Exocrine Pancreatic Pathology in Female Harlan Sprague-Dawley Rats after Chronic Treatment with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and Dioxin-like Compounds

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We evaluated the effect of chronic exposure to dioxin and dioxin-like compounds on the pancreas in female Harlan Sprague-Dawley rats. This investigation represents part of an ongoing National Toxicology Program initiative to determine the relative potency of chronic toxicity and carcinogenicity of polychlorinated dioxins, furans, and biphenyls. Animals were treated by gavage for up to 2 years with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), or a toxic-equivalency-factor (TEF) mixture of these agents; control animals received corn oil–acetone vehicle alone. A complete necropsy was performed on all animals, and a full complement of tissues was collected and examined microscopically. Administration of each of the four compounds was associated with increased incidences of several nonneoplastic changes in the exocrine pancreas, including cytoplasmic vacuolation, chronic active inflammation, atrophy, and arteritis. Low incidences, but higher than those in the historical database, of pancreatic acinar adenoma and carcinoma were seen in the TCDD, PeCDF, and TEF-mixture groups. These results indicate that the pancreatic acini are target tissues for dioxin and certain dioxin-like compounds. *Key words:* carcinogenesis, dioxin, furans, inflammation, pancreas, polychlorinated biphenyls. *Environ Health Perspect* 112:903–909 (2004). doi:10.1289/ehp.6869 available via *http://dx.doi.org/* [Online 4 March 2004]

In the United States, pancreatic cancer ranks as the fifth most common cause of cancer death in humans of both sexes (American Cancer Society 2003). Risk factors for pancreatic cancer include heritable, germline mutations in genes such as *p16* (Hruban et al. 1999) and *BRCA2* (Risch et al. 2001); cigarette smoking [International Agency for Research on Cancer (IARC) 1986]; and a diet consisting of an increased intake of meat or cholesterol (Howg and Burch 1996). Recently, Risch (2003) proposed that the risk of pancreatic cancer is also increased by prolonged excessive gastric/duodenal acidity and frequent or repeated exposure to *N*-nitroso compounds or their precursors. Of particular concern are observations suggesting that chronic pancreatitis may predispose individuals to cancer of the pancreas.

Chronic pancreatitis, an irreversible process with permanent loss of pancreatic function due to fibroinflammatory changes originating from various factors (Maisonneuve and Lowenfels 2002), often precedes the development of pancreatic malignancies. This chronic condition occurs in tropical regions or it may result from metabolic or hereditary disorders. Patients suffering from tropical calcifying pancreatitis (a form of nonalcoholic calcific pancreatitis in adolescents or young adults with no proven etiologic factor) have a significantly increased risk of developing pancreatic cancer (Chari et al. 1994), suggesting that chronic pancreatitis is a premalignant disease. Two independent epidemiologic studies calculated a 7- to 50-fold increased risk of pancreatic cancer in patients with hereditary pancreatitis (Lowenfels et al. 1997). Chronic pancreatic inflammation may also be induced by alcohol, congenital defects such as cystic fibrosis, some infectious diseases, drugs, and radiation therapy. Epidemiologic studies support the concept that inflammation associated with glandular destruction is a risk factor for exocrine pancreatic cancer. Lowenfels et al. (1993) studied 2,015 subjects with chronic pancreatitis and concluded that the risk of pancreatic cancer is significantly increased in this population and appears to be independent of sex, country, and type of pancreatitis. In addition, several environmental agents have been proposed as causal for chronic pancreatitis and pancreatic carcinomas and are associated with the wood and pulp industry, the dry cleaning business, and gasoline production and use (Foster et al. 1993; Lin and Kessler 1981; Milham and Demers 1984).

Polyhalogenated aromatic hydrocarbons (PHAHs) comprise a large class of compounds including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes, and polybrominated diphenyl ethers. Certain PCDDs, PCDFs, and coplanar PCBs have the ability to bind to the aryl hydrocarbon receptor (AhR) and exhibit biologic actions similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); they are commonly referred to as dioxin-like compounds (DLCs). Exposure of humans to high levels of TCDD has been implicated in the development of diabetes. According to one study (Bertazzi et al. 2001), an increase of diabetes mellitus was suggestively time related among females in all exposure groups. Exposure of female Sprague-Dawley rats of the Spartan strain to 100 ng/kg TCDD for 2 years was associated with atrophy, fibrosis, and periarteritis of the pancreas (Kociba et al. 1978), whereas TCDD administered intraperitoneally to hamsters at the dose of 100 μm/kg induced hepatocytic transdifferentiation of the acinar pancreatic cells (Rao et al. 1988). Others, however, have observed little or no correlation between diabetes and dioxin exposure (Steenland et al. 1999).

The National Toxicology Program (NTP) has recently conducted multiple 2-year lifetime rat bioassays to evaluate the chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. Given the known mode of action of DLCs acting through the AhR, one of the hypotheses tested in these studies was that both individual compounds and mixtures would elicit a similar spectrum of neoplastic and nonneoplastic responses after chronic exposure. Our work describes the incidences and morphologic aspects of pancreatitis and exocrine pancreatic cancer related to these

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DLCs, as observed in the 2-year toxicity and carcinogenicity studies.

