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Increase in Cardiovascular Pathology in Female Sprague-Dawley Rats Following Chronic Treatment with 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and 3,3',4,4',5-Pentachlorobiphenyl

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Abstract

The effects of chronic exposure to dioxin (2,3,7,8,-tetrachlorodibenzo-p-dioxin [TCDD]) and a dioxin-like compound (3,3',4,4',5-pentachlorobiphenyl [PCB126]) on the cardiovascular system were evaluated in female Harlan Sprague-Dawley rats as part of an ongoing National Toxicology Program investigation. The animals were gavage treated 5 d per week with up to 1000 ng of PCB126 per kilogram of body weight per day or up to 100 ng of TCDD per kilogram of body weight per day for up to 2 yr. The control animals received only a corn oil/acetone vehicle (99:1 mixture). The corresponding stop-study groups received the highest doses for 31 wk and then received only the vehicle for the remainder of the study. After a full necropsy of all animals, a complete set of tissues was examined microscopically. Administration of each compound was associated with treatment-related increases in the incidences of degenerative cardiovascular lesions. Cardiomyopathy and chronic active arteritis increased in a dose-related manner in all groups treated with PCB126 or with TCDD. Increased incidences were also observed in the stop-study groups, indicating that a shorter term exposure may produce some effects. The average severity of cardiomyopathy was minimal or slightly greater in all dose groups, including the controls. Chronic active arteritis occurred primarily in the mesentery and pancreas, although the rectum, liver, heart, ovary, uterus, and glandular stomach in the PCB126 study and the liver and ovary in the TCDD study were affected in a few of the dosed animals. The authors' investigations indicate that the rat cardiovascular system is a target for dioxin toxicity, which increases the incidence of spontaneous cardiomyopathy and arteritis.

Key Words: Dioxin; heart; cardiomyopathy; artery; vasculitis; polychlorinated biphenyls.

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Introduction

Although some epidemiologic studies implicate dioxin exposure in the increased severity of ischemic heart disease and in the incidence of mortality caused by ischemic heart disease, other studies show conflicting results or offer no definitive conclusions *(1, 2)*. Animal studies indicate that exposure to dioxins can produce effects on the cardiovascular system. For example, Kociba et al. *(3)* report that rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have hemorrhages in the brain and spinal cord, an increase above background levels in the incidence of mesenteric/thoracic periarteritis accompanied by thrombosis, and, in females only, an increase above background levels in the incidence of myocardial degeneration. Brewster et al. *(4)* note that macrophage-like structures in the intima and media, considered to be preatherosclerotic lesions, occurred in TCDD-treated rabbits. Dioxin treatment of C57Bl/6J mice produces an elevated mean tail-cuff blood pressure and an increase in the urinary excretion of vasoactive eicosanoids, and exposure of *Apoe* (–/–) mice to dioxins causes a trend toward an earlier onset and greater severity of atherosclerotic lesions *(5)*. Riecke et al. *(6)* note diffuse myocardial interstitial collagen deposition in marmosets, which they suggest is mediated through transforming growth factor β-1.

Dioxins have also been shown to induce adverse cardiovascular effects in fish and chick embryos. Exposure of zebrafish embryos to TCDD during early development results in reduced blood flow in the mesencephalic vein, which may be related to aryl hydrocarbon receptor (AHR) activation, induction of cytochrome P-450 (CYP1A), and oxidative stress *(7)*. Exposure of chick embryos to TCDD is associated with an increase in the normally occurring level of apoptosis in the heart, a decreased myocyte proliferation with a thinner ventricular wall, and a reduction in coronary artery development, which is demonstrated by decreased coronary artery size and number. The decrease in coronary artery development may be a result of the decreased myocyte proliferation and thinner ventricular wall, which, in turn, may be influenced by increased apoptosis *(8)*.

Research into the potential mechanisms involved in dioxin-related cardiovascular pathology suggests a mediation of the binding of these compounds in cytosolic AHR activation *(7,9)*. However, lung vasculitis was observed in AHR-deficient mice when a high dose of TCDD was applied (2000 μg/kg) but not when lower doses were used (200 μg/kg). These observations suggest a pathway leading to toxicity that is unrelated to AHR when high doses are used *(10)*. No TCDD-related lesions were observed in other tissues, including the heart, in the AHR-deficient mice. Another suggested mechanism for cardiovascular pathology is a reduction in local circulation involving CYP1A induction associated with the release of reactive oxygen species and oxidative stress, resulting in increased vascular permeability *(7)*.

Because humans are exposed to mixtures of dioxin-like compounds, the toxic equivalence factor (TEF) has been developed to characterize their toxicities. To assess the suitability of TEFs for cancer risk, the National Toxicology Program (NTP) has studied the relative chronic toxicity and carcinogenicity of TCDD and 3,3',4,4',5-pentachlorobiphenyl (PCB126).

