Carcinogenicity of individual and a mixture of dioxin–like compounds in female Harlan Sprague Dawley rats.

Nigel Walker[1], Abraham Nyska[1], Patrick Crockett[2], Amy Brix[3], Micheal Jokinen[4], James Hailey[1], Joseph Haseman[1], Donald Sells[5], Michael Wyde[1], Cynthia Smith[1], Denise Orzech[1], John Bucher[1]

[1]National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
[2]Constella Group, Research Triangle ParK, NC, USA
[3]Experimental Pathology Laboratories, Research Triangle Park, NC, USA
[4]Pathology Associates, Durham, NC, USA
[5]Battelle Columbus Laboratories, Columbus, OH, USA

Introduction

The human health risk posed by exposure to persistent organochlorine pollutants (POPs), including polychlorinated-dioxins (PCDDs), -furans (PCDFs) and –biphenyls (PCBs), present in the food and the environment is one of widespread concern throughout the industrialized world. The dioxin Toxic Equivalency Factor (TEF) approach is currently the most feasible interim approach for assessing and managing the risk posed by exposure to mixtures of these compounds and has been formally adopted by regulatory bodies in many countries, the International Programme on Chemical Safety and the World Health Organization[1].

The TEF methodology is a relative potency scheme that estimates the total exposure and biological effects of a mixture of chemicals based on a common mechanism of action involving an initial binding of the compound to the Aryl hydrocarbon receptor (AhR)[2,3]. An implicit assumption of the TEF methodology is that the combined risk of effects of the different congeners is dose additive. Therefore, the total dioxin toxic equivalents (TEQs) of a mixture of PCDDs, PCDFs, and PCBs may be estimated by the summation of the mass of each compound in the mixture after adjustment for its potency relative to that of 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (TCDD). While dose additivity is supported for certain mixtures for some biological endpoints in some experimental models, this has never been evaluated for cancer risk.

Here we present a summary of four chronic rodent bioassay conducted by the National Toxicology Program (US Department of Health and Human Services) that evaluated the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3.3',4,4',5- pentachlorobiphenyl (PCB126) and 2,3,4,7,8 pentachlorodibenzofuran (PeCDF) and a mixture of these three dioxin-like compounds in female Harlan Sprague Dawley rats. Data from these studies will be used to test the hypothesis of dose-additivity of carcinogenicity by a defined mixture of dioxin-like compounds.

Materials and Methods

These studies were conducted in female Harlan Sprague Dawley rats only, based on the prior observations of the carcinogenicity of TCDD in Spartan Sprague Dawley rats[4]. The animal studies were conducted at Battelle Columbus Laboratories (Columbus, OH). Animals were obtained from Harlan (Indianapolis, IN) and upon receipt were approximately 6 weeks of age. They were held under quarantine for approximately 2 weeks for health screening and were approximately 8 weeks old at the start of the study. After quarantine, the animals were randomly assigned to control or treated groups and permanently identified by tail tattoo. They were housed 5 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 hours of light and 12 hours 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 and once/month for formal clinical signs of toxicity; 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 male sentinel rats that were placed in the study rooms. Serum samples remained negative for any significant rodent pathogen. Animal husbandry and handling were conducted in accordance with the National Institutes of Health Guidelines.

TCDD was supplied by IIT Research Institute (Chicago, IL), PCB126 by AccuStandard, Inc. (New Haven, CT) and PeCDF was purchased from Cambridge Isotope Laboratories (Cambridge, MA). 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; nuclear magnetic resonance spectroscopy; and gas chromatography using flame ionization detection (PCB 126), electron capture detection (TCDD), proton and carbon-13 nuclear magnetic spectroscopy (PeCDF), and GC/MS (TEF mixture). Purities of TCDD, PCB126, PeCDF, and the TEF mixture were determined to be approximately 98%, 99.51%,

97%, respectively, with no change in purity observed over the duration of the studies. Dose formulations were prepared for gavage administration by mixing the test chemical in a corn oil vehicle containing 1% USP-grade acetone. The corn oil was analyzed by potentiometric titration and the acetone by infrared spectroscopy. Homogeneity and stability studies of dose formulations indicated that 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. For the mixture, the dose formulations were prepared by mixed volumes of the TCDD, PeCDF, and PCB 126 formulations.