Materials and Methods

Study design. These studies were conducted by the NTP (2004) as part of an ongoing series of chronic 2-year rat bioassays examining the relative potencies for carcinogenicity of individual dioxins and mixtures of DLCs. In these studies, TCDD, 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and a mixture of TCDD, PCB-126, and PeCDF were tested at levels based on their toxic equivalency factors (TEFs); the studies were conducted with female Harlan Sprague-Dawley rats because these had been used in prior investigations of DLCs and because the female rat is more sensitive to the effects of TCDD than the male (Kociba et al. 1978). The same study design was used for the TCDD, PCB-126, and PeCDF studies and included interim evaluation groups, 2-year study groups, and a single stop-study group that received the highest dose of chemical. The stop-study group was added to investigate the potential reversibility of various pathologic effects induced by these compounds upon withdrawal of daily administration and to evaluate whether lesions seen at the end of the 2 years of study required persistent lifetime exposure (Walker et al. 2000).

The investigation of the tertiary mixture did not include a stop-study group. Interim evaluations of 10 animals/group were conducted at weeks 14, 31, and 53 of the study. The stop-study groups contained 50 animals, whereas each 2-year study group contained 53. Animals were dosed once daily for 5 days/week by oral gavage using the test compound mixed in a corn oil:acetone vehicle (99:1 vol:vol). 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 weeks and then given the vehicle only until study termination at 2 years.

TCDD is the most potent DLC and the reference compound to which all DLCs are compared in the TEF methodology. Doses for the TCDD study were 0, 3, 10, 22, 46, and 100 ng/kg/day; the stop-study dose was 100-stop ng/kg/day. These doses were based on the TEF values selected by the World Health Organization (WHO) (Van den Berg et al. 1998). PCB-126 is a non-*ortho*-substituted PCB with high bioaccumulation in the food chain and a TEF value of 0.1. Selected doses of PCB-126 were 0, 30, 100, 175, 300, 550, 1,000, and 1,000-stop ng/kg/day. PeCDF is a dioxin-like PHAH with high bioaccumulation in the food chain and a TEF value of 0.5. Selected PeCDF doses were 0, 6, 20, 44, 92, 200, and 200-stop ng/kg/day.

The TEF mixture of TCDD, PCB-126, and PeCDF was designed to test for dose additivity of induced effects seen for the DLCs with the highest potency in each of the three classes of PHAHs covered by the TEF methodology. Based on average human tissue levels of these compounds, they represent approximately 43% of the human tissue burden of the TCDD toxic equivalent (TEQ). The TEF mixture was composed of equal ratios (1:1:1) of TEQs for TCDD, PCB-126, and PeCDF. The TEQ, calculated by multiplying the TEF value of each specific compound by the concentration of that compound in the mixture, results in the TCDD equivalent of that compound. For the TEF mixture, selected doses were 0, 10, 22, 46, and 100 ng TEQ/kg/day using the WHO TEF values of 1.0 for TCDD, 0.1 for PCB-126, and 0.5 for PeCDF. Specific doses used in the TEF-mixture study were 10 ng TEQ/kg (3.3 ng/kg TCDD, 6.6 ng/kg PeCDF, 33.3 ng/kg PCB-126), 22 ng TEQ/kg (7.3 ng/kg TCDD, 14.5 ng/kg PeCDF, 73.3 ng/kg PCB-126), 46 ng TEQ/kg (15.2 ng/kg TCDD, 30.4 ng/kg PeCDF, 153 ng/kg PCB-126), and 100 ng TEQ/kg (33 ng/kg TCDD, 66 ng/kg PeCDF, 333 ng/kg PCB-126).

Chemicals. TCDD (lot no. CR82-2-2) was supplied by IIT Research Institute (Chicago, IL), and PCB-126 (lot no. 130494) by AccuStandard, Inc. (New Haven, CT). PeCDF (lot 080196) was purchased from Cambridge Isotope Laboratories (Cambridge, MA). For the TEF mixture, we used the same chemicals as in the single-compound studies. The dose formulations were prepared by mixed volumes of the TCDD, PeCDF, and PCB-126 formulations. Each chemical was received in one lot that was used for the entire study. Purity was determined several times during the study by gas chromatography/mass spectroscopy; by nuclear magnetic resonance spectroscopy; and by gas chromatography using flame ionization detection (PCB-126), electron capture detection (TCDD), proton and ^{13}C nuclear magnetic spectroscopy (PeCDF), and gas chromatography/mass spectrometry (TEF mixture). Purities of TCDD, PCB-126, and PeCDF, were determined to be approximately 98%, 99.51%, 97%, respectively, with no change in purity observed over the duration of the studies. The corn oil was analyzed by potentiometric titration, and the acetone by

Table 1. Incidence and average severity of selected pancreatic lesions in rats treated with TCDD.

