and/or precursor (preneoplastic) lesions in the mucosal cells of the trachea in young Syrian golden hamsters following administration of NDEA alone or after a combined administration of NDEA and PBB. It was hoped that these lesions, if any, would correlate with the tumor promoting ability of PBB in the tracheas. If indeed, chemicals, such as PBB, can promote tumors in the respiratory tract, results of this study are of special concern because people and animals are continually at risk for exposure to a wide variety of chemicals, such as N-nitrosamines, which may occur in the food chain. Many of these chemicals can also be inhaled and can locally affect the respiratory tract, particularly the nasal and tracheal epithelial cells. Also, these chemicals may selectively accumulate in these cells after any systemic route of exposure. Therefore, persistent environmental chemicals, such as PBB, PCB and TCDD, which accumulate in the food chain and have been classified as hepatic carcinogens, may also promote tumors in nonhepatic sites, such as the respiratory tract. This study should provide valuable information to those concerned about environmental chemicals throughout the world.

Materials and Methods - Experiment 1

Experimental Design

One hundred and twenty male Syrian golden hamsters[a] were used. Hamsters were 5-6 weeks old and weighed approximately 82 g when the experiment was started. Hamsters were acclimated for 7 days prior to NDEA[b] administration and were fed a basal diet[c] and tap water ad libitum.

The hamsters were randomly alloted to 4 groups of 30 each. Hamsters were given a single dose of 0 (groups A and D) or 80 (groups B and C) mg NDEA/kg body weight subcutaneously. Seven days later, hamsters were fed a basal diet containing 100 mg PBB[d]/kg of diet (groups C and D) which was continued for 140 days. The other hamsters were fed the basal diet (groups A and B) until the experiment was terminated on day 273 as illustrated in Table 1.

The hamsters were housed 6 per cage in stainless wire-top, plastic cages and the bedding was changed twice a week. Cages containing PBB-treated hamsters were placed in filtered laminar flow units[e].

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[a]Charles River Breeding Laboratories, Inc., Portage, Michigan.

[b]Eastman Kodak Company, Rochester, New York.

[c]Wayne Rodent Blox, Research Animal Diets, Chicago, Illinois.

[d]Firemaster BP-6, Michigan Chemical Co., St. Louis, Michigan.

[e]Contamination Control, Inc., Lansdale, Philadelphia.

Table 1. Experimental design.

Group[a]	Treatment	Diets (day)		
		0-7	7-147	147-273
A	Control	basal diet	basal diet	basal diet
B	NDEA[b]	basal diet	basal diet	basal diet
C	NDEA PBB	basal diet	PBB-basal diet	basal diet
D	PBB[c]	basal diet	PBB-basal diet	basal diet

[a]Each group consisted of 30 hamsters and experiment was terminated on day 273.

[b]NDEA was given as a single subcutaneous dose of 80 mg/kg bw.

[c]PBB was given at 100 mg/kg of diet for 140 days beginning 7 days after NDEA administration.

N-nitrosodiethylamine Administration

To establish the desired dose of NDEA used in this experiment, the toxic effects of a single dose of NDEA on nasal tissues of Syrian golden hamsters were evaluated in a pilot study. Twenty-four male weanling Syrian golden hamsters at 3-4 weeks of age were used. After a 3-day acclimation, hamsters were randomly assigned to 8 groups of 3 each and given a single subcutaneous injection of NDEA in the dorsal region. NDEA solution was freshly dissolved in 0.9% NaCl[f] at the time of each injection. Each dose consisted of 0.5 ml of 0.9% NaCl in which 0.2, 0.4, 0.8, 1.6, 2.4, 3.2 or 4.0 mg of NDEA had been dissolved, corresponding to 5, 10, 20, 40, 60, 80 or 100 mg NDEA/kg bw; the average body weight being approximately 40 g. The control hamsters received a single injection of 0.5 ml of 0.9% NaCl. Twenty-four hours after NDEA administration, hamsters were killed with CO_2. At necropsy, the nasal tissues were collected and fixed in 10% neutral buffered formalin. Nasal tissues were then decalcified, and multiple frontal sections of the nasal cavity were made (Young, 1981). Processed portions of the nasal cavity were embedded in paraffin, sectioned with a microtome at 5 µm and stained with hematoxylin and eosin. Serial sections of the nasal cavity were also stained with Alcian blue-periodic acid-Schiff (AB/PAS).

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[f]Abbott Laboratories, North Chicago, Illinois.

Results of this study demonstrated that normal amounts of AB/PAS-stained glycoprotein in cells of Bowman's glands were observed in nasal cavities of control hamsters (Figure 1). Hamsters given single doses of 40, 60, 80 or 100 mg NDEA/kg bw had the most extensive inhibition of glycoprotein synthesis in cells of Bowman's glands in the olfactory region of the nasal cavities as determined by severe loss of AB/PAS staining material (Figure 2). At 20 mg NDEA/kg bw, this histochemical change was similar to, but less marked than that in hamsters given 40, 60, 80 or 100 mg NDEA/kg bw. At 5 or 10 mg NDEA/kg bw, there appeared to be inhibition of AB/PAS staining material in some cells of Bowman's glands in the olfactory region of the nasal cavity. Histologic lesions in cells of Bowman's glands were not seen in nasal cavities of control hamsters (Figure 3). Necrosis of cells in Bowman's glands occurred only in hamsters given 100 mg NDEA/kg bw. Changes in cells of Bowman's glands in hamsters given 80 mg NDEA/kg bw included individualization of cells with increased eosinophilia of the cytoplasm and condensation of nuclear chromatin, but necrosis was not evident (Figure 4). Histologic changes were not evident in hamsters given lower doses of NDEA. A single dose of 80 mg NDEA/kg bw was therefore used as the initiation dose in the present study because histopathologic changes in cells in Bowman's glands without necrogenic effects occurred in hamsters given this dose. Larger doses of NDEA may sufficiently destroy cells of Bowman's glands so that there

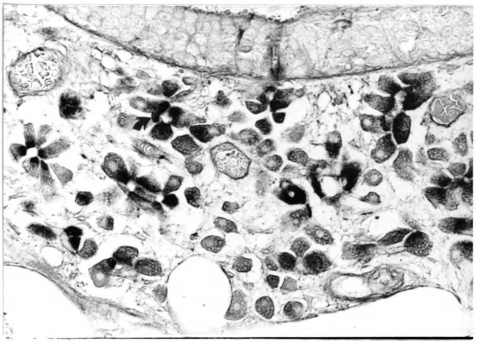

Figure 1. Photomicrograph of nasal cavity from a control hamster. Notice glycoprotein staining as dark AB/PAS-positive granules in cells of Bowman's glands (arrow). (AB/PAS, 900x.)