In the present study, female Harlan Sprague-Dawley (SD) rats were gavage treated 5 d per week for up to 2 yr with up to 1000 ng/kg PCB126 or up to 100 ng/kg TCDD. The vehicle was a 99:1 mixture of corn oil/acetone. The animals were evaluated pathologically at 14 wk, 31 wk, 53 wk, or 2 yr. A complete gross evaluation was performed on all animals, including those that died prior to the time of scheduled study termination. To ensure consistency across studies, the same pathologist conducted the microscopic pathology evaluations. Furthermore, a complete NTP peer review of the pathology findings was conducted by independent pathologists. The same pathologists conducted the peer review of both studies to ensure consistency. Our work describes the morphological aspects of the increases in PCB126-related and TCDD-related incidences of cardiomyopathy and arteritis observed in these 2-yr toxicity and carcinogenicity studies.

Materials and Methods

Study Design

These studies were conducted by the NTP (http://ntpserver.niehs.nih.gov) as part of an ongoing series of chronic 2-yr rat bioassays examining the relative potencies for carcinogenicity of individual dioxins and mixtures of dioxin-like compounds. The studies

for TCDD and PCB126 were conducted using female Harlan SD rats because these animals had been used in prior investigations of dioxin compounds and because the female rat is more sensitive to compound effects than is the male *(3)*. The same study design was used for both studies and included interim evaluation groups, 2-yr study groups, and a single stop-study group that received the highest dose of chemical. The stop-study group was added to (1) investigate the potential reversibility of various pathological effects induced by these compounds on withdrawal of the treatment and (2) attempt to distinguish between genotoxic and nongenotoxic mechanisms of carcinogenesis *(11)*. Interim valuations were conducted at 14 wk, 31 wk, and 53 wk; 10 animals per group were evaluated at 14 wk and at 31 wk; and 8 animals per group were evaluated at 53 wk. No interim evaluations were conducted on the stop-study animals. These animals were evaluated at the end of the 2-yr study along with the 2-yr study animals. The stop-study groups contained 50 animals, whereas the 2-yr study groups contained 53. Animals were dosed once daily for 5 d/wk by oral gavage using the test compound mixed in a corn oil/acetone vehicle (99:1 mixture). The control animals received the vehicle only. All animals were dosed for the duration of the study except for the stop-study animals, which were dosed for 31 wk and then given only the vehicle until study termination at 2 yr. Doses for the TCDD study were 0, 3, 10, 22, 46, and 100 ng/kg/d. The doses used for the PCB126 study, based on the World Health Organization (WHO) TEF value of 0.1 for PCB126 *(12)*, were 0, 30, 100, 175, 300, 550, and 1000 ng/kg/d.

Chemicals

TCDD (Lot Number CR82-2-2) was supplied by IIT Research Institute (Chicago, IL), and PCB126 (Lot no. 130494) was supplied by AccuStandard, Inc. (New Haven, CT). Each chemical was received in one lot that was used for the entire study. Purity was determined at several times during the study by gas chromatography/mass spectroscopy, nuclear magnetic resonance spectroscopy, and gas chromatography using flame ionization detection (PCB126) or electron capture detection (TCDD). Purities of TCDD and PCB126 were determined to be approx 98% and 99.51%, respectively, with no change in purity observed over the duration of the studies. The corn oil was analyzed by potentiometric titration, and the acetone was analyzed by infrared spectroscopy. Dose formulations were prepared for gavage administration by mixing the test chemical in a corn oil vehicle containing 1% USP-grade acetone. Homogeneity and stability studies of dose formulations indicated that both chemicals could maintain an acceptable homogeneity for dosing and stability for 35 d when stored at room temperature.

Animals

The animal studies were conducted at Battelle Columbus Laboratories (Columbus, OH). Animals were obtained from Harlan SD (Indianapolis, IN) and, upon receipt, were approx 6 wk of age. They were held under quarantine for approx 2 wk for health screening and were approx 8 wk old at the start of the study. After quarantine, the animals were randomly assigned to control or treated groups and were permanently identified by tail tattoo. They were housed five to a cage in solid-bottom polycarbonate cages (Lab Products, Inc., Maywood, NJ) suspended on stainless steel racks. Filtered room air underwent at least 10 changes per hour. Animal rooms were maintained at 69–75°F with 35%–65% relative humidity and 12 h of subdued light daily. Irradiated NTP-200 pelleted feed (Zeigler Bros., Inc., Gardners, PA) and water were available ad libitum. All animals were observed twice daily for clinical signs of toxicity, and moribund animals were euthanized and necropsied. The health status of the animals was monitored by serological analysis of serum samples collected from the study animals and from female sentinel rats that were placed in the study rooms. Serum samples remained negative for *Mycoplasma pulmonis, Mycoplasma arthritidis*, Sendai virus, pneumonia virus of mice, parvovirus, and rat coronavirus/sialodacryoadenitis virus for the duration of the study. Animal husbandry and handling were conducted in accordance with the National Institutes of Health (NIH) guidelines *(13)*.

