MOLECULAR PHARMACOLOGY, 10, 349-359

Comparison of 2,3,7,8-Tetrachlorodibenzo-p-dioxin, a Potent Inducer of Aryl Hydrocarbon Hydroxylase, with 3-Methylcholanthrene

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(Received December 3, 1973)

SUMMARY

POLAND, ALAN, AND GLOVER, EDWARD: Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* **10**, 349-359 (1974).

The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the induction of microsomal oxygenase were studied in the rat. TCDD was 3×10^4 times more potent than 3-methylcholanthrene (MC) as an inducer of hepatic aryl hydrocarbon hydroxylase. Half-maximal induction occurred at a dose of 0.85 nmole/kg. TCDD and MC produced parallel log dose-response curves for the induction of hepatic aryl hydrocarbon hydroxylase, with the same maximal response. The simultaneous administration of maximally inducing doses of both drugs produced no greater response than either drug alone. TCDD, like MC, induced a spectrally distinct type of cytochrome P-450, with a shift in the absorption maximum of the carbon monoxide difference spectrum from 450 nm to 448 nm, and an increase in the ratio of the 455 nm:430 nm absorption maxima of the ethyl isocyanide difference spectra. Following a single dose of TCDD (31.1 nmoles/kg), hepatic aryl hydrocarbon hydroxylase activity and microsomal hemoprotein remained elevated for over 35 days. Both TCDD and MC had little effect on hepatic aminopyrine *N*-demethylase and NADPH-cytochrome *c* reductase. The dose of TCDD which produced half-maximal induction of hepatic aryl hydrocarbon hydroxylase in the rat, chick embryo, and several strains of mice was similar, varying from 0.4 to 1.2 nmoles/kg.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is an extraordinarily toxic contaminant produced during the commercial synthesis of the herbicide and defoliant 2,4,5-trichlorophenoxyacetic acid (1-4). The widespread environmental dispersion of TCDD,[1] its resistance to biodegradation, and its toxicity have generated considerable concern about the potential public health hazard.

Recent studies from this laboratory on the biochemical effects of TCDD have shown that in the chick embryo it is a potent in-

This study was supported in part by National Institutes of Health Special Postdoctoral Fellowship 5 FO3 ES46196, Ford Motor Company Grant for Toxicology and National Institutes of Health Center Grant for Toxicological Research and Training 2P11-GM-15190-06A1. A preliminary report of this work was given at the National Institute of Environmental Health Sciences Conference on Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans, Research Triangle Park, N. C., April 2-3, 1973.

[1] The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dixoin; MC, 3-methylcholanthrene.

ducer of hepatic δ-aminolevulinic acid synthetase (5, 6), the rate-limiting enzyme in heme synthesis, and aryl hydrocarbon hydroxylase (6), a microsomal mixed-function oxidase activity. Microsomal oxygenase is a membrane-bound, multicomponent enzyme complex which is responsible for the oxidative metabolism of many drugs and a number of endogenous compounds (7). The terminal component and active enzyme site of microsomal oxygenase is the hemoprotein(s) termed cytochrome P-450. A considerable body of evidence suggests that this cytochrome consists of a mixture of at least two hemoproteins and that the type of cytochrome present determines the substrate specificity (8, 9).

Drugs that induce microsomal oxygenase have been classified in two groups (7). One group of inducing drugs, typified by phenobarbital, enhances the metabolism of most substrates; the other group, of which 3-methylcholanthrene is the prototype, enhances the metabolism of only a few substrates and stimulates the formation of a spectrally distinct microsomal hemoprotein, cytochrome P_1-450.

In this report, we compare the effects of TCCD and MC as inducers of microsomal oxygenase and cytochrome P_1-450 in the rat.

METHODS

NADP, NADPH, NADH, the disodium salt of glucose 6-phosphate, isocyanide ethyl ester (ethyl isocyanide), bovine serum albumin, glucose 6-phosphate dehydrogenase (*Torula* yeast, type XI), cytochrome *c* (type III from horse heart), Tris, 3-methylcholanthrene, and benzo[*a*]pyrene were all purchased from Sigma Chemical Company. The benzo[*a*]pyrene was recrystallized from warm benzene by the addition of cold methanol. Sodium phenobarbital was purchased from Merck & Company.