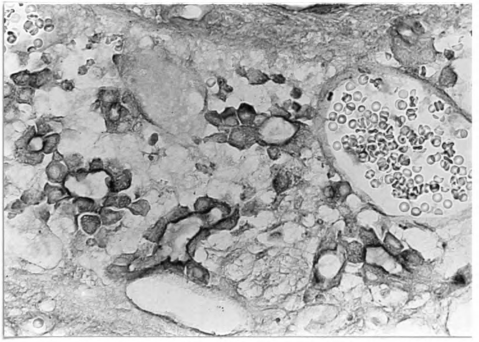

Figure 2. Photomicrograph of nasal cavity from a hamster given 80 mg NDEA/kg bw. Notice a dramatic decrease in the amount of glycoprotein in cells of Bowman's glands. (AB/PAS, 900x.)

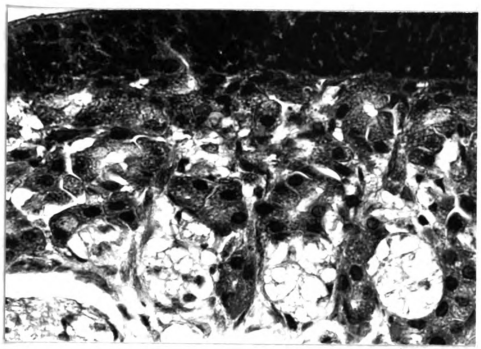

Figure 3. Photomicrograph of nasal cavity from a control hamster. Notice normal cells of Bowman's glands. (H & E stain, 1125x.)

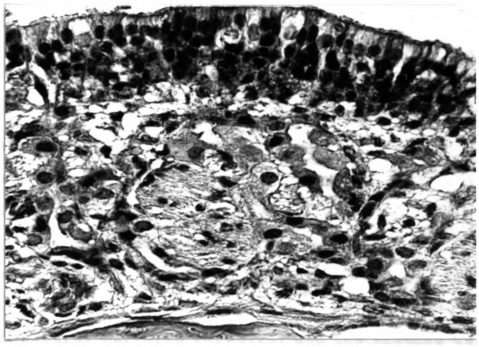

Figure 4. Photomicrograph of nasal cavity from a hamster given 80 mg NDEA/kg bw. Notice individualized cells of Bowman's glands with condensed nuclear chromatin. (H & E stain, 1125x.)

is no longer a sensitive population of cells at risk for its carcinogenic effects. Doses smaller than 80 mg NDEA/kg bw may not result in effective concentration of NDEA in target tissues.

Diet Preparation

The diet was prepared by adding appropriate amounts of PBB in corn oil to a basal diet. A premix containing 2 g of PBB/2 kg of feed was prepared by dissolving 2 g of PBB in 10 mg of corn oil by gentle heating and stirring[g], and the dissolved PBB was then added to 2 kg of feed. Diet containing 100 mg PBB/kg was prepared from the premix by mixing 400 g of the premix that contained 400 mg of PBB with 3.6 kg of feed.

Necropsy and Histopathologic Procedures

Hamsters were observed daily for clinical signs, and body weights were recorded every 2 months. The moribund and dead hamsters were necropsied immediately after discovery. The experiment was terminated on day 273. Hamsters were weighed and killed with CO_2, and all organs were routinely examined for gross lesions.

Specimens of nasal cavity, larynx, trachea, lung, liver and brain were fixed in 10% neutral buffered formalin. The lungs were perfused intratracheally with approximately 3 ml of 10% neutral buffered formalin, and the trachea was then

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[g]Thermolyne Type 1000 Stir Plate, Dubuque, Iowa.

ligated. After being fixed, tracheas were incised mediosagittally, the mucosae were carefully observed, and papillomas were counted using a dissecting microscope. At necropsy, the nasal cavities were infused with approximately 3 ml of 10% neutral buffered formalin through the posterior opening of the nasal pharynx and were fixed for 7 days. Nasal tissues were decalcified in a formic acid-sodium citrate solution for 9 days with 3 changes of the solution. Tissues were then neutralized for 6 hours in a sodium sulfate solution to enhance staining quality. Following decalcification and neutralization, the nasal cavities were washed overnight in running tap water and then returned to 10% neutral buffered formalin. Multiple frontal sections of the nasal cavity were made. The method for preparation of the nasal tissues was adopted from the procedures described for the rat (Young, 1981).

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

Statistical Evaluation

The data for body weight were analyzed using the one-way analysis of variance. For tumors of the larynx and trachea, the number of papillomas was calculated and the calculation was based on grossly observed papillomas at

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[h]Histomatic, Model 166, Fisher Scientific Co., Pittsburg, Pennsylvania.

necropsy. Differences in the number of papillomas between groups B and C were evaluated by one-way analysis of variance. For tumors of the nasal cavity, tumor incidence was calculated on the basis of results of histopathologic examination. Differences in the tumor incidence between groups B and C were analyzed by chi-square. The differences were considered significant at the level of $p<0.05$ (Gill, 1981).

Results

Clinical Signs

Adverse clinical signs were observed only in certain individual hamsters treated either with 80 mg NDEA/kg bw alone (group B) or with a combined treatment of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet (group C). These hamsters had tumors in the trachea. Clinical signs included rough hair coat, decreased activity and severe weight loss, and prior to death, the hamsters breathed with difficulty. Although certain hamsters in groups B and C lost weight, overall body weights were not significantly different from control values (Table 2).

Prior to day 273, 8 hamsters from group B and 10 hamsters from group C died. The cause of death was apparently due to asphyxia resulting from the obstruction of the respiratory tract by tracheal papillomas. The first hamster from these groups died on day 180. Six hamsters given 100 mg PBB/kg alone (group D) also died prior to day

Table 2. Body weights of Syrian golden hamsters (g).

