Design of 2-year bioassays with dioxin-like compounds in female Sprague Dawley rats

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Introduction

Dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) have a common mechanism of action: an initial binding to the aryl hydrocarbon (Ah) receptor. This receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals[1)]. In addition, these compounds bioaccumulate, are structurally related, and result in dioxin-like effects. These four criteria are used for the inclusion of dioxin-like compounds in the toxic equivalency factor (TEF) methodology[2)]. TEF values are consensus relative potency values derived from all available studies which compared the TEF chemical to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This methodology is currently used to calculate the total dioxin-activity of mixtures of this class of compounds[2-9)]. In this TEF methodology, the response of each compound that acts through the Ah receptor is expressed as a potency value relative to TCDD, the most potent dioxin-like compound which is assigned a TEF value of one. Multiplying the TEF value of a specific compound by the concentration of that compound in a mixture results in the TCDD equivalent (TEQ) of that compound. The sum of all TEQs for every dioxin-like compound in a mixture gives the total TEQ of that specific mixture.

The TEF-concept is based on additivity of the individual dioxin-like congeners in mixtures. Additivity has been reported for mixtures of dioxin-like compounds or dioxin-like and non-dioxin-like compounds for tumor promotion, lethality, body weight loss, teratogenicity, fish early life stage mortality, immunotoxicity, and cytochrome P450 induction[5,6,10-14)]. However, non-additive effects have been reported after co-administration of dioxin-like compounds or dioxin-like and non-dioxin-like compounds for various endpoints. Synergistic interactions have been reported for cytochrome P450 induction, tumor promotion, lethality, thyroid hormone metabolism, hepatic porphyrin accumulation, and *in vitro* mutagenicity[5,6,15)]. These synergistic effects have been attributed to multiple mechanisms and/or are associated with alterations in distribution of the administered compounds. In addition, antagonistic effects have been reported for cytochrome P450 induction, immunotoxicity, and teratogenicity[5,6)]. For immunotoxocity it has been shown that two competing mechanisms are involved, leading to functional antagonism[16)]. In addition, several studies suggest that some antagonistic effects are related to alterations in the tissue distribution of the administered compounds.

The 2-year carcinogenicity study of Kociba and co-workers[17)] and the 3-generation study of Murray *et al.*[18)] form the basis for the risk assessment of TCDD in most countries using a safety factor approach. In the United States carcinogenicity has driven the risk assessment of TCDD[19)]. TCDD is carcinogenic in mice, rats, hamsters, and humans[1,20)]. Mixtures of dioxins or PCBs have also shown to be carcinogenic[21-23)]. However, it is not clear whether the TEF concept holds for carcinogenicity. Moreover, no animal cancer data are available for individual PCDF and PCB congeners.

This extended abstract describes the study design of 2-year bioassays in female Sprague Dawley rats with dioxin-like compounds to be conducted by the National Toxicology Program. Female Sprague Dawley rats will be used since this rat has been used frequently in chronic and subchronic studies with dioxin-like compounds for supporting the calculation of TEF values. The tested compounds reflect the most potent congeners from each class of dioxin-like chemicals: TCDD, 2,3,4,7,8-pentachlorodibenzofuran (4-PnCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126, a planar PCB), and 2,3',4,4',5-pentachlorobiphenyl (PCB 118, a mono-ortho substituted PCB). These four dioxin-like compounds reflect about 80% of the total TEQ of human and environmental samples using the current TEF values (see Table 1). Furthermore, a di-ortho substituted PCB will be evaluated for carcinogenicity: 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), since this PCB is the most abundant PCB in environmental and human samples.

To test the additivity of the TEF concept for carcinogenicity, two mixture studies have been designed. The first mixture consists of three planar compounds: TCDD, 4-PnCDF, and PCB 126. The dose levels of each compound in this mixture are chosen based on the current TEF values of these compounds (Table 1) and also considering the maximum detectable sensitivity for possible interactive effects, *i.e.*, they are tested in equal (expected) TEQ ratios (1:1:1). This ratio is very close to the actual ratio of these compounds to which humans are exposed, *i.e.*, 1:1:4.5 for TCDD:4-PnCDF:PCB 126. The second mixture consists of a planar PCB (PCB 126) and a di-ortho substituted PCB (PCB 153). In this way the ability of a non-dioxin-like PCB to alter the carcinogenic potency of a planar PCB can be evaluated. The ratios used in this binary study cover the ratio of these compounds to which humans are exposed (PCB 126:PCB 153 1:1000).

In addition, a stop study with each chemical is proposed since a recent study with TCDD resulted in different tumors when dosed for 30 weeks compared to those dosed for 2 years[24].

Aim

A) To test the carcinogenic potency of dioxin-like and non-dioxin-like compounds: TCDD, 4-PnCDF, PCB 126, PCB 118, and PCB 153.
B) To test the TEF concept in a mixture containing three planar compounds (TCDD, 4-PnCDF, and PCB 126).
C) To test the ability of a non-dioxin like PCB (PCB 153) to alter the carcinogenic potency of a planar PCB (PCB 126).