Dr. Harry Gelboin, National Cancer Institute, generously provided a sample of 3-hydroxybenzo[*a*]pyrene.

Radioactive aminopyrine (4-[$^{14}CH_3$]$_2$-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), 11.6 mCi/mmole, was specially synthesized by Amersham/Searle Corporation (10). [^{14}C]Formaldehyde, 44.3 mCi/mmole, was purchased from International Chemical and Nuclear Corporation (10).

Dr. Albert Pohland, Food and Drug Administration, Washington, D. C., generously provided a sample of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (lot F-883) (11). Special precautions were used in the handling of TCDD, as previously outlined (6).

All other compounds used were of reagent grade purity or better.

Animals. Male Sprague-Dawley rats, 70–100 g, purchased from Blue Spruce Farms, Altamont, N.Y., were housed in hanging metal cages and exposed to a 12-hr light-dark cycle.

C3H/HeN female mice were generously provided by Dr. Daniel Nebert, National Institute of Child Health and Human Development. C57BL/6J and BALB/cJ female mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. The mice were housed in plastic cages with hardwood bedding. Both rats and mice were given a standard chow diet and water ad libitum and fasted for 24 hr before death.

The rats were given a single intraperitoneal injection of *p*-dioxane (0.3 ml/kg), TCDD in *p*-dioxane, corn oil (2 or 8 ml/kg), MC in corn oil, or sodium phenobarbital in 0.9% saline. The mice received an intraperitoneal injection of *p*-dioxane (0.4 ml/kg) or TCDD in the same volume of solvent. Both *p*-dioxane and TCDD were administered with a Hamilton microsyringe.

Tissue preparation. The animals were killed by a blow to the head and decapitated. The livers—and in some experiments other tissues—were dissected free, weighed, and homogenized in isotonic potassium chloride. The homogenates were centrifuged at 10,000 × *g* for 20 min, and the postmitochondrial supernatant fraction was used to assay aryl hydrocarbon hydroxylase and aminopyrine *N*-demethylase. The 10,000 × *g* supernatant fraction was centrifuged at 104,000 × *g* for 1 hr to prepare microsomes. For spectral studies of cytochrome P-450, the microsomes were resuspended in isotonic potassium chloride and resedimented to remove any hemoglobin present.

Enzyme assays. Aryl hydrocarbon hydroxylase was assayed as previously described in

chick embryo liver (6), with the following modifications. In assaying rat liver, the buffer was 50 mM Tris, pH 7.2 (at 37°); in other rat tissues and mouse liver the buffer was 50 mM potassium phosphate, pH 7.2. The assay was performed on the 10,000 × *g* supernatant fraction, equivalent to 2.5 mg (wet weight) of liver in the rat, 1 mg of liver in the mouse, and 5 mg (wet weight) of other tissues in the rat. All other conditions were as previously described. Each assay was performed in duplicate; duplicates usually varied less than 10%. Under the conditions described, the assay was linear for enzyme concentration and time and at the pH optimum.

One unit of aryl hydrocarbon hydroxylase was defined as that amount of enzyme catalyzing the formation of hydroxylated product per minute at 37° equivalent to 1 pmole of 3-hydroxybenzo[*a*]pyrene. Activity was expressed as units per milligram of tissue, wet weight.

Aminopyrine *N*-demethylase was assayed by a slight modification of the radiometric assay previously described (10), which measures the rate of [^{14}C]formaldehyde formation from radioactive aminopyrine. The reaction was performed in a total volume of 1 ml, containing 0.5 μmole of NADP, 15 μmoles of glucose 6-phosphate, 1 Kornberg unit of glucose 6-phosphate dehydrogenase, 5 μmoles of $MgCl_2$, 10 μmoles of semicarbazide, 55 μmoles of potassium phosphate buffer (pH 7.4), 2 μmoles of aminopyrine containing 4.96×10^5 cpm of [^{14}C]aminopyrine, and the 10,000 × *g* supernatant fraction equivalent to 10 mg of rat liver, wet weight. The reaction was incubated for 10 min at 37° in a shaker water bath and then terminated by the addition of 8 ml of cold chloroform and 0.5 ml of 0.2 N NaOH. The mixture was briefly shaken to extract the substrate into the chloroform, leaving the formaldehyde in the aqueous phase. Following centrifugation at 1000 × *g* for 5 min, 1 ml of the aqueous phase was again extracted with 8 ml of chloroform. A 0.5-ml sample of the aqueous phase from this second extraction was added to 10 ml of Aquasol (New England Nuclear Corporation) and counted on a Packard Tri-Carb scintillation counter, model 3375.