Pathology

Animals from interim evaluations, those that survived to study termination, and those found in a moribund condition were humanely killed by CO_2 asphyxiation. All animals, including those animals that died prior to the time of scheduled study termination, were necropsied, and tissues from all animals were exam-

Table 1
Survival in Rats Treated with 2,3,7,8,-Tetrachlorodibenzo-p-Dioxin (TCDD) or 3,3',4,4',5-Pentachlorobiphenyl (PCB126) for 2 Yr

TCDD								
Dose (ng/kg/d)	0	3	10	22	46	100	100 stop	
Number of animals in group	53	54	53	53	53	53	50	
2-yr survival	25	21	23	19	22	21	21	
Percent survival	47	39	43	36	42	40	42	
PCB126								
Dose (ng/kg/d)	0	30	100	175	300	550	1000	1000 stop
Number of animals in group	53	55	53	53	53	53	53	50
2-yr survival	15	25	26	22	16	23	7	28
Percent survival	28	45	49	42	30	43	13	56

ined microscopically. Animals found dead were necropsied as soon as possible after discovery. At necropsy, all tissues were examined grossly, any lesions observed were recorded, and a full complement of tissues was removed and fixed in 10% neutral-buffered formalin for microscopic evaluation. After fixation, the tissues were trimmed, processed, embedded in paraffin, sectioned at a thickness of 5 µ, stained with hematoxylin and eosin (H&E), and examined microscopically. The severity of lesions was graded on a 4-point scale, with 1 = *minimal*, 2 = *mild*, 3 = *moderate*, and 4 = *marked*. To maintain diagnostic consistency, the same pathologist evaluated both studies. The pathology findings from both studies were subjected to a full NTP peer review that was performed by the same pathologists.

Statistical Analysis

Incidences of lesions in the study animals were evaluated statistically by the Poly-3 test of Bailer and Portier *(14,15)*, which adjusts for survival differences among groups. Incidences of lesions in animals from each of the interim evaluations and from the 2-yr study were analyzed separately. For animals in the 2-yr studies, the total lesion incidences, including findings from animals that survived until study termination and from early death animals, were included in the analysis.

Results

Survival data for the TCDD and PCB126 studies are given in Table 1. Microscopically, increased incidences of cardiomyopathy and chronic active arteritis, occurring primarily in the mesentery and pancreas, were seen in treated 2-yr animals from both the TCDD and PCB126 studies. The incidences and average severities of cardiomyopathy and arteritis from the 2-yr studies are listed in Table 2 for TCDD and Table 3 for PCB126. As can be seen in these tables, because the average severities of cardiomyopathy were quite low, the range of severities of cardiomyopathy in affected animals in each group is given below the average severities. The incidence figures include findings from the animals that survived to study termination and the early death animals. Because neither cardiomyopathy nor arteritis was observed in any of the interim evaluation groups, these groups are not included in the tables. Gross examination occasionally revealed discolored nodules, enlarged mesenteric arteries, or thickened areas occurring in the mesentery in treated animals in the studies of each chemical. These gross observations correlated with the microscopic finding of arteritis. Findings for each chemical are discussed in detail below.

2,3,7,8,-Tetrachlorodibenzo-p-Dioxin

Survival to study termination at 2 yr was similar among control and treated groups and averaged approx 40% (Table 1). Based on microscopic evaluation, cardiomyopathy was not considered to be a cause of morbidity or mortality, whereas arteritis was sometimes considered to be the possible cause of death in early death animals. The incidences of cardiomyopathy were increased in the 2-yr treated groups, with the highest incidences occurring in the highest

Table 2
Incidences and Average Severities of Selected Cardiovascular Lesions
in Rats Treated with 2,3,7,8,-Tetrachlorodibenzo-p-Dioxin (TCDD) for 2 Yr

Dose (ng/kg/d)	0	3	10	22	46	100	100 stop
Number of animals examined	53	54	53	52	53	52	50
Cardiomyopathy [a]	10	12	22[b]	25[b]	32[b]	36[b]	22[b]
	(1.3)	(1.0)	(1.0)	(1.1)	(1.1)	(1.4)	(1.4)
	(1–2)	(1)	(1)	(1–2)	(1.2)	(1–3)	(1–3)
Arteritis							
Mesentery	0	1	0	0	4	7[b]	1
		(3.0)			(2.3)	(2.7)	(3.0)
Pancreas	0	1	1	2	2	7[b]	2
		(3.0)	(2.0)	(2.5)	(3.0)	(2.5)	(2.5)
Other sites	0	0	0	0	0	2	0

[a] Total number of animals affected with lesion; average severity (number in parentheses below incidence) based on severity grades of 1, *minimal*; 2, *mild*; 3, *moderate*; and 4, *marked*; range of severities (numbers in parentheses under average severity).
[b] $p < 0.01$ vs controls (poly-3 test).