Group	No. of hamsters	Treatment	Day 0	56	112	168	224	273
A	30	Control	82.1 ±6.3	146.2 ±13.9	151.9 ±16.4	155.8 ±20.8	160.8[c] ±20.8	159.9[c] ±18.4
B	30	DEN[a]	81.0 ±5.9	141.3 ±13.5	148.1 ±16.5	148.7 ±17.2	149.8[c] ±22.8	145.0[c] ±22.8
C	30	DEN PBB	82.2 ±5.1	134.2 ±12.3	152.0 ±13.9	134.7 ±20.4	136.4[c] ±25.2	132.8[c] ±24.6
D	30	PBB[b]	81.3 ±7.1	138.7 ±14.4	153.6 ±16.4	139.6 ±12.9	141.3[c] ±20.0	151.9[c] ±16.3

Data expressed as mean ± SD.

[a]NDEA was given as a single subcutaneous dose of 80 mg/kg bw.

[b]PBB was given at 100 mg/kg of diet for 140 days beginning 7 days after NDEA administration.

[c]Hamsters that died before day indicated were not included in data.

There were no significant differences among the groups of hamsters.

273. The cause of death was not apparent. Two hamsters from the control (group A) died at days 175 and 231, respectively. One of these hamsters had an extensive abscess in the small intestine and the other had chronic glomerulonephritis.

Gross Lesions

Gross lesions in the nasal cavity were found only when multiple frontal sections of the nasal cavities were made. The lesions were firm, whitish-yellow masses arising at multiple sites. Lesions frequently projected from the ethmoturbinates and occasionally perforated the nasal septa and involved the opposite side of the nasal cavities. However, none extended into the brain or caused marked swelling of the nose, orbital region, face or head.

Hamsters treated either with 80 mg NDEA/kg bw alone (group B) or with a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet (group C) had papillomas in the larynx and trachea. The papillomas were multiple, soft and papillary nodular masses that were 1-3 mm in diameter. In the tracheas of many hamsters in group C, the papillomas were located throughout the organ and often obstructed its lumen (Figure 5). The number of papillomas in the trachea of hamsters in group C was significantly higher than in group B ($p<0.05$). There was no significant difference in the number of papillomas of the larynx between groups C and B. Papillomas were not found in hamsters from the control (group A) or in hamsters given 100 mg PBB/kg alone (group

D). The number of papillomas in the larynx or trachea is illustrated in Table 3.

One hamster from group B and 2 hamsters from group C each had a firm, brown hepatic nodule that was approximately 1 cm in diameter. A splenic nodule was observed in 1 hamster from the control (group A). One hamster from this group that died had an extensive abscess in the region of the small intestine. The brain of 1 hamster from group B had a cyst filled with yellow-whitish fluid. Hamsters from group D that died had no significant gross lesions.

Histopathology

Papillomas were observed only in the anterior region of the nasal cavity, and they appeared to arise in the respiratory epithelium lining the nasoturbinates and maxilloturbinates. One hamster in group B and 9 hamsters in group C had these tumors. Histopathologically, the tumors were characterized by papillary growths and consisted of infolded masses of squamous cells, with intact basement membranes and minimal stroma (Figure 6). Adenomas (2 in group B; 1 in group C), adenocarcinomas (7 in group B; 2 in group C) and squamous cell carcinomas (2 in group C; 1 in group D) were mainly observed in the posterior region of the nasal cavity. Adenomas were composed of well differentiated neoplastic cells with a well defined glandular pattern (Figure 7), whereas adenocarcinomas were composed of poorly differentiated anaplastic cells with round or fusiform

Table 3. A comparison of papillomas in the larynx or trachea of Syrian golden hamsters treated either with NDEA alone or with a combination of NDEA and PBB.

	NDEA[a] (n=28)		NDEA + PBB (n=27)	
	Larynx	Trachea	Larynx	Trachea
	0	2	1	9
	0	1	1	4
	0	1	0	2
	0	0	1	3
	0	1	1	5
	0	1	0	5
	1	3	1	3
	1	2	0	5
	1	2	0	3
	0	3	0	4
	1	4	1	11
	2	2	0	3
	0	1	1	7
	0	1	0	5
	1	3	0	5
	0	1	0	3
	1	0	0	5
	0	0	0	7
	1	2	0	3
	2	1	1	3
	1	3	0	5
	1	2	0	3
	0	3	2	5
	1	3	0	1
	0	1	0	3
	1	0	1	3
	1	0	0	2
	1	1		
Total	17	44	11	117

[a]NDEA was given as a single subcutaneous dose of 80 mg/kg bw. PBB was given at 100 mg/kg of diet for 140 days beginning 7 days after NDEA treatment. Basal diet was fed from day 140 to day 273.

Total number of tracheal papillomas in hamsters treated with a combination of NDEA and PBB was significantly greater ($p<0.05$) than in hamsters treated with NDEA alone.

hyperchromatic nuclei, scanty cytoplasm and indistinct cell boundaries (Figure 8). In squamous cell carcinomas, there were areas of poorly differentiated squamous cells with keratinization and epithelial pearl formation (Figure 9). Other histopathologic features of the carcinomas included abnormal mitotic figures, debris of necrotic cells and inflammatory cells. The site of origin of the carcinomas was difficult to determine because of the widespread involvement of the tissues. In 1 hamster each from groups B and C, multiple types of tumors, such as papillomas and adenomas were also noted. The total incidence of nasal cavity tumors was not significantly different between groups B and C (Table 4). Tumors were not found in control hamsters (group A).

Papillomas developed only in the larynx and trachea of hamsters treated either with 80 mg NDEA/kg bw alone (group B) or with a combined treatment of 80 mg NDEA/kg bw and 100 mg PBB/kg (group C). Tumors were characterized by papillary growths consisting of squamous cells (Figure 10). Some of the tumors were markedly vascular. There was no evidence of invasiveness by any of these papillomas. In hamsters from group C, the tracheal papillomas appeared much more extensive and severe when compared to those from group B. The papillomas often almost completely filled the tracheal lumen (Figure 11).

Papillomas of bronchiolar origin were observed only in 2 hamsters from group C. The histopathologic features were

Table 4. Tumors in the nasal cavity of Syrian golden hamsters.

Group	No. of hamsters	Treatment	Tumors					
			sc	ac	a	p	p+a	Total
A	30	Control	0	0	0	0	0	0
B	30	NDEA[a]	0	7	2	1	1	11
C	30	NDEA PBB	2	2	1	9	1	15[c]
D	30	PBB[b]	1	0	0	0	0	1

[a]NDEA was given as a single subcutaneous dose of 80 mg/kg bw.