		TCDD dose (ng/kg/day)					
	Vehicle control	3	10	22	46	100	100-stop
14-Week interim evaluation							
No. of animals examined	10	10	10	10	10	10	
Inflammation, chronic active	0	0	0	0	0	2	
(no. with lesion)						(1.5)	
Acinus, atrophy	0	0	0	0	0	2	
(no. with lesion)						(1.5)	
31-Week interim evaluation							
No. of animals examined	10	10	10	10	10	10	
Acinus, vacuolation cytoplasmic	0	0	0	0	0	5*	
(no. with lesion)						(1.0)	
53-Week interim evaluation							
No. of animals examined	8	8	8	8	8	8	
Acinus, vacuolation cytoplasmic	0	0	0	0	0	7**	
(no. with lesion)						(1.0)	
Inflammation, chronic, active	0	0	0	0	0	2	
(no. with lesion)						(2.0)	
Acinus, atrophy	0	0	0	0	0	2	
(no. with lesion)						(2.0)	
2-Year evaluation							
Probability of survival (%) at end of study (Kaplan-Meier determinations)	47	39	49	36	42	40	42
No. of organs examined	51	54	52	53	53	51	49
Acinus, vacuolation cytoplasmic	1	0	0	1	15**	42**	0***
(no. with lesion)	(2.0)			(1.0)	(1.1)	(1.8)	
Inflammation, chronic active	0	0	2	2	3	6*	4
(no. with lesion)			(1.5)	(1.0)	(1.3)	(2.0)	(1.5)
Acinus, atrophy	1	2	4	4	4	9*	4
(no. with lesion)	(1.0)	(1.5)	(1.5)	(1.5)	(1.5)	(2.2)	(1.8)
Artery, inflammation, chronic active	0	1	1	2	2	7*	2
(no. with lesion)		(3.0)	(2.0)	(2.5)	(3.0)	(2.3)	(2.5)
Acinus, adenoma[a] (no. with lesion)	0	0	0	0	0	1	0
Acinus, carcinoma[b] (no. with lesion)	0	0	0	0	0	2	1

Lesion severity is shown in parentheses. Grades of lesion severity: 1, minimal; 2, mild; 3, moderate; 4, marked.
[a]Historical incidence (pooled control incidence from the four studies) for 2-year gavage studies with Sprague-Dawley vehicle control group; 1 of 207; range, 0–2%. [b]Historical incidence: 0 of 207. *Significantly different ($p \le 0.05$) from vehicle control group by Fisher exact test (interim evaluations) or Poly-3 test (2-year study). **Significantly different ($p \le 0.01$) from vehicle control group by Fisher exact test (interim evaluations) or Poly-3 test (2-year study). ***Significantly different ($p \le 0.01$) from the 100-ng/kg study group by the Poly-3 test.

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 all study chemicals could maintain an acceptable homogeneity for dosing and stability for 35 days when stored at room temperature. Dose formulations analyzed were within 10% of the target concentrations.

Animals. The animal studies were conducted at Battelle Columbus Laboratories (Columbus, OH). Female Sprague-Dawley rats, approximately 6 weeks of age, were obtained from Harlan (Indianapolis, IN). The animals were held under quarantine for approximately 2 weeks for health screening and were approximately 8 weeks of age at the start of the study. After quarantine, the animals were randomly assigned to control or treatment groups and permanently identified by tail tattoo. They were housed five per cage in solid-bottom polycarbonate cages (Lab Products, Inc., Maywood, NJ) suspended on stainless steel racks. Filtered room air underwent at least 10 changes/hour. Animal rooms were maintained at 69–75°F with 35–65% relative humidity and 12 hr of light and 12 hr of dark. Irradiated NTP-2000 pelleted feed (Zeigler Bros., Inc., Gardners, PA) and water were available *ad libitum*. All animals were observed twice daily for morbidity checks and once each month for formal clinical signs of toxicity; moribund animals were euthanized and necropsied. The health status of the animals was monitored by serologic analysis of serum samples collected from both the study animals and male sentinel rats placed in the study rooms. Serum samples remained negative for any significant rodent pathogen. Animal husbandry and handling were conducted in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources 1996).

Pathology. At necropsy, all tissues were examined grossly, any observed lesions 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 µm, stained with hematoxylin and eosin (H&E), and examined microscopically. The severity of lesions was graded on a four-point scale: 1, minimal; 2, mild; 3, moderate; and 4, marked. The pathology findings from all studies were subjected to a full NTP peer review. For assuring the consistency of the histopathologic diagnoses among the TEF dioxin projects, the same study pathologist, quality assurance pathologist, pathology working group chairperson, NTP pathologist, and members of the pathology working group served in all studies to peer-review the study pathology findings.

Statistical analysis. Incidences of lesions in the study animals were evaluated statistically by the Poly-3 test of Portier and Bailer (1989) and Bailer and Portier (1988), which makes adjustments for survival differences among groups. Incidences of lesions in animals from each of the interim evaluations and from the 2-year study were analyzed separately. For animals in the 2-year studies, the incidences of total lesions, including findings from animals that survived until study termination and from early-death animals, were included in the analysis.

Results

Survival data for the 2-year exposures to TCDD, PCB-126, PeCDF, and the TEF-mixture studies are given in Tables 1–4.

We observed no significant reductions in survival of test animals relative to control animals in any of the four studies. Although none of the individual dose groups showed a significant reduction in survival relative to controls, in the PCB-126 and mixture studies there was a significant ($p < 0.05$) decreasing overall trend in survival, due primarily to a marginally increased mortality in the highest dose group relative to the other groups.

Administration of the four compounds was associated with increased incidences of

Table 2. Incidence and average severity of selected pancreatic lesions in rats treated with PCB-126.