Table 3
Incidences and Average Severities of Selected Cardiovascular Lesions
in Rats Treated with 3,3',4,4',5-Pentachlorobiphenyl (PCB126) for 2 Yr

Dose (ng/kg/d)	0	30	100	175	300	550	1000	1000 stop
Number of animals examined	52	54	53	53	53	51	51	50
Cardiomyopathy[a]	9	16	17	16	24[c]	28[c]	32[c]	15
	(1.0)	(1.1)	(1.1)	(1.2)	(1.1)	(1.1)	(1.2)	(1.2)
	(1)	(1–2)	(1–2)	(1–2)	(1–2)	(1–2)	(1–2)	(1–3)
Arteritis								
Mesentery	0	0	2	2	6[b]	10[c]	7[b]	0
			(3.0)	(3.5)	(3.2)	(2.6)	(3.0)	
Pancreas	0	4	2	4	8[c]	15[c]	11[c]	0
		(2.0)	(3.0)	(3.0)	(2.7)	(2.5)	(2.9)	
Other sites	0	0	1	2	1	2	1	1

[a] Total number of animals affected with lesion; average severity (number in parentheses below incidence) based on severity grades of 1, *minimal*; 2, *mild*; 3, *moderate*; and 4, *marked*; range of severities (numbers in parentheses under average severity).
[b] $p < 0.05$ vs controls (poly-3 test).
[c] $p < 0.01$ vs controls (poly-3 test).

dose groups (Table 2). In the stop-study group, the incidence was similar to that of the 10- and 22-ng/kg/d dose groups in the 2-yr study. The incidences were significantly increased ($p < 0.01$) in all treated groups compared with controls, except in the low-dose group that received 3 ng/kg/d. The average severity was low and varied from 1.0 to 1.4 in all groups, which indicates that there was no compound-related effect on the overall average severity of cardiomyopathy. Chronic active arteritis occurred in one to seven animals in each of the 2-yr treated groups, with the highest incidence occurring in the highest dose group. The sites primarily affected were the pancreas and mesentery. The heart, liver, and ovary were sporadically affected in a small number of animals.

3,3',4,4',5-Pentachlorobiphenyl

Survival of the 1000-ng/kg/d group to study termination at 2 yr was approximately half of that of the control groups (13% vs 28%, respectively). Terminal survival in the other treated groups was similar to or greater than that of controls (Table 1). As with

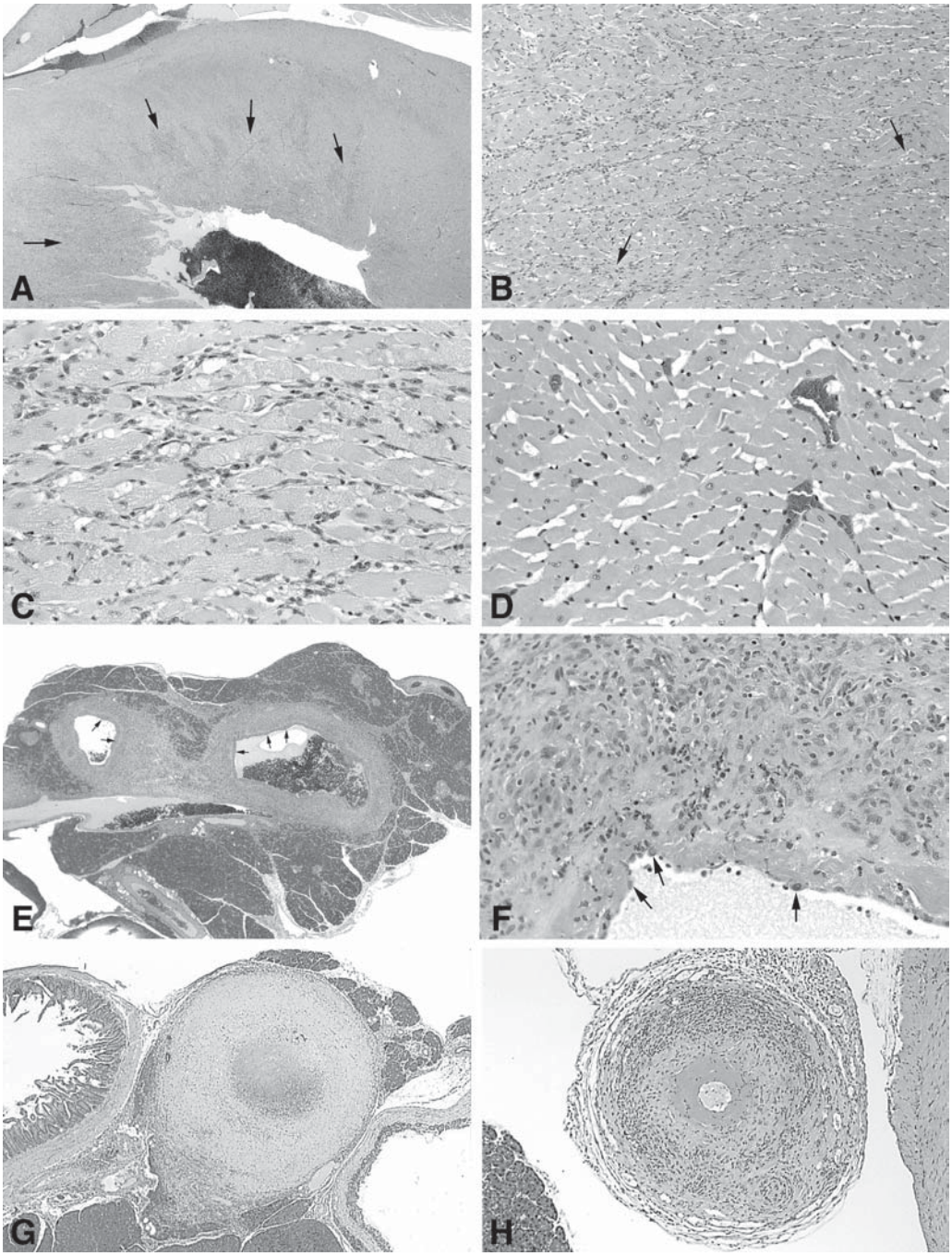
TCDD, based on microscopic evaluation, cardiomyopathy was not considered to be a cause of morbidity or mortality, although arteritis was considered to be the possible cause of expiration in some early death animals. The incidences of cardiomyopathy in the 2-yr study were increased in all treated groups, with the highest incidences occurring in the highest dose groups. The exception to this finding was the stop-study group, in which the incidence was similar to that of the low-dose group (Table 3). The incidences were significantly increased ($p < 0.01$) in treated groups receiving 300, 500, or 1000 ng/kg/d. The average severity was low, varying from 1.0 to 1.2 in all groups, including controls, which indicates there was not a compound-related effect on the overall average severity of cardiomyopathy. Chronic active arteritis occurred in 2–15 animals in each of the 2-yr treated groups, with the highest incidences occurring at the highest dose levels. Only 1 treated animal from the stop-study was affected. The most commonly affected site was the pancreas, followed by the mesentery. The following other sites were sporadically affected in a small number of animals: the heart, liver, large intestine, glandular stomach, ovary, and uterus.