Animals were treated by daily gavage, 5 days per week for up to 2 years. Target doses used for the individual compound studies were TCDD (3, 10, 22, 46, 100 ng/kg); PCB126 (30, 100, 175, 300, 550, 1000 ng/kg); PeCDF (6, 20, 44, 92, 200 ng/kg). The TEF mixture was comprised of equal ratios (1:1:1) of TCDD equivalents (TEQ) for TCDD, PCB126, 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, doses were formulated for comparison to the 0, 10, 22, 46, and 100 ng TCDD/kg/day group by using the WHO TEFs of 1.0 for TCDD, 0.1 for PCB126, and 0.5 for PeCDF. Specific target 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). Control animals received corn oil:acetone vehicle (2.5ml /kg) alone. A high dose stop-exposure group was included in the individual compound studies. In this group, dosing was for 31 weeks after which time animals received vehicle alone for the remainder of the study. Dose selection for TCDD 3-100 ng/kg/day was based on the prior range used in the Kociba et al study and on the demonstrated induction of liver neoplasms over this dose range.

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 5µm, stained with hematoxylin and eosin (H&E), and examined microscopically. The pathology findings from all studies were subjected to a full pathology peer review. For assuring the consistency of the histopathological diagnoses among the TEF dioxin projects, the same study pathologist, Quality Assurance (QA) pathologist,

Pathology Working Group (PWG) chairperson, NTP pathologist, and members of the PWG served in all studies.

Results and Discussion

There was a significant increase in the incidences of non-neoplastic lesions in multiple tissues including the lung, liver, oral mucosa, heart, thymus, adrenal gland, thyroid gland, and pancreas. There was a significant increase in the incidence of neoplastic effects in multiple tissues in each study. Most notable were increased incidences of four specific neoplasms seen in common across multiple studies. These were cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. (Table 1). The data on the incidence of all neoplastic and non-neoplastic lesions in each of these studies is available on the U.S. National Toxicology Program web server (http://ntp.niehs.nih.gov). These data were obtained according to GLP and underwent extensive pathology peer review. Hepatocellular proliferative lesions were also reviewed in a special pathology workshop and overall conclusions of the complete studies were also peer reviewed by an NTP external expert panel advisory board.

In the TCDD study, at 2 years, the incidence of hepatocellular adenoma was significantly increased in the 100 ng/kg study group and exceeded the pooled vehicle control range (comprised of the control data from all four studies). Dose-related increased incidences of cholangiocarcinoma were seen in the rats administered 22 ng/kg or greater. The highest incidence of cholangiocarcinoma was seen in the 100 ng/kg dosed group and included a significant number of animals with multiple cholangiocarcinomas. No cholangiocarcinomas occurred in the pooled vehicle controls in these studies. Two cholangiocarcinomas and two hepatocellular adenomas were seen in the 100 ng/kg stop-exposure group. Two hepatocholangiomas were also seen in the 100 ng/kg study group, and one cholangioma occurred in a 100 ng/kg stop-exposure rat (data not shown). No hepatocholangiomas or cholangiomas occurred in the pooled vehicle controls. In the lung at 2 years, the incidence of cystic keratinizing epithelioma (CKE) was significantly increased in the 100 ng/kg group, and multiple epitheliomas occurred in two animals in the 100 ng/kg group. There were no epitheliomas in the 100 ng/kg stop-exposure group, and cystic keratinizing epitheliomas did not occur in the pooled vehicle controls. The epitheliomas ranged from relatively small to very large lesions that replaced much of the normal lung parenchyma. In the oral mucosa there was a positive trend in the incidence of gingival squamous cell carcinoma, and the incidence in the 100 ng/kg group was significantly increased.

In addition, the incidences in the 46 ng/kg and 100 ng/kg and stop-exposure groups exceeded the pooled vehicle control range.

The site specificity of these increased neoplasms is consistent with prior observation of the carcinogenicity of TCDD in the dosed feed study of Kociba et al. that was conducted in the Spartan Sprague-Dawley rat[4]. In that study they observed specific increased incidences of neoplasms in the liver, lung and hard palate/nasal turbinates. This present study further shows that related dioxin-like compounds PCB126 and PeCDF and a mixture of TCDD, PCB 126 and PeCDF qualitatively show a similar pattern (Table 1). Significant increases in incidences of hepatocellular adenoma, cholangiocarcinoma, cystic keratinizing epithelioma and gingival squamous cell carcinoma were also seen in the PCB126 study. A significant increased trend in incidence of hepatocellular adenoma, and cholangiocarcinoma was observed in the PeCDF study. A significant increased incidence of hepatocellular adenoma, cholangiocarcinoma, and cystic keratinizing epithelioma was seen in the mixture study. Marginally increased incidences of gingival squamous cell carcinoma were seen in the PeCDF and TEF mixture studies but they did not reach statistical significance. Although not statistically significant, the incidence of squamous cell carcinoma of the oral cavity in the high dose (200 ng/kg) group from the PeCDF study were outside the pooled vehicle control range, and were considered treatment related.