		PCB-126 dose (ng/kg/day)							
	Vehicle control	10	30	100	175	300	550	1,000	1,000-stop
31-Week interim evaluation									
No. of animals examined	10	10	9	10	10	10	10	10	
Inflammation, chronic active	0	0	0	0	0	0	0	1	
(no. with lesion)								(1.0)	
Acinus, atrophy	0	0	0	0	0	0	0	1	
(no. with lesion)								(1.0)	
53-Week interim evaluation									
No. of animals examined	8	8	7	8	8	8	8	8	
Acinus, vacuolation cytoplasmic	0	0	0	0	0	0	1	6*	
(no. with lesion)							(1.0)	(1.0)	
Inflammation, chronic active	0	0	0	0	0	1	0	1	
(no. with lesion)						(1.0)		(1.0)	
Acinus, atrophy	0	0	0	0	0	1	0	1	
(no. with lesion)						(1.0)		(1.0)	
2-Year evaluation									
Probability of survival (%) at end of study (Kaplan-Meier determinations)	31		48	49	42	31	43	13	57
No. of animals examined	51		55	53	53	53	52	51	48
Acinus, vacuolation cytoplasmic	0		0	1	4	9**	20**	23**	1***
(no. with lesion)				(1.0)	(1.5)	(1.0)	(1.1)	(1.4)	(1.0)
Inflammation, chronic active	5		1	3	4	4	6	13*	4***
(no. with lesion)	(1.6)		(1.0)	(2.0)	(1.3)	(1.8)	(2.3)	(2.2)	(1.5)
Acinus, atrophy	5		3	2	7	2	11	18**	7***
(no. with lesion)	(2.0)		(1.3)	(2.5)	(1.6)	(2.5)	(2.2)	(2.1)	(1.4)
Artery, inflammation, chronic active	0		4	2	4	8**	15**	11**	1***
(no. with lesion)			(2.0)	(3.0)	(2.5)	(2.5)	(2.5)	(2.9)	(2.0)
Acinus, adenoma (no. with lesion)	1		0	0	0	2	0	0	0
Acinus, carcinoma (no. with lesion)	0		0	0	1	0	0	1	0

Lesion severity is shown in parentheses. Grades of lesion severity: 1, minimal; 2, mild; 3, moderate; 4, marked.
*Significantly different ($p \leq 0.05$) from vehicle control group by Poly-3 test (2-year evaluation) or Fisher exact test (53-week interim evaluation). **Significantly different ($p \leq 0.01$) from vehicle control group by Poly-3 test. ***Significantly different ($p \leq 0.01$) from 1,000 ng/kg study group by Poly-3 test.

nonneoplastic changes of the exocrine pancreas, including cytoplasmic vacuolation, chronic active inflammation, atrophy, and arteritis, variably observed in the 14-, 31-, and 53-week interim sacrifices and seen in the 2-year studies (Tables 1–4, Figures 1–8). In addition, low incidences of acinar adenoma and carcinoma were also seen in the TCDD, PeCDF, and TEF-mixture studies. The general histologic characteristics were comparable for all chemicals.

Cytoplasmic vacuolation consisted of small, clear, discrete vacuoles within pancreatic acinar cells (Figures 2 and 3). Occasionally a single large vacuole was noted. The severity of the change was determined by the degree of vacuolization per cell and the amount of tissue involved.

Table 3. Incidence and average severity of selected pancreatic lesions in rats treated with PeCDF.

		PeCDF dose (ng/kg/day)					
2-Year evaluation	Vehicle control	6	20	44	92	200	200-stop
Probability of survival (%) at end of study (Kaplan-Meier determinations)	47	42	47	48	38	43	30
No. of animals examined	53	53	53	52	52	52	49
Acinus, vacuolation cytoplasmic (no. with lesion)	0	0	0	0	2 (1.0)	23** (1.1)	2*** (1.0)
Artery, inflammation, chronic active (no. with lesion)	1 (1.0)	2 (2.0)	1 (1.0)	2 (2.5)	4 (2.3)	11** (3.3)	1*** (2.0)
Acinus, adenoma (no. with lesion)	0	0	0	0	1	0	1
Acinus, carcinoma (no. with lesion)	0	0	0	0	1	0	1

Lesion severity is shown in parentheses. Grades of lesion severity: 1, minimal; 2, mild; 3, moderate; 4, marked.
**Significantly different ($p \leq 0.01$) from vehicle control group by Poly-3 test. ***Significantly different ($p \leq 0.01$) from 200 ng/kg study group by Poly-3 test.

Table 4. Incidence and average severity of selected pancreatic lesions in rats treated with TEF mixture.