[b]PBB was given at 100 mg/kg of diet for 140 days beginning 7 days after NDEA treatment.

[c]Not significantly different from group B.

sc=squamous cell carcinoma; ac=adenocarcinoma; a=adenoma; p=papilloma.

similar to those described for the nasal cavity, larynx and trachea (Figure 12). One hamster from group B and 2 hamsters from group C had adenomas in the lungs. The adenomas appeared to originate in the alveoli. In one tumor, there were diffuse peripheral proliferative lesions with adenomatoid structures developing around the bronchiole (Figure 13). In adenomas located in the lung parenchyma, there were small areas of early adenomatoid structures in the alveoli (Figure 14). In addition, 1 hamster in group C had a papilloma and an adenoma.

The hepatic nodules observed in 1 hamster from group B and 2 hamsters from group C consisted of focal areas of large acidophilic hepatocytes with enlarged nuclei and prominent nucleoli. A cavernous hemangioma in the spleen was seen in 1 hamster from the control group. No tumors were observed in other organs, including the brain, heart, kidney, adrenal gland, stomach, small intestine and pancreas.

Materials and Methods - Experiment 2

Experimental Design

One hundred and forty-four male weanling Syrian golden hamsters, weighing approximately 42 g at 3-4 weeks of age were used. Hamsters were acclimated for 24 hours. The hamsters were randomly divided into 4 groups of 36 each and were given a single dose of 0 (groups A and D) or 80 (groups

B and C) mg NDEA[i]/kg body weight subcutaneously. Beginning three days after NDEA injection, hamsters were fed a basal diet (groups A and B) or a basal diet containing 100 mg PBB/kg of diet (groups C and D) throughout the experiment. Twelve hamsters in each group were euthanatized using CO_2 on days 21, 42 and 63, respectively, after NDEA treatment.

The hamsters were housed 6 per cage in stainless wire-top, plastic cages and the bedding was changed twice a week. The water was given ad libitum. Procedures for diet preparation were as described in Experiment 1.

Necropsy and Histopathologic Procedures

Hamsters were observed daily for clinical signs. At necropsy, tracheas were fixed in 10% neutral buffered formalin. Tracheas were then transversely divided into 3 cross sections at 2 mm in thickness at the upper, middle and lower portions. Each of these portions was processed for pathologic examination, sectioned at 5 µm and stained with hematoxylin and eosin. The remaining portions of incised tracheas were opened medio-longitudinally, and mucosae were observed for lesions using a dissecting microscope.

Results

Clinical Signs and Gross Lesions

There were no adverse clinical signs or gross lesions observed in any of the hamsters throughout the study.

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[i]Sigma Chemical Co., St. Louis, Missouri.

Histopathology

At 21 days, tracheas from the hamsters in all groups were histologically normal. At 42 days, 1 of 12 hamsters in group B had an area of focal epithelial cell hyperplasia with squamous cell features without keratin formation in the trachea (Figure 15). Hamsters from the other groups did not have tracheal changes at this time. At 63 days, 1 of 12 hamsters in group B and 2 of 12 hamsters in group C had early evidence of papillomas in the tracheas. Histopathologically, the tumors were similar to those described in Experiment 1, but the degree of mucosal changes was less severe (Figure 16).

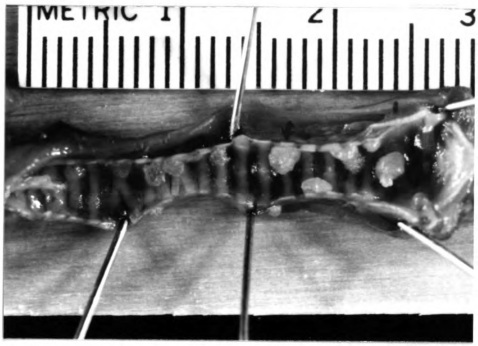

Figure 5. Papillomas of the trachea from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice multiple nodular lesions on the mucosal surface of the trachea (arrow).

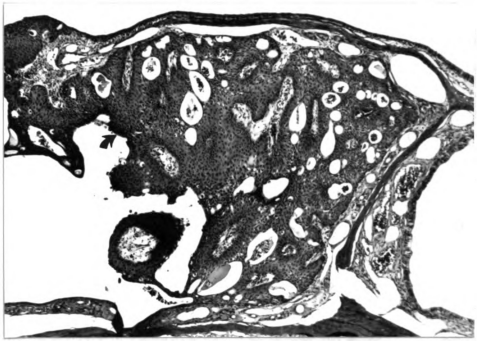

Figure 6. Papilloma of the nasal cavity from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice infolding of squamous cells arising from maxilloturbinates (arrow). (H & E stain, 144x.)

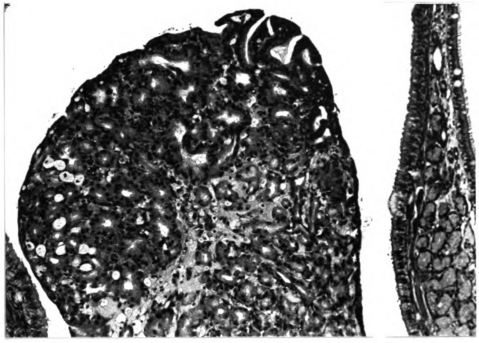

Figure 7. Adenoma of the nasal cavity from a hamster given 80 mg NDEA/kg bw. Notice glandular structures lined by cuboidal cells. Tumor had arisen from the surface of an endoturbinate. (H & E stain, 281x).

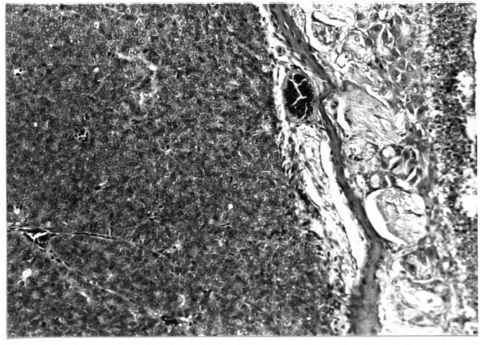

Figure 8. Adenocarcinoma of the nasal cavity from a hamster given 80 mg NDEA/kg bw. Notice a solid area of poorly differentiated cells with few glandular structures. (H & E stain, 360x.)