Histopathology Findings

The microscopic appearances of cardiomyopathy and arteritis were similar in all affected animals in all groups—in both treated and control animals and in both studies (Fig. 1A–H). In the treated and control rats, the microscopic appearance of cardiomyopathy, which is a common spontaneous change in rats, was typical of the appearance that has been described for this change *(16)*. Cardiomyopathy was characterized by a slight alteration consisting of multiple foci of myocardial degeneration scattered within the ventricular walls, most commonly the left ventricle and the interventricular septum. The earliest changes, seen in only a few animals in these studies, consisted of small foci of myocardial fiber necrosis, characterized by deeply eosinophilic myocardial fibers with pyknotic nuclei and diffusely infiltrated with lymphocytes and macrophages and an occasional neutrophil. As lesion development progressed, the necrotic myocardial fibers were replaced by proliferating interstitial connective tissue cells producing several small, irregular, lightly eosinophilic areas that contained numerous connective tissue nuclei lying between myocardial fibers. In addition, areas of accumulation of small to moderate amounts of lightly basophilic, homogeneous material—presumably ground substance—that lay between and separated adjacent myocardial fibers were also commonly seen. This was the stage of lesion development that was observed most prevalently in these studies. In a few animals, lesion development progressed even further, producing a somewhat more severe change that consisted of moderate-sized foci of complete replacement of myocardial fibers by connective tissue. Lesion severity was graded according to the amount of myocardium affected. In most animals, because the amount of affected myocardium was relatively small, the severity of cardiomyopathy was graded as minimal.

Arteritis occurred in small, medium, and large arteries, although it was considerably more prominent in the larger ones and was characterized by a spectrum of changes. These changes included the following: circumferential fibrinoid necrosis of part of or the full thickness of the tunica media, proliferation and thickening of the tunica media with haphazard disorganization of medial nuclei, proliferation of adventitial connective tissue with adventitial thickening, and infiltration of the adventitia and, occasionally, the media, with small to moderate numbers of lymphocytes, macrophages, and, sometimes, neutrophils. Endothelial cell proliferation and occasional thrombosis were also seen. The lesions appeared to follow this progression: medial fibrinoid necrosis, adventitial proliferation, medial proliferation in cases without full-thickness necrosis of the tunica media, inflammation, and, occasionally, endothelial cell proliferation. Thrombosis infrequently occurred in necrotic arteries, presumably secondary to the damage to the

Fig. 1. *(Opposite page)* (**A**) Mild cardiomyopathy in rat that received 1000 ng/kg/d 3,3',4,4',5-pentachlorobiphenyl (PCB126) for 2 yr is evident in the multifocal distribution (arrows) within the left ventricular wall (hematoxylin and eosin [H&E], ×4). (**B**) Higher magnification of A, showing fibrosis and mononuclear cell infiltrate between myocardial fibers and accumulation of ground substance (arrows) (H&E, ×25). (**C**) Higher magnification of B, showing myocardial fiber degeneration, fibrosis, mononuclear cell infiltrate, and ground substance accumulation (H&E, ×66). (**D**) Heart from control animal that received vehicle for 2 yr (H&E, ×66). (**E**) Arteritis in pancreatic artery in rat that received 1000 ng/kg/d