		TEF dose (ng TEQ/kg/day)			
	Vehicle control	10	22	46	100
14-Week interim evaluation					
No. of animals examined	10	10	10	10	10
Inflammation, chronic active (no. with lesion)	0	0	0	2 (1.0)	0
Acinus, atrophy (no. with lesion)	0	0	0	0	1 (1.0)
31-Week interim evaluation					
No. of animals examined	10	10	10	10	10
Acinus, vacuolation cytoplasmic (no. with lesion)	0	0	0	0	5* (1.0)
Acinus, atrophy (no. with lesion)	1 (1.0)	1 (1.0)	2 (1.0)	0	0
53-Week interim evaluation					
No. of animals examined	8	8	8	8	8
Acinus, vacuolation cytoplasmic (no. with lesion)	0	0	0	2 (1.0)	7** (1.6)
Acinus, atrophy (no. with lesion)	0	0	0	1 (2.0)	1 (2.0)
Inflammation, chronic active (no. with lesion)	0	0	0	1 (3.0)	1 (1.0)
2-Year evaluation					
Probability of survival (%) at end of study (Kaplan-Meier determinations)	30	43	46	44	16
No. of animals examined	52	53	53	53	51
Artery, inflammation, chronic active (no. with lesion)	0	6* (2.2)	3 (1.7)	8** (2.6)	14** (2.9)
Acinus, vacuolation cytoplasmic (no. with lesion)	1 (1.0)	0	3 (1.3)	15** (1.0)	30** (1.1)
Acinus, atrophy (no. with lesion)	3 (1.3)	2 (1.0)	7 (1.7)	7 (1.7)	20** (2.0)
Inflammation, chronic active (no. with lesion)	3 (1.7)	1 (3.0)	6 (1.3)	7 (1.7)	16** (1.8)
Duct, dilatation (no. with lesion)	0	0	0	0	5* (3.0)
Acinus, adenoma (no. with lesion)	0	0	2	0	0
Acinus, carcinoma (no. with lesion)	0	1	0	2	0

Lesion severity is shown in parentheses. Grades of lesion severity: 1, minimal; 2, mild; 3, moderate; 4, marked.
*Significantly different ($p \leq 0.05$) from vehicle control group by Fisher exact test (interim evaluations) or Poly-3 test (2-year study). **Significantly different ($p \leq 0.01$) from vehicle control group by Fisher exact test (interim evaluations) or Poly-3 test (2-year study).

Atrophy was a focal to multifocal to diffuse change consisting of a reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue and dilatation of the ducts (Figures 4, 5, and 7). Chronic active inflammation was generally seen in association with atrophy and consisted of an infiltrate of mononuclear cells with occasional neutrophils within the stroma. The islets of Langerhans were morphologically normal (Figures 2–4), dispersed throughout the affected acinar tissue and without reduction in their number.

Arterial chronic active inflammation was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries, with infiltration into the muscular layers of the artery (Figure 1) (Jokinen et al. 2003). Fibrinoid necrosis of the vessel occurred often, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number. This inflammatory reaction often extended into the surrounding parenchyma.

Adenoma of the acinar cells was characterized microscopically by a discrete mass consisting of tubular and acinar structures composed of small acinar cells with brightly eosinophilic cytoplasm lacking zymogen granules. In contrast, a single case of carcinoma exhibited a

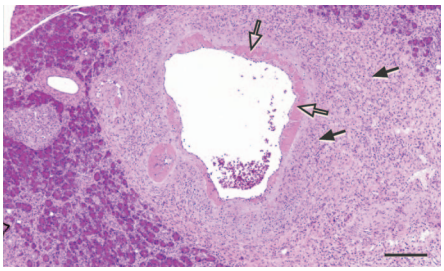

Figure 1. Photomicrograph showing chronic active inflammation of the pancreatic artery from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. This inflammation is characterized by transmural infiltration of mixed inflammatory cells (solid arrows) and fibrinoid necrosis (open arrows). H&E; bar = 500 µm.

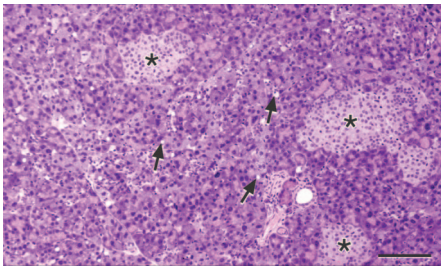

Figure 2. Low magnification photomicrigraph of pancreatic tissue from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. Cytoplasmic vacuolation (arrows) consists of small, clear, discrete vacuoles within pancreatic acinar cells. Apparently normal islets of Langerhans are indicated by asterisks. H&E; bar = 300 µm.

large, multinodular lesion with moderate amounts of dense fibrous stroma. Carcinomas appeared composed of densely packed clusters of poorly formed acinar structures consisting of small acinar cells with prominent vesicular nuclei and small amounts of eosinophilic cytoplasm with indistinct borders. Scattered solid areas composed of densely packed, highly pleomorphic, round to ovoid acinar cells with large vesicular nuclei and scant cytoplasm also occurred.

In the TCDD study, treatment-related nonneoplastic changes were seen at the 31-week interim sacrifice and became progressively more prominent at the other sacrifice periods (Table 1). The incidences of the nonneoplastic lesions in the stop-exposure group were less than those in the 100-ng/kg study group. One acinar adenoma and two acinar carcinomas were seen in the 100-ng/kg study group. The incidences of acinar cell carcinoma and adenoma or carcinoma (combined) exceeded those within the historical control range (overall incidence of acinar cell adenoma, 1 of 207 or 0.5%, range 0–2%; overall incidence of acinar cell carcinoma, 0%). A single acinar cell carcinoma was seen in the stop-exposure group.