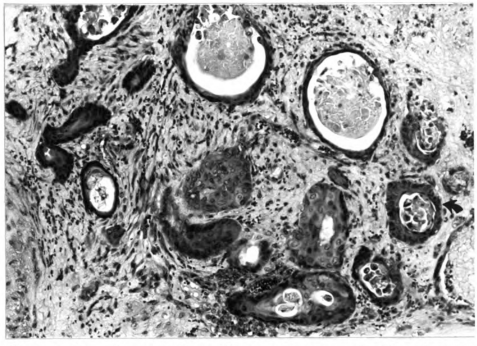

Figure 9. Squamous cell carcinoma of the nasal cavity from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice an area of poorly differentiated squamous cells with keratinization and epithelial pearl formation (arrow). (H & E stain, 281x.)

Figure 10. Papilloma of the trachea from a hamster given 80 mg NDEA/kg bw. Notice papillary growths consisting of squamous cells with a connective tissue stalk. Tumor had arisen from mucosal epithelial cells. (H & E stain, 112x.)

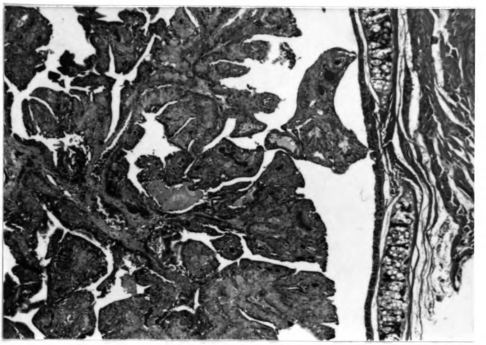

Figure 11. Papilloma of the trachea from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice papillary growths consisting of squamous cells which almost completely obstruct the lumen of the trachea. (H & E stain, 112x.)

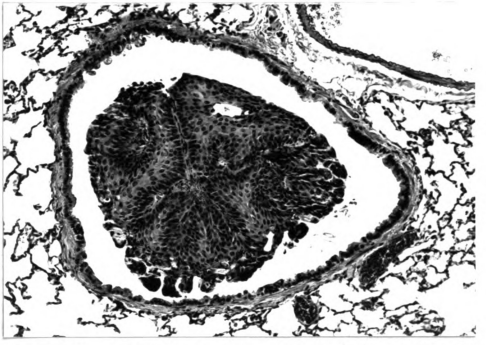

Figure 12. Papilloma in a bronchiole from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice squamous cells. (H & E stain, 281x.)

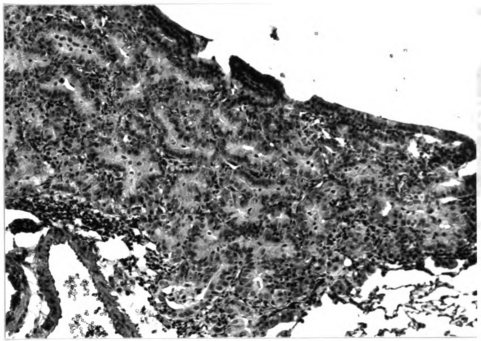

Figure 13. Adenoma of the lung from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice adenomatoid structures peripheral to the bronchiole. (H & E stain, 281x.)

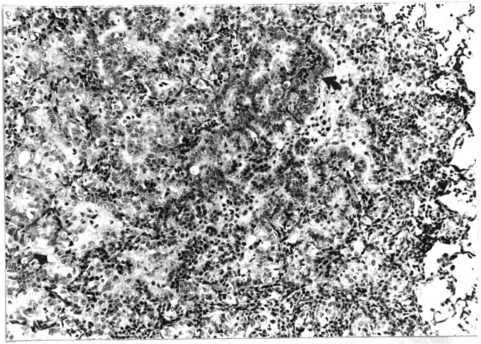

Figure 14. Adenoma of the lung from a hamster given a combination of 80 mg NDEA/kg bw and 100 mg PBB/kg of diet. Notice adenomatoid structures in the alveoli (arrow). (H & E stain, 281x.)

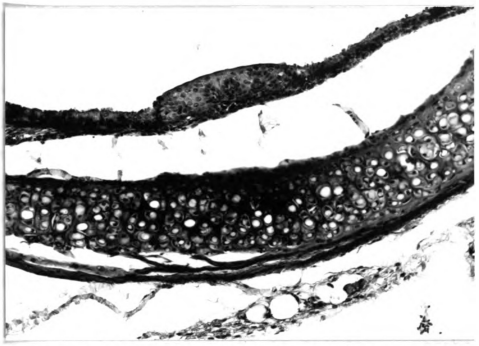

Figure 15. Trachea of a hamster fed a commercial diet for 42 days after administration of 80 mg NDEA/kg bw. Notice focal epithelial cell hyperplasia with squamous cell features without keratin formation. Detachment of mucosal epithelial cells is an artifact. (H & E stain, 281x.)

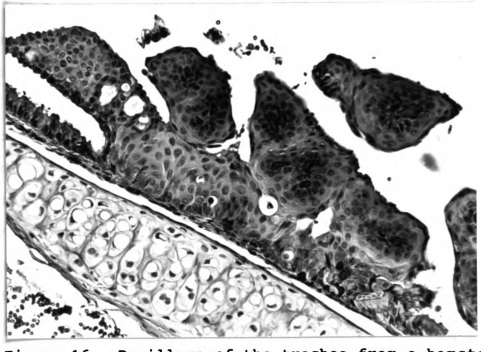

Figure 16. Papilloma of the trachea from a hamster fed a diet containing 100 mg of PBB/kg for 63 days after administration of 80 mg NDEA/kg bw. Notice papillary growths consisting of squamous cells. (H & E stain, 450x.)