Methods

Chemicals: TCDD, 2,3,4,7,8-pentachlorodibenzofuran (4PnCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) will be used as the test chemicals in the oral gavage studies.

Animals and treatment: Female Sprague-Dawley rats (Harlan) (8 weeks old) will be given water and food *ad libitum*. The rats will be assigned to the treatment groups (see Table 2-4) and housed 5 per cage. Rats will be exposed to the chemicals or mixtures (in corn oil) by gavage for 5 days a week for up to 104 weeks. Fifty animals per dose group (including controls) will be dosed for 104 weeks for evaluation of carcinogenicity. Interim evaluations will be conducted at 13, 30, and 52 weeks using an additional 10 animals per dose group (including controls) per time point. In addition, stop studies will be included with 50 rats at one concentration. These rats will be dosed up to 30 weeks and will receive vehicle only from week 30 until week 104. Furthermore, an additional 20 rats will be included in each treatment and control group and dosed for up to 104 weeks. These rats will be used for optional special studies.

Endpoints: The interim sacrifices at 13, 30, and 52 weeks will be evaluated for the endpoints as mentioned in Table 5. Histopathologic evaluations will be performed on a select number of tissues (Table 6) at each interim time point. For the animals on study until 104 weeks (including the stop study), an expanded list of organs (Table 7) will be evaluated histopathologically. Body weights will be recorded on day one on test, at weekly intervals for the first 13 weeks, at 4 week intervals thereafter, and at terminal sacrifice. In addition, selected tissues will be weighed.

Table 1. Current TEF values.

Compound	TEF
TCDD	1
4-PnCDF	0.5[3)]
PCB 126	0.1[2)]
PCB 118	0.0001[2)]

Table 2. Dose levels of the single congeners in the 2-year bioassays in female Sprague Dawley rats.

Compound	Dose levels
TCDD	0, 3, 10, 22, 46, and 100 ng/kg/day
4-PnCDF	0, 6, 20, 44, 92, and 200 ng/kg/day
PCB 126	0, 10, 30, 100, 175, 300, 550, and 1000 ng/kg/day
PCB 118	0, 100, 220, 460, 1000, 4600 µg /kg/day
PCB 153	0, 10, 100, 300, 1000, and 3000 µg /kg/day

Table 3. Dose levels of the planar mixture in the 2-year bioassay in female Sprague Dawley rats.

dose (ng TEQ/kg/day)	TCDD dose (ng/kg/day)	PnCDF dose (ng/kg/day)	PCB 126 dose (ng/kg/day)	estimated TEQ ratio
10	3.3	6.6	33.3	1:1:1
22	7.3	14.5	73.3	1:1:1
46	15.2	30.4	153	1:1:1
100	33	66	333	1:1:1

Table 4. Dose levels of the binary mixture in the 2-year bioassay in female Sprague Dawley rats.

PCB 126 (ng/kg/day)	PCB 153 (µg/kg/day)	Ratio PCB 153:PCB 126
10	10	1000
100	100	1000
300	100	333
300	300	1000
300	3000	10000
1000	1000	1000

Table 5. Interim evaluations in female Sprague Dawley rats (week 13, 30, and 52).

Organ	Analyses
Liver	CYP1A1, CYP1A2, CYP1B1, T4-UGT, UGT1A1, UGT1A2, retinoid levels, PGST, TGFα, in situ end labeling, CK19, cell proliferation, porphyrins, tissue concentration (also at 104 weeks)
Lung	CYP1A1, CYP1B1, tissue concentration (also at 104 weeks)
Blood	TT4, FT4, TT3, TSH, tissue concentration (also at 104 weeks)
Adipose tissue	Tissue concentration (also at 104 weeks)
Ovaries	Ah receptor

Table 6. Histopathologic evaluations of tissues at interim evaluations (week 13, 30, and 52).

Tissue
Adrenal glands
Duodenum
Liver
Lung
Mammary gland
Ovary
Pituitary
Thyroid
Uterus
Vagina

Table 7. Histopathologic evaluations of tissues at the end of the 2-year studies (including the stop studies).

Organ/tissue	Organ/tissue
Adrenal glands	Nose (nasal cavity and nasal turbinates)
Brain	Ovaries
Clitoral glands	Pancreas
Esophagus	Parathyroid glands
Femur	Pituitary gland
Gross lesions	Salivary glands
Heart and aorta	Spinal cord and sciatic nerve
Intestine, large (cecum, colon, rectum)	Spleen
Intestine, small (duodenum, jejunum, ileum)	Stomach (forestomach and glandular)
Kidneys	Thymus
Liver	Thyroid glands
Lungs and mainstem bronchi	Trachea
Lymph nodes: mandibular and mesenteric	Urinary bladder
Mammary gland with adjacent skin	Uterus
Muscle	

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