In the PCB-126 study, treatment-related nonneoplastic changes observed at the 31-week sacrifice period became progressively more prominent at the other sacrifice periods (Table 2). We found fewer nonneoplastic lesions in the stop-exposure group than in the 1,000 ng/kg study group. In the 2-year study group, exocrine adenomas and carcinomas were observed sporadically in treated groups but were not related to exposure with PCB-126. Only one adenoma was observed in the control group.

At the 2-year sacrifice of the PeCDF portion of this investigation, we observed one acinar adenoma and one acinar carcinoma in both the 92-ng/kg group and the 200-ng/kg stop-exposure group (Table 3). An increased incidence of acinar cytoplasmic vacuolation and increased incidence and severity of arteritis also occurred in the 92-ng/kg and 200-ng/kg study groups; the incidence in the 200-ng/kg stop-exposure study group was significantly decreased compared with the 200-ng/kg study group.

In the TEF-mixture group, treatment-related nonneoplastic changes were seen at the 14-week sacrifice period and became progressively worse at the other sacrifice periods (Table 4). At 2 years, sporadic incidences of acinar adenoma and acinar carcinoma were observed in all dosed groups except those administered 100 ng/kg. The incidence of acinar adenoma in the 22-ng/kg group and the incidences of acinar carcinoma in the 10-ng/kg and 46-ng/kg groups exceeded the historical control ranges (overall incidence of acinar cell adenoma, 1 of 207 or 0.5%, range 0–2%; overall incidence of acinar cell carcinoma, 0%).

Discussion

In these studies, we evaluated the effects of chronic exposure to dioxin and multiple DLCs on the pancreas in the female Harlan Sprague-Dawley rat. Our data indicate that the pancreatic exocrine acini are a target tissue of the DLCs, inducing mainly degenerative, inflammatory, and atrophic lesions and possibly also sporadic acinar adenomas and carcinomas. Despite the low incidences, we found one pancreatic tumor effect that was statistically significant ($p < 0.001$): the increasing trend in pancreatic acinar cell adenoma/carcinoma in the TCDD study. Although the marginal increases in the other studies would not be particularly noteworthy when considered individually, it is the consistency of the effects in all four studies that is important. Only a single control animal (of 207) had a pancreatic

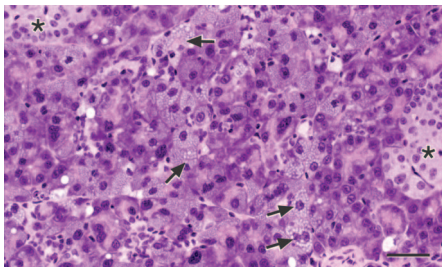

Figure 3. High magnification photomicrigraph of pancreatic tissue from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. Cytoplasmic vacuolation (arrows) consists of small, clear, discrete vacuoles within pancreatic acinar cells. Apparently normal islets of Langerhans are indicated by asterisks. H&E; bar = 100 µm.

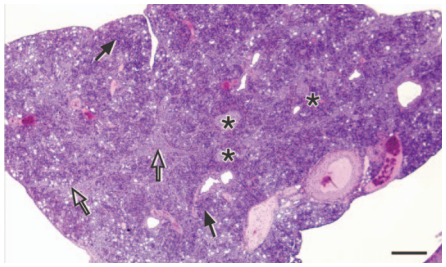

Figure 4. Low magnification photomicrograph of pancreas affected by diffuse chronic active inflammation (open arrows) and acinar atrophy (solid arrows) from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. Chronic active inflammation consists of mixed inflammatory cells throughout the parenchyma; atrophy is characterized by reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue and dilatation of ducts. Apparently unaffected islets of Langerhans are present throughout affected acinar tissue (asterisks). H&E; bar = 1,000 µm.

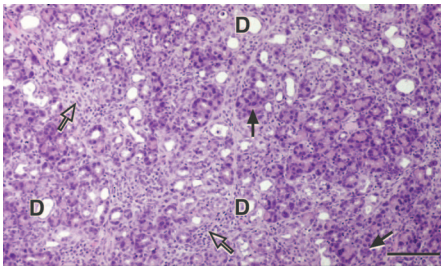

Figure 5. Higher magnification photomicrograph of pancreas affected by diffuse chronic active inflammation (open arrows) and acinar atrophy (solid arrows) from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. Dilated ducts (D), occasionally containing sloughed cells, are present throughout the gland. H&E; bar = 300 µm.

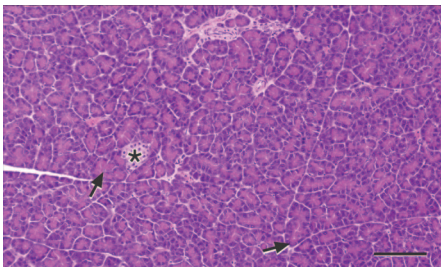

Figure 6. Photomicrograph showing unaffected acinar tissue (arrows) from a control rat from the PCB-126 2-year study. A normal islet of Langerhans is indicated by the asterisk. H&E; bar = 300 µm.