Discussion

Results of this research indicate that PBB at a dietary concentration of 100 mg/kg increased the number of papillomas in the tracheas of Syrian golden hamsters initiated with a single dose of 80 mg NDEA/kg bw. This was determined by quantitating grossly observed papillomas under a dissecting microscope. The mechanism whereby PBB promotes the development of tracheal papillomas is unknown. Berenblum (1944) and Friedewald and Rous (1944) postulated that chemicals may act as promoters by causing chronic or recurrent toxicity resulting in necrosis, regenerative stimuli of initiated cells and subsequent neoplasia. Jensen et al. (1983) reported that hepatic tumor promotion by 3,3',4,4',5,5'-hexabromobiphenyl (345-HBB) as determined by enhancement of γ-glutamyl transpeptidase (GGT) enzyme-altered foci (EAF) occurred only at a dietary concentration (1.0 mg/kg) that was toxic. They proposed that the selective necrosis of hepatocytes caused by 345-HBB followed by an endogenous regenerative stimulus could promote the progressive growth of initiated cells. In the present study, however, necrosis was not evident in the tracheas in noninitiated hamsters given PBB for 140 days. Thus, cytotoxicity as a possible mechanism whereby PBB enhances development of tracheal papillomas is unlikely. Chronic dietary administration of as much as 100 mg PBB/kg of diet appeared to be relatively nontoxic as evidenced by gross and histologic appearance of the liver. This finding provides

further evidence that toxicity *per se* is not important in PBB carcinogenicity in the present study.

At the cellular level, PBB perhaps interact with a specific cell membrane receptor on the epithelial cells and subsequently reduce the high-affinity binding of epidermal growth factor (EGF) to the cell membrane receptor resulting in alteration in the process of cellular differentiation. There is evidence that TCDD causes a significant reduction in EGF receptor binding in *in vivo* and *in vitro* studies (Matsumura *et al.*, 1984; Moriya *et al.*, 1986). Action of TCDD on the EGF receptor may cause a hyperplastic response among epithelial cells and appears to be related to its tumor-promoting ability (Madhukar *et al.*, 1984). Poland *et al.* (1982) have found that TCDD and PBB promote papillomas in the skin of hairless mice initiated with N-methyl-N'-nitro-N-nitrosoguanidine.

Gap junction-mediated metabolic cooperation could account for normal cell growth, differentiation and development (Hooper and Subak-Sharpe, 1981). PBB could have acted as a promoter in tracheal carcinogenesis by inhibiting gap junction-mediated metabolic cooperation since PBB have been shown to inhibit metabolic cooperation *in vitro* at noncytotoxic doses (Tsushimoto *et al.*, 1982; Evans, 1987; Kavanagh *et al.*, 1987). Indeed, Kavanagh *et al.* (1987) suggested that at cytotoxic doses (Jensen *et al.*, 1983) the mechanism of promotion by PBB may be related to indirect interference with metabolic cooperation since lack of gap

junctions on hepatocyte membranes was found during cellular regeneration (Yee and Revel, 1978; Meyer et al., 1981).

Alternatively, perhaps, a mechanism of tracheal tumor promotion by PBB is through activation of a transforming gene. Boutwell (1974) proposed that initiation results in the formation of permanent and heritable unexpressed changes in the cell genome. Promotion causes the phenotypic expression of these changes in genotype as altered metabolism and later as altered cell morphology and ultimately as a tumor. If promoters regulate gene transcription, then treatment with a promoter would lead to increased synthesis of RNA and protein and these, in part, might come from regions of the genome not normally expressed. The best characterized function regulated in this manner is induction by TCDD of cytochrome P_1-450 enzymes, including aryl hydrocarbon hydroxylase (AHH) which appears to involve binding of an Ah receptor-TCDD complex to specific gene regions (Jones et al., 1985). Induction of cytochrome P_1-450 (AHH) by TCDD has been demonstrated in the nasal cavity (Bond, 1983) and lung (Roberts et al., 1986). PBB are close chemical relatives of TCDD. A regulation of gene activation by PBB may occur by mechanism(s) similar to those described with TCDD. Thus, induction of tracheal cytochrome P_1-450 (AHH) by PBB could be important as an indicator of its tumor promoting ability. This study was the first demonstration that PBB apparently acts as a promoter in tracheal carcinogenesis, and, therefore, further

in vivo and *in vitro* studies are needed to assess the tumor promoting potential and mechanism(s) of tumor promotion by PBB in tracheal carcinogenesis.

The major target organs for a single subcutaneous dose of 80 mg NDEA/kg bw in Syrian golden hamsters appeared to be the trachea and nasal cavity. Tumor incidence was low in the liver and lung. Similar results were described in previous studies (Montesano and Saffiotti, 1970; Li *et al.*, 1979). Herrold and Dunham (1963), however, demonstrated an increased incidence of tumors of the livers after intragastric administration of NDEA in Syrian golden hamsters. This difference could be due to the route of exposure since intradermal, intraperitoneal and topical (Herrold, 1964a) and subcutaneous administration (Montesano and Saffiotti, 1968; Harada *et al.*, 1985) produced no or few tumors in the liver. It is suggested that NDEA is metabolized by cytochrome P-450 to an active intermediate (e.g. ethyldiazonium hydroxide) (Montesano and Bartsch, 1976; Hecht *et al.*, 1983). The ethyldiazonium hydroxide may covalently modify nucleophilic groups in cellular macromolecules to generate alkylating intermediates (DNA adducts) (Hecht *et al.*, 1983). It is thought that the marked organ specificity of tumor initiation by NDEA may be partly determined by the extent of the level of DNA alkylation in that particular organ (Magee, 1968).

Expression of a malignant phenotype occurs late in the carcinogenic process (Farber, 1984b). None of the tracheal

tumors was malignant. All tumors were papillomas and were characterized by papillary growths consisting of squamous cells with no infiltrative growth into the subepithelial tissue. These papillomas obstructed the trachea of several hamsters and caused them to die before the tumors became malignant. In a previous study, squamous cell carcinoma of the trachea was observed in only 2 hamsters during a lifetime study (Reznik-Schuller, 1980). In the present study, malignant tumors (e.g. squamous cell carcinomas and adenocarcinomas) developed only in the nasal cavities.

Another possibility for lack of malignancy is that a single dose of 80 mg NDEA/kg bw could favor the formation of benign tumors. In two-stage models, repeated exposure to initiators has been shown to increase malignant conversion of skin and liver tumors in mice and rats, respectively (Hennings et al., 1983; Scherer and Emmelot, 1983).