PCB126 for 2 yr is seen in enlargement of artery with a thickened, hypercellular tunica media and subintimal fibrinoid necrosis (arrows) (H&E, ×5). (**F**) Higher magnification of same artery in E, showing discontinuous endothelial layer with endothelial cell hypertrophy (arrows), subintimal fibrinoid necrosis, and medial thickening with cellular proliferation and infiltrate in the tunica media (H&E, ×66). (**G**) Arteritis in pancreatic artery in rat that received 100 ng/kg/d TCDD for 2 yr is seen in thickened, hypercellular tunica media and thrombus filling lumen (H&E, ×8). (**H**) Arteritis in a mesenteric artery in rat that received 100 ng/kg/d TCDD for 2 yr is shown in pronounced hypercellularity and severe thickening of tunica media with subintimal fibrinoid necrosis (H&E, ×25).

arterial wall. As the lesions progressed, the necrosis of the vessel wall resolved and was replaced by varying degrees of medial thickening, which, in some cases —primarily in large arteries—resulted in a medial thickness that was several times greater than that seen in the normal wall. The thickened media generally contained increased numbers of nuclei arranged in a somewhat disordered pattern, and small amounts of homogeneous, lightly basophilic material, presumably ground substance, were present between smooth muscle fibers. The lumina of larger arteries were often dilated. The thickening of the arterial wall, together with the luminal dilatation seen in the larger arteries, led to an overall increase in the size of the artery, which accounted for the grossly observed lesions. Irregular focal remnants of the necrotic media were often seen within the thickened walls of large arteries, indicating that fibrinoid necrosis was resolved through replacement by medial proliferation. Some degree of adventitial proliferation, as well as inflammatory cell infiltrate within the adventitia, was still present. In many cases, the adventitial thickening and inflammatory cell infiltrate were the only indications of arterial change.

Discussion

Administration of TCDD or PCB126 to female Harlan SD rats in this study significantly increased the incidences of cardiomyopathy in a dose-related manner; however, the average severity of cardiomyopathy was unaffected by either compound and remained at a minimal or only slightly greater level across the control and treated groups. Thus, both chemicals increased the incidences of cardiomyopathy without increasing the average severities. That none of the interim evaluation animals, including those evaluated at 53 wk, was observed to have cardiomyopathy indicates that the lesions developed later in life. Cardiomyopathy occurred in the stop-study groups that received the high dose of compound for 31 wk and vehicle thereafter until study termination at 2 yr. However, at 2 yr, the incidences in the stop-study groups were greater than those in the respective control groups—indicating a chemical-related effect—but less than those in the high-dose groups that had received chemical for 2 yr. These findings suggest that a shorter term exposure to the compounds can result in a higher incidence of cardiomyopathy than in controls. Furthermore, longer exposure times and higher doses of chemical can lead to the occurrence of cardiomyopathy in an even greater number of animals. Because myocardial degeneration of cardiomyopathy heals by fibrosis and leaves a permanent lesion, microscopic examination should have revealed whether cardiomyopathy had ever been present in the stop-study animals, even if lesion development had ceased after compound administration was discontinued. Thus, the lack of any lesions in a given stop-study animal indicates that cardiomyopathy was never present in that animal. The lower incidence in the stop-study groups apparently occurred because fewer animals developed cardiomyopathy. In a 2-yr study in which SD rats were administered up to 0.1 µg/kg/d of TCDD in the feed, Kociba et al. *(3)* also reported an increase of myocardial degenerative change above background levels in females only, which suggests a possible sexual predisposition. In the present studies, determination of a possible sexual predilection was not possible because only females were used.

Myocardial degeneration can result from spontaneously occurring cardiomyopathy, by anoxia that is secondary to vascular changes, or from direct toxicity to myocardial cells *(16)*. The microscopic appearance of cardiomyopathy was the same in both the control and treated animals and the TCDD and PCB126 studies and was typical of that described for spontaneous lesions. This finding may suggest that exposure to the chemicals increased the occurrence of the spontaneous change. According to Greaves *(17)*, however, the presence of fibrosis is a common end-stage finding with myocardial degeneration and results from different etiologies and pathogeneses (i.e., initial, direct, chemically induced insult to the myocardium; exaggeration of spontaneous disease after treatment-related increase in circulatory demand; or other chemically induced changes). Determining the precise cause of myocardial degeneration using microscopic examination alone is thus difficult.