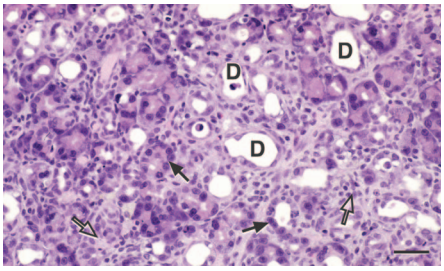

Figure 7. Higher magnification photomicrograph of pancreas affected by diffuse chronic active inflammation (open arrows) and acinar atrophy (solid arrows) from a female rat administered 1,000 ng/kg/day PCB-126 by gavage for 2 years. Note the relatively small area of acinar-cell cytoplasm compared with those in Figure 8 and the interstitial mixed inflammatory cells. Dilated ducts (D), occasionally containing sloughed cells, are present throughout the gland. H&E; bar =100 µm.

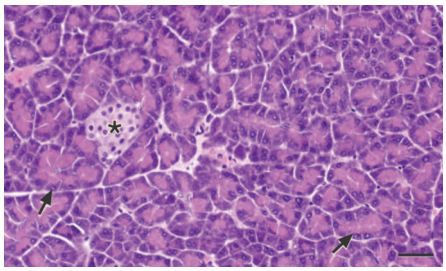

Figure 8. Photomicrograph showing unaffected acinar tissue (arrows) from a control rat from the PCB-126 2-year study. A normal islet of Langerhans is indicated by the asterisk. H&E; bar = 100 µm.

acincar cell tumor (an adenoma), confirming that this tumor is relatively uncommon. Yet in the TCDD, PCB-126, PeCDF, and TEF-mixture groups, we found four, four, four, and five pancreatic acinar cell tumors, respectively, including two or more carcinomas in each study. It is this consistency of effect for these DLCs for a relatively uncommon tumor that is impressive. The potential copromotional effect of corn oil on the pancreatic tumors has been considered (Eustis and Boorman 1985); however, this association was primarily reported as a male rat phenomenon, and we found only 1 adenoma in 362 female rats treated with corn oil for 2 years and used as controls in our TEF studies. Majeed (1997) reported that the historical incidence of exocrine pancreatic tumors in aged CD female rats was 0.1% for adenomas and 0.02% for carcinomas. Two collections of historical data dealing with the Sprague-Dawley rats reported zero incidence of pancreatic acinar tumors (Kaspareit and Rittinghausen 1999; McMartin et al. 1992). We observed low incidences, but higher than historical levels, of pancreatic acinar adenoma and carcinoma in the TCDD, PeCDF, and TEF-mixture studies (Tables 1, 3, and 4). Both the adenomas and carcinomas observed in these studies were exclusively of exocrine acinar cell origin. Therefore, regarding the pancreatic tumors, we conclude that there was a marginal increase in this uncommon tumor in all four studies.

The acinar cell adenomas we observed in the rats were characterized by being a discrete mass consisting of tubular and acinar structures composed of small acinar cells with brightly eosinophilic cytoplasm and lacking zymogen granules. In contrast, the acinar cell carcinoma was a large, multinodular lesion, with moderate amounts of dense fibrous stroma. Carcinomas were composed of densely packed clusters of poorly formed acinar structures consisting of small acinar cells with prominent vesicular nuclei and small amounts of eosinophilic cytoplasm with indistinct borders. We also observed scattered solid areas composed of densely packed, highly pleomorphic, round-to-ovoid acinar cells with large vesicular nuclei and scant cytoplasm.

Acinar cell carcinomas do occur rarely in humans, approximately 1% of all pancreatic tumors (Solcia et al. 1997); however, the existence of a benign counterpart, acinar cell adenoma, has not been clearly established (Lack 2003a, 2003b). Excluding the rare cases in children, the average age of adults with acinar cell carcinoma in two series was 62 years (Klimstra et al. 1992) and 55 years (Hoorens et al. 1993). These tumors typically appear to be well circumscribed and often exhibit coarse lobulation on cut section, simulating the lobular character of the normal human pancreas. Microscopically, the tumor lobules are highly cellular with scanty stroma, and the tumor cells may be arranged in acinar, solid, trabecular, or glandular patterns. These are aggressive neoplasms, as indicated by the presence of metastases in about 50% of the patients at the time of diagnosis, and patients have a 5-year survival rate of < 10% (Lack 2003b).

In contrast to tumor origin in the Sprague-Dawley rat, the 80% of all pancreatic tumors in humans are ductal in origin (ductal adenocarcinomas). When variants of ductal adenocarcinoma are included (mucinous noncystic carcinoma, adenosquamous carcinoma, and undifferentiated carcinoma), about 90% of pancreatic tumors are ductal (Solcia et al. 1997). Most ductal adenocarcinomas are well to moderately differentiated and, in contrast to acinar tumors, secrete mucin and elicit a desmoplastic stromal reaction. Invasion of peripancreatic retroperitoneal fatty tissue occurs early in the course of the disease, and despite surgery, most patients die of tumor recurrence within 1–2 years (Solcia et al. 1997). Unfortunately, this is not only the most common of the human pancreatic tumors but also the most malignant, with an overall 5-year survival rate in most series of ≤ 2% (Rosai 1996).