Tumor promoters are compounds that lack significant carcinogenic activity but induce the development of tumors when given continously after administration of a subcarcinogenic dose of a known carcinogen (initiator) (Berenblum, 1941; Williams et al., 1981). In the present study, a single dose of 80 mg NDEA/kg bw was used as the initiation dose because histopathologic changes in cells of Bowman's glands in the areas of the olfactory region of the nasal cavity without necrogenic effects occurred in hamsters given this dose. However, the minimal single dose of NDEA needed to induce tracheal papillomas was estimated to be

only 1.03 mg/kg bw (Li et al., 1979). Thus, in an initiation-promotion model for tracheal carcinogenesis, one could use a single low dose of NDEA as an initiator. Relatively few tracheal papillomas should occur in NDEA-initiated animals. Therefore, it would be easier to define the tumor promoting ability of chemicals such as PBB in an initiation-promotion model.

Nasal tumors occurred at approximately the same incidence in hamsters treated with NDEA as in those treated with NDEA and PBB. An apparent increase in the incidence of papillomas of the nasal cavity in hamsters treated with NDEA and PBB may suggest tumor promotion. However, the total number of papillomas in the nasal cavities could not be documented because gross observation of the cut surface of the nasal tissue during trimming did not allow for the precise quantitation of papillomas which would be necessary to more clearly define the tumor promotion ability of PBB.

A previous study with NDEA has demonstrated that a single administration of 2 mg of NDEA to pregnant Syrian golden hamsters is sufficient to induce tracheal papillomas in the dam and her offspring. The earliest detectable tumor was seen on day 56 in the offspring (Mohr et al., 1966). In another study, Montesano and Saffiotti (1968) reported that the first tracheal papilloma appeared on day 119 in an adult hamster which received a total dose of 60 mg NDEA/kg bw.

Basal cells are proposed as the origin for neoplastic development in the trachea. The precursor lesions observed in hamsters treated with NDEA were areas of hyperplastic tracheal epithelium, and the cells appeared to be transitional in their differentiation between basal and mucous cells (Reznik-Schuller, 1980). Precursor lesions in the mucosal cells of the trachea have been seen consistently in tracheal organ cultures from Syrian golden hamsters at 7 days following exposure to benzo(a)pyrene (BaP) or asbestos, and these lesions were more extensive in tracheas from younger animals than in those from older animals (Mossman et al., 1977; Placke et al., 1986). PBB have been shown to enhance the formation of enzyme-altered foci in the livers of rats previously initiated with NDEA (Jensen et al., 1982; Jensen and Sleight, 1986b). Altered hepatic foci are proposed precursors for hepatic nodules and hepatocellular carcinomas in the liver (Scherer, 1984; Schulte-Hermann, 1985).

It was decided to do a short term sequential study in young Syrian golden hamsters to determine if the presence of precursor lesions could be demonstrated by days 21, 42 or 63 following NDEA or NDEA and PBB exposure. If so, there would be a more complete understanding of the nature of precursor lesions and their relevance to promotion assessment. During the time frame of this study, no precursor lesions were observed that could be correlated with the tumor promoting ability of PBB on the trachea.

Several potential explanations for the lack of precursor lesions exist. In this study, we examined sections from the upper, middle and lower portions of the tracheas, and precisely the same areas were examined in all animals to assure a standardized procedure. It is possible that tracheas from the treated hamsters may have had precursor lesions in portions of the tissue not sectioned. One might attempt, therefore, to take serial portions of the whole trachea so as to include all possible lesions on slides to be examined histologically. It is also possible that the precursor lesions could not be identified with the routine histologic examination. Therefore, an alternative approach would be to employ enzyme-histochemical procedures. For example, these methods have been employed to determine cells within the nasal tissues which contain certain enzymes involved in the metabolism of inhaled chemicals, such as acetaldehyde, glycol ether acetates and acrylate esters (Bogdanffy et al., 1986; Bogdanffy et al., 1987). Another possibility for lack of precursor lesions is that the length of time of this experiment (63 days from the time of NDEA administration) may not have been long enough for adequate development of the lesions. One would probably be able to define these lesions if an experiment were of longer duration.

Tracheal organ culture models are probably the best way to determine early morphologic responses of trachea to NDEA or NDEA and PBB exposure. Tracheal organ culture has proven

to be a useful assay in determining precursor lesions in tracheas exposed to BaP or asbestos (Mossman et al., 1977; Placke et al., 1986). Whether the precursor lesions are a critical determinant of susceptibility to promotion ability in tracheal carcinogenesis remains to be determined.

CHAPTER II

THE PROMOTING EFFECT OF TETRACHLORODIBENZO-p-DIOXIN AND 2,2',4,4',5,5'-HEXACHLOROBIPHENYL ON NASAL CAVITY TUMORS IN SPRAGUE-DAWLEY RATS

CHAPTER II

THE PROMOTING EFFECT OF TETRACHLORODIBENZO-p-DIOXIN AND 2,2',4,4',5,5'-HEXACHLOROBIPHENYL ON NASAL CAVITY TUMORS IN SPRAGUE-DAWLEY RATS

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 interest in and importance of neoplasms in the nasal cavity of man and animals have recently 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; Buchmann et al., 1986; Jensen and Sleight, 1986b). 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 assess hepatic tumor promotion, demonstrated 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, acccumulate 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; Cordle, 1983). Therefore, this study may have important public health implications 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[j] initially weighing 180-200 g at 5-6 weeks old were used. Rats were acclimated for 7 days and were fed a basal diet[k] 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 controls were not PH or given NDEA. Thirty days after the PH, rats were randomly allotted into 12 groups of 24 or 12 each. The experimental design is illustrated in Table 5.

Diets were prepared by adding appropriate 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 maintained 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, 3 or 6 rats per cage, and the

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[j]Charles River Breeding Laboratories, Inc., Portage, Michigan.

[k]Certified Rodent Chow 5002, Ralston Purina Co., St. Louis, Missouri.

Table 5. 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.

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) and were as previously described in Chapter I, Experiment 1.

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

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 6). 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 died 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 17). Adenocarcinomas were composed of poorly differentiated cells

Table 6. 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.

with mostly solid areas and with prominent nuclear features of malignancy, such as pleomorphism, hyperchromatism and abnormal mitotic figures. Evidence of a glandular pattern was occasionally present (Figure 18). Tumors were mainly observed in the lining epithelium of posterior regions of the nasal cavities.