Cardiomyopathy is a common, spontaneously occurring degenerative change of myocardial fibers that is seen in rats as they age. Its cause in the rat is unknown, but age of onset and severity are affected by diet, environment, and stress *(16)*. The caloric and protein content of the diet, having a positive association with the development of cardiomyopathy, and fiber content, displaying a negative association, have

been reported to influence the severity of spontaneous cardiomyopathy *(18,19)*. In the SD rat, spontaneous cardiomyopathy is more common in males *(20, 21)*, in which it can appear as early as 3–4 mo of age. Sites of predilection include the myocardium of the apex, the region below the fibrous rings, the papillary muscles, and the free wall of the left ventricle. The changes are focal or diffuse, consisting of progressive mononuclear cell infiltration, fibrosis, myofiber degeneration/necrosis, and atrophy *(21)*. Early lesions of spontaneous cardiomyopathy are focal to multifocal and tend to enlarge and become more extensive as they increase in severity. In general, myocardial lesions resulting from anoxia tend to be focal, whereas toxic lesions are usually generalized. Cardiomyopathy may, therefore, be secondary to focal anoxia, possibly resulting from slight vascular changes. In these studies, however, no vascular lesions were observed in the hearts affected with cardiomyopathy, which suggests that any vascular changes must be functional rather than pathological. Treatment of rats with catecholamines, which are vasoactive compounds, can result in multifocal myocardial necrosis *(16)*. TCDD, through the activation of AHR, may be able to influence blood pressure in mice through the production of vasoactive eicosanoids *(5)*. This suggestion introduces the possibility that vasoactive effects of the test compounds affected the occurrence of cardiomyopathy; however, information is lacking concerning potential vasoactive effects of dioxin compounds in rats.

In addition, TCDD has been shown to cause contractile defects and impairment of the capacity of the avian heart sarcoplasmic reticulum to sequester Ca^{2+} *(22)*. Exposure of isolated rat ventricular myocytes to TCDD prolonged the action-potential duration and caused abnormal triggered-afterdepolarization *(23)*. That this effect may lead to clinical cardiac arrhythmia was suggested, especially when susceptible subjects were stressed by elevated sympathetic activity or suffered from other cardiomyopathies that coincided with Ca^{2+} overload. Exposure of pregnant mice to 6 μg/kg of TCDD induced a significant reduction of proliferating cardiomyocytes and was associated with reduced expression of genes involved in cardiomyocytic proliferation (e.g., CDK2, cyclin A1, Cdc2, Cdc25C, and cyclin C1) *(24)*. Taken together, the functional and molecular data indicate that the heart is a direct target for TCDD.

The result of increased incidences of cardiomyopathy at the 2-yr evaluation after 31 wk of exposure in the TCDD stop-study group is difficult to explain if only a constant daily dosing scenario is considered; however, TCDD has an approximate 4-wk half-life. After cessation of treatment, therefore, the chemical exhibits a slow decrease in body burdens over the remainder of the study to low levels that are equivalent to those seen with lower dose exposure (data not shown). Thus, exposure per se does not stop; rather, it persists after cessation of daily dosing. By simply considering the total weekly averaged exposure over the course of the study, one sees that the stop group has a higher total of administered dose than a low-dose treated group in which increases in cardiomyopathy were observed (10 ng/kg/d). For example, dosing at 100 ng/kg/d for 5 d a week for 31 wk, averaged over 104 wk, yields an average of 149 ng/kg/wk or 21 ng/kg/d. The greater incidences of cardiomyopathy in the stop-study groups may, therefore, indicate that a time-integrated dose metric (such as total area under the curve of exposure) may be a better measure of dose for evaluating cardiomyopathy. Clearly, though, these data indicate that cardiomyopathy does not require continuous high-dose levels over the entire course of the study; rather, early exposure can lead to effects later. Alternatively, chance occurrence unrelated to treatment may have played a role, but this possibility seems unlikely, as the incidences in both stop-study groups exceeded those in both control groups and were similar to those seen in treated groups. Thus, at present, the mechanism by which TCDD and PCB126 increased the incidences of cardiomyopathy remains undetermined and requires elucidation.

These studies were conducted as part of an NTP initiative evaluating the relative potency of dioxin-like compounds for inducing tumors in the female Sprague-Dawley rat model. Although no formal modeling of the relative potency for induction of cardiomyopathy by TCDD and PCB126 is presented in this article, the data do indicate that, qualitatively and quantitatively, the two chemicals yielded similar responses. At the highest dose (100 ng TCDD equivalents/kg), both compounds caused significant increases in incidences (63% in PCB126 and 69% in TCDD). At the 10-ng TCDD equivalence/kg dose, TCDD gave a slightly higher response (42% incidence) than PCB126 (32%), despite being an "equivalent"

dose based on a similar WHO TCDD equivalent (TEQ). (Multiplying the TEF value by the concentration of a specific compound in the mixture results in the TEQ of that compound.) These data suggest that the potency of PCB126 for inducing cardiomyopathy may be less than that predicted by the current WHO TEF of 0.1. However, a formal modeling of the appropriateness of WHO TEFs and the additivity of relative potency factors for nonneoplastic changes (including cardiomyopathy) and neoplastic changes observed in these and other studies will be presented elsewhere.