Several compounds have been shown to induce pancreatic tumors in rats, including nitrofen [National Cancer Institute (NCI) 1978], 3,2′-dimethyl-4-aminobiphenyl (Shirai et al. 1989), and azaserine (Woutersen et al. 1989). Induction of pancreatic inflammation after ethionine treatment in rats promoted the transformation of benign pancreatic acinar tumors into malignant tumors, and this transformation was associated with the appearance of mutated p53 protein (Bednarz and Olewinski 2002).

The generation of the DLC-related pathology in the pancreas may be related to a variety of factors [e.g., the induction of drug-metabolizing enzymes such as cytochrome P450 isoenzymes, down-regulation of cholecystokinin (CCK), perturbations in the vitamin A homeostasis, or other mechanisms] that act either independently or concomitantly to promote the development of pancreatic damage.

An increase in CYP1A1 and CYP1A2 is a hallmark response to DLCs and is directly linked to binding and activation of the AhR by DLCs (Whitlock 1993). This is mediated by binding of the AhR complex to dioxin response elements present in the 5′ flanking region of the gene. In one study, the AhR was expressed in all tissues examined (Dolwick et al. 1993) with a definite tissue specificity in level of expression and diversity of response, indicating that DLCs may be likely to exert some effect in every tissue. In the same study, Northern blot analysis indicated that human AhR mRNA is highly expressed in the pancreas. One must remember, however, that even with the same receptor and the same ligand, both qualitative and quantitative differences exist in the response to TCDD, depending on the tissue and species involved.

A relationship among exposure to environmental lipophilic chemicals, elevated levels of drug-metabolizing enzymes in the pancreatic exocrine and endocrine cells, and increased incidences of pancreatic cancer and chronic pancreatitis may exist in humans (Foster et al. 1993; Standop et al. 2002). Future studies of samples from our DLC studies in the rat are aimed at investigating the potential involvement of cytochrome P450 induction in the pathogenesis of the pancreatic acinar pathology.

The observed acinar atrophy of the pancreas may be related in part to the down-regulation of CCK, an important regulator of pancreatic growth and function (Baldwin 1995; Varga et al. 1998). As shown by Lee et al. (2000) in samples from the present PCB-126 study, levels of intestinal CCK are reduced by PCB-126 exposure. Down-regulation of CCK is likely due to a general endocrine effect resulting from the reduction in body weight gain observed with exposure to DLCs. Previous studies have shown that increased apoptosis and pancreatic acinar atrophy occur in Otsuka Long-Evans Tokushima Fatty rats that lack the *CCK-A* receptor gene (Jimi et al. 1997). In addition, antagonism of CCK action by chemical (devazepide) blockage of the CCK-A receptor can lead to reduced pancreatic proliferation (Ohlsson et al. 1995).

TCDD influences and disturbs vitamin A dynamics and metabolism; vitamin A metabolites are known to affect the endocrine and exocrine glandular cell integrity (Fattore et al. 2000; Nilsson and Hakansson 2002; Schmidt et al. 2003). Certain synthetic retinoids, administered in the diet for 1 year at a dose of 0.5–2 mmol/kg, have chemopreventive potential, reducing the progression of pancreatic carcinomas induced in rats by five weekly injections of azaserine (Longnecker et al. 1983). More studies are required to determine the presence or absence of disturbed vitamin A metabolism in Sprague-Dawley rats exposed to DLCs; the biologic relevance of disturbed vitamin A metabolism to the pathogenesis of exocrine-gland pathology remains to be elucidated.

Chronic inflammation is associated with oxidative stress, which leads to DNA damage and cancer promotion in experimental studies and clinical cases. In a study of inhalation exposure to indium phosphide, a pulmonary carcinogen, severe pulmonary inflammation occurred that correlated with the infiltration of reactive oxygen-generating immune cells, and macrophages exhibited high levels of the inducible forms of nitric oxide synthase (i-NOS) and cyclo-oxygenase (COX-2)

(Gottschling et al. 2001). Nitric oxide promotes tumor progression (Orucevic et al. 1999), and similarly, COX-2–derived prostanoids contribute to the progression of hyperplasia in nearby epithelial cells (Fosslien 2000). In humans, chronic pancreatitis has been implicated in 3–4% of pancreatic cancers (Maisonneuve and Lowenfels 1999, 2002). In our investigation, however, the severity of the range of nonneoplastic changes (e.g., degeneration and inflammation) in the exocrine pancreas of treated animals with acinar cell tumors did not indicate a higher grade of severity compared with other animals in the same groups without such tumors. Our data, therefore, do not to support the notion that chronic inflammation in the pancreas may promote carcinogenesis in this organ.

Our studies were not designed to measure the levels of islet-related hormones in the blood, and the relative number of islets/area of pancreatic tissue was not evaluated. Other studies in rats, however, have shown that TCDD treatment (25 and 125 ng/kg) reduces the serum levels of insulin and glucagon. Because the damage in the pancreas of animals exposed to DLCs was widespread, including alterations to cell membranes and arteritis, we speculate that the functionality and integrity of the islets were probably also affected (Ebner et al. 1993; Gorski et al. 1988). No treatment-related histopathologic change was observed in the islets of Langerhans in rats treated with these DLCs; however, the hormonal function of the islets was not monitored in these studies. Because of the widespread involvement of the exocrine pancreas, as well as the observed damage to the blood vessels, we can speculate that alterations in islets of Langerhans–derived hormonal levels in the serum may have occurred.

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