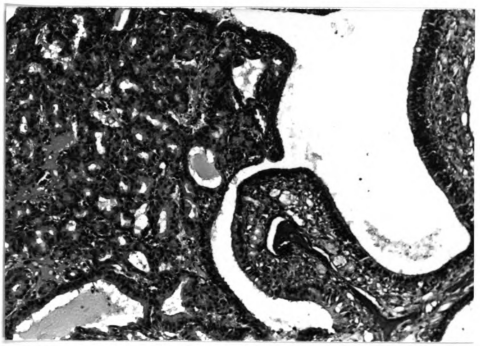

Figure 17. Adenoma of the nasal cavity from a NDEA-initiated rat fed a diet containing 100 ppt TCDD plus 245-HCB for 140 days and killed on day 420. Notice well differentiated cells have formed glandular structures with a papillary growth pattern. (H & E stain, 218x.)

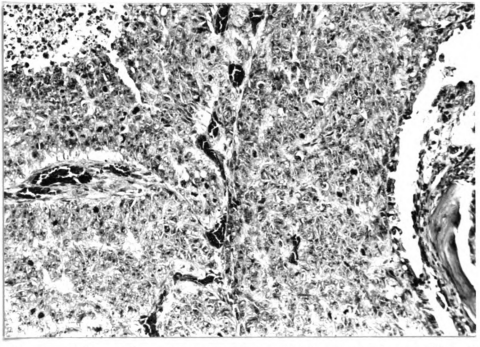

Figure 18. Adenocarcinoma of the nasal cavity from a NDEA-initiated rat fed a diet containing 100 ppt TCDD plus 245-HCB for 140 days. Rat died on day 323. Notice solid areas of poorly differentiated cells with few glandular structures. (H & E stain, 360x.)

Discussion

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 water, soil and food products, such as fish. Concentrations of TCDD as high as 30 ppt and with an average value of 25 ppt were found in edible portions of salmonoid fish, such as 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; Buchmann et al., 1986). It is important to understand, however, their carcinogenic potential at nonhepatic sites since it is becoming apparent that TCDD or related compounds may 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 attempt 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.

Results of this experiment indicate that dietary exposure of rats to diets containing both TCDD and 245-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 initiators (Berenblum, 1941; Pitot et al., 1981). Since TCDD has no properties of known tumor initiators (e.g. 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. A metabolized PBB congener, 3,3',4,4'-tetrabromobiphenyl has been shown to have a weak initiation ability in a two-stage model of hepatocarcinogenesis (Dixon et al., 1985).

Since, as a normal entrance to the respiratory tract, the nasal cavity is the first target for airborne irritants and the first physical barrier impeding their progress to the lower respiratory tract and, since the duration of this experiment was long (420 days), it might be asked whether the increased incidence of nasal tumors is related to susceptibility to chronic airborne particles of compounds rather than via systemic exposure. It should be emphasized that the histologic appearance of the nasal cavities from the controls was apparently normal (e.g. no inflammation). Moreover, in the nasal cavity, tumors were mainly confined to the posterior part (olfactory region) of the nasal cavity, and even within this tissue they appeared only at certain sites. Also, since the doses were low, if the insult persists, it is almost certainly very minor.

In this study, there were 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 245-HCB may decrease the latency time for nasal carcinomas to develop as well as increase the incidence of hepatocellular carcinomas.

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 nonhepatic 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 interactions between such chemicals.

SUMMARY AND CONCLUSIONS

Polybrominated biphenyls (PBB) at 100 mg/kg, when fed to Syrian golden hamsters in the diet, apparently act as a tracheal tumor promoter as evidenced by a significant increase in the number of tracheal papillomas after initiation with a single dose of NDEA. Although none of the tracheal neoplasms was malignant, deaths occurred in 8 of 30 hamsters treated with 80 mg NDEA/kg bw and in 10 of 30 hamsters given NDEA (as an initiator) and PBB (as a promoter) in the diet. The cause of death appeared to be related to obstruction of the tracheas by the papillomas. Tracheal papillomas were not seen in hamsters fed the basal diet or in hamsters fed diets containing PBB. No significant increase in nasal tumors was observed in hamsters fed diets containing PBB after a single dose of NDEA.

The upper respiratory tract appeared to be the main target area for carcinogenic effects of a single subcutaneous dose of 80 mg NDEA/kg bw. The trachea was more frequently affected than other segments of the upper respiratory tract. Tumors observed in the respiratory tract were papillomas (trachea, larynx, nasal cavity and lung), adenomas (nasal cavity and lung), adenocarcinomas and squamous cell carcinomas (nasal cavity).

With one exception, precursor tracheal lesions such as epithelial hyperplasia or metaplasia were not seen by day 63 in the hamsters given a single dose of 80 mg NDEA/kg bw or in the hamsters fed diets containing PBB after a single dose of NDEA. Small tracheal papillomas had developed in a few hamsters from these groups by this time.

The nasal carcinogenic effects of low doses of TCDD and 245-HCB were assessed and the additive or potentiating effects of combined exposure to these chemicals were evaluated in a long-term sequential study in Sprague-Dawley rats. Initiation consisted of a 70% partial hepatectomy and a single intraperitoneal administration of 10 mg NDEA/kg bw. Diets containing the potential promoting agents were fed from days 0 to 140 beginning 30 days after the initiation. By 140 days, nasal tumors were not observed among groups of rats fed diets containing TCDD and/or 245-HCB. A nasal adenoma was evident by 210 days in 1 rat each from the groups given a diet containing 100 ppt TCDD or 100 ppt TCDD and 245-HCB. By 420 days, nasal adenomas and adenocarcinomas were observed. The incidence of nasal tumors was significantly higher in NDEA-initiated rats given either TCDD or a combination of TCDD and 245-HCB than in NDEA-initiated rats given a basal diet. However, there was no apparent additive or potentiating effect on the incidence of nasal tumors caused by simultaneous exposure to TCDD and 245-HCB. Five of 10 rats that had nasal adenocarcinomas died. The earliest deaths occurred in 2 of 12 rats given a

combination of 100 ppt TCDD and 245-HCB at 288 and 323 days, respectively. The results suggest that TCDD may promote nasal tumors and the combined treatment of 100 ppt and 245-HCB may, in some instances, decrease the latency time for the development of nasal carcinomas.

Further studies are needed to develop and evaluate an initiation-promotion model for the comparative assessment of the ability of environmental chemicals to cause respiratory tract tumors in the nasal cavity and trachea.

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