Arteritis occurred in one or more animals in each of the treated groups, including the stop-study groups, but not in the controls. The incidences were higher in the PCB126-treated animals than in the TCDD-treated animals, except for the stop-study groups, in which the incidence was greater in the TCDD study. The incidences increased with increasing doses, but the average severity, which generally fell between mild to moderate, was similar across dose groups. That arteritis was not observed in any of the interim evaluation animals indicates that, like cardiomyopathy, it developed later in life. Arteritis was observed in a small number of animals in the stop-study groups that received the high dose of compound for 31 wk and vehicle thereafter until study termination at 2 yr. The incidences were fewer than those of the high-dose groups that received the chemical for 2 yr and were higher in the TCDD group than in the PCB126 stop-study group, in which only a single animal was affected. These findings suggest that, as in cardiomyopathy, shorter term exposure to the chemical may predispose the animal to the development of arteritis, but longer exposure times and higher doses of the chemical may lead to the occurrence of arteritis in a greater number of animals. Arteritis affected primarily the mesenteric and pancreatic arteries, occasionally the arteries in other abdominal organs, and, in one animal, the heart. In their 2-yr study of TCDD, Kociba et al. *(3)* also reported an increase above background levels in mesenteric and thoracic periarteritis, with accompanying changes, including thrombosis, in males and females. In the present studies, arteritis in the heart, which occurred in one stop-study animal from the PCB126 study, was its only occurrence in an extra-abdominal location. That thrombosis was occasionally observed in affected arteries is in agreement with the findings of Kociba et al. *(3)*.

Because arteritis heals by fibrosis of the affected arterial wall, microscopic examination should have revealed the presence of arteritis in the stop-study animals, even if lesion development had stopped once compound administration stopped. The lack of lesions in any stop-study animal seems to indicate that arteritis was never present; thus, the lower incidence in the stop-study groups apparently occurred because fewer animals developed arteritis.

The morphology and location of the arteritis seen in the rats in these studies were consistent with other descriptions for spontaneous polyarteritis in rats *(25)*. Spontaneous polyarteritis—synonymously termed *periarteritis nodosa*, *polyarteritis nodosa*, *chronic arteritis*, *necrotizing arteritis*, and *necrotizing vasculitis*—occurs occasionally in the various rat strains, including the SD rat, and is characterized in the acute stage by fibrinoid necrosis with intense inflammatory cell infiltrate and then medial and adventitial fibrosis, leading to a thickening of the arterial wall. The sites of predilection are the mesenteric, pancreatic, and spermatic arteries, but other muscular arteries sometimes are affected. The etiology of spontaneous polyarteritis is unknown, although it resembles immune-mediated arteritis in other species *(25)*.

Arteritis in the rat can be caused by treatment with various vasoactive compounds, particularly vasodilator compounds *(25,26)*. Vasodilation has been suggested to damage the arterial wall by producing increased blood flow with increased intramural shear stress, leading to an increase in endothelial permeability and medial necrosis *(27)*. The mesenteric and pancreatic arteries have been reported to be especially susceptible to the effects of vasodilators *(28,29)*, possibly resulting from a lack of anatomic supporting structures for these vessels and/or the presence of specific receptors for the vasodilator in the vessel wall *(27,30,31)*. A recent study in F344/N rats of theophylline, a nonspecific phosphodiesterase inhibitor that produces excessive vasodilation, resulted in periarteritis of the mesenteric and pancreatic arteries that appeared morphologically similar to the arteritis in the present studies *(26)*. This finding suggests the possibility that the arteritis observed in our investigation was caused by the vasoactive effects of the compounds on the affected arteries, although the potential vasoactive effects of these compounds in rats are unknown. Dioxin has been reported to increase

mean tail-cuff blood pressure in mice *(5)*, but this increase implies vasoconstrictor rather than vasodilator activity. The involvement of possible vasoactive effects does not address the presence of arteritis in the stop-study animals. Also, explaining how 31 wk of exposure in stop-study groups early in the studies resulted in the occurrence of arteritis at the 2-yr evaluation is difficult. The arteritis seen in the stop-study animals may have been spontaneous and unrelated to treatment, but this possibility appears unlikely because arteritis was not seen in any control from either study. Thus, the mechanism by which PCB126 and TCDD caused the occurrence of arteritis is undetermined and requires elucidation.

Morphologically, the cardiomyopathy and arteritis seen in these studies resembled some lesions observed in humans. Multiple foci of myocardial fibrosis can be seen in humans with idiopathic or toxic cardiomyopathy *(32)*, whereas the arteritis seen in the treated rats in these studies resembled that of spontaneous polyarteritis nodosa of humans *(33)*. In addition, 50% of cases of polyarteritis in humans affect the gastrointestinal tract; thus, human polyarteritis shares a site of predilection with the arteritis reported in our investigation.

The results of our studies with TCDD and PCB126 indicate that these compounds produce an increase in degenerative cardiomyopathy in the heart, with arteritis occurring primarily in the mesenteric and pancreatic arteries. These findings provide further evidence to show that dioxin compounds can produce pathologic cardiovascular lesions. Although chemical-related vasoactive effects may possibly be involved in producing these lesions, further studies are required to determine the precise mechanisms by which these lesions occur.

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