The observation of a similar site specificity across these studies is consistent with the hypothesis that these three compounds have a common mechanism of action that involves initial binding to the AhR[5]. Quantitative differences between the studies in the specific incidences of specific neoplasms suggest a deviation of the actual relative potency factor for specific sites from the current WHO TEF values[1]. Current efforts are using data from these studies for the statistically based quantitative determination of specific relative potency factors for carcinogenicity at these sites and how they relate to the WHO TEF values, and a statistically based evaluation of the dose-additivity of the effects of these individual compounds when present in a mixture.

Acknowledgements

The authors would like to thank all those involved in the design, conduct, reporting and interpretation of these NTP studies. These studies could not have been completed without the hard work of a large number of people. While space constraints limit acknowledgement of each and every person, we would like to give special thanks to Angelique Braen, Milton Hejtmancik, Michael Easterling, Charles Alden and Susan Gunnels for their hard work and efforts.

References

1. Van den Berg, M.; Birnbaum, L.; Bosveld, A.T.C.; Brunstrom, B.; Cook, P.; Feeley, M.; Giesy, J.P.; Hanberg, A.; Hasegawa, R.; Kennedy, S.W.; Kubiak, T.; Larsen, J.C.; van Leeuwen, F.X.; Liem, A.K.; Nolt, C.; Peterson, R.E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Waern, F.; Zacharewski, T. Environ Health Perspect 1998, 106, 775-792.
2. Ahlborg, U.G.; Brouwer, A.; Fingerhut, M.A.; Jacobson, J.L.; Jacobson, S.W.; Kennedy, S.W.; Kettrup, A.A.; Koeman, J.H.; Poiger, H.; Rappe, C.; Safe, S.H.; Seegal, R.F.; Tuomisto, J.; van den Berg, M. Eur J Pharmacol 1992, 228, 179-199.
3. Safe, S.H. Environ Health Perspect 1998, 106 Suppl 4, 1051-1058.
4. Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M.; Wade, C.E.; Dittenber, D.A.; Kalnins, R.P.; Frauson, L.E.; Park, C.N.; Barnard, S.D.; Hummel, R.A.; Humiston, C.G. Toxicol. Appl. Pharmacol. 1978, 46, 279-303.
5. Birnbaum, L.S. Prog Clin Biol Res 1994, 387, 139-154.
6. Bailer, A.J.; Portier, C.J. Biometrics 1988, 44, 417-431.

Table 1. Summary of survival adjusted neoplasm incidences (%).

Study	Dose (ng/kg) [a]	Cholangio-carcinoma	Hepatocellular adenoma	Cystic keratinizing epithelioma	Gingival squamous cell carcinoma
TCDD	0	0 [b]**	0**	0**	2.5**
	3	0	0	0	5.7
	10	0	0	0	2.6
	22	2.9	0	0	0
	46	10.3	2.6	0	10.2
	100	54.9**	29.9**	21.1**	22.0**
PCB 126	0	0	3.2**	0**	0**
	30	0	5.2	0	2.6
	100	2.5	2.5	0	2.5
	175	0	0	0	2.7
	300	13.6*	5.5	2.7	5.4
	550	14.0*	9.7	26.0**	4.7
	1000	60.3**	20.9*	83.5**	20.2**
PeCDF	0	0*	2.4**	0	2.4
	6	0	0	0	5.2
	20	0	2.7	0	2.7
	44	2.6	0	0	0
	92	2.8	5.5	0	2.8
	200	5.4	10.9	***2.7***	8.1
Mixture [c]	0	0**	0**	0**	2.7
	10	0	2.5	0	2.5
	22	4.8	2.4	0	0
	46	17.4	2.5	5.1	0
	100	26.0**	31.0**	54.7**	6.0

[a] Animals were treated with each compound/mixture with each respective dose, 5 days per week for, up to 104 weeks, n = 52-54/group

[b] Values represent the poly-3 adjusted neoplasm incidence (%) after adjustment for intercurrent mortality

[c] Mixture of TCDD, PCB126 and PeCDF. Doses represented as ng TEQ/kg.

* $P<0.05$. ** $P<0.01$ by the Poly 3 test[6]. Asterices for the control group indicate a significant trend in incidence.