Each sample was assayed in duplicate with an appropriate blank, which consisted of the entire reaction mixture inactivated by the addition of chloroform before the addition of the radioactive substrate. To correct for formaldehyde lost during the extraction procedure, we added [^{14}C]formaldehyde (7.5×10^4 cpm) to samples lacking only the radioactive substrate. The recovery of added formaldehyde was 91%. The counting efficiency, as determined by automatic external standardization, was virtually identical in all samples (85%).

The quantity of formaldehyde formed was calculated from the net counts per minute observed (sample minus blank counts per minute) corrected for the total volume of the aqueous phase and corrected for formaldehyde recovery. The total corrected net counts per minute were converted to nanomoles of product from the specific activity of the substrate.

Under the conditions employed, the assay was linear with time and the amount of enzyme activity.

NADPH–cytochrome *c* reductase was assayed in microsomes by the method of Masters *et al.* (12).

Spectral studies on cytochrome P-450. Cytochrome P-450 was measured by the method of Omura and Sato (13) on a Cary model 118C recording split-beam spectrophotometer in cuvettes of 1-cm optical path. The microsomal preparation, 1–2 mg of protein per milliliter in 0.1 M potassium phosphate buffer (pH 7.4), was placed in both sample and reference compartments and reduced with a small amount of solid sodium dithionite, and the baseline spectrum was recorded. Carbon monoxide (Matheson Gas Products, East Rutherford, N. J.) was bubbled into the sample cuvette for at least 10 sec, and the CO difference spectrum was recorded. The concentration of cytochrome P-450 was calculated from the difference in absorption between the Soret maximum and 490 nm, using an extinction coefficient of 91 mM^{-1} cm^{-1} (13).

The ethyl isocyanide difference spectrum was obtained on dithionite-reduced microsomes by the addition of 10 μl of a 0.36 M solution of the ligand to 3 ml of the micro-

somal suspension in the same cuvette. The final ligand concentration, 1.2 mM, was saturating because the addition of more ethyl isocyanide produced no greater absorbance. The difference spectrum was recorded, and the results were expressed as the ratio of the two peaks, $\Delta A_{455-490}:\Delta A_{430-490}$. Because the absolute absorbance and the ratio of the peak heights are influenced by pH and ionic strength (14, 15), these studies were always performed in 0.1 M potassium phosphate buffer, pH 7.4.

Protein determination. The microsomal protein concentrations were determined by the method of Lowry *et al.* (16) with bovine serum albumin as a standard.

RESULTS

Dose-response relationship. The administration of TCDD produced a dose-related increase in aryl hydrocarbon hydroxylase in rat liver (Fig. 1). At the lowest dose tested, 0.31 nmole/kg, there was nearly a 5-fold induction of hydroxylase activity, and maximal induction was produced by a dose of 31 nmoles/kg. The dose that evoked half-maximal induction (ED_{50}) was estimated to be 0.85 nmole/kg (0.27 μg/kg). This is roughly one-hundredth the reported oral LD_{50} in the male rat (22 μg/kg) (4).

The potency of TCDD as an inducer of hepatic aryl hydrocarbon hydroxylase is compared with that of MC, the most commonly employed inducing agent, in Fig. 1. It can be seen that TCDD is considerably more potent. The slopes of the midportion of the two log dose-response curves are parallel, and the maximal response elicited by both drugs is the same (see Table 1). Analysis of the data by a computer program for bioassay (17) showed that TCDD was 28,640 times as potent as MC on a molar basis.[2] The index of precision, λ, was 0.18.

TCDD and MC produced the same maximal induction of aryl hydrocarbon hydroxylase, and the simultaneous administration of both drugs evoked no greater response than that produced by either drug alone (Table 1). The change in the total microsomal CO-binding cytochrome, and the ratio of the maxima of the ethyl isocyanide difference spectra of the microsomal hemoprotein, were no greater with combined TCDD and MC administration than produced by either drug alone.

Time course of response. Previous studies in chick embryos suggested that TCDD

[2] The 95% confidence interval of the potency ratio is 2.07–3.95 × 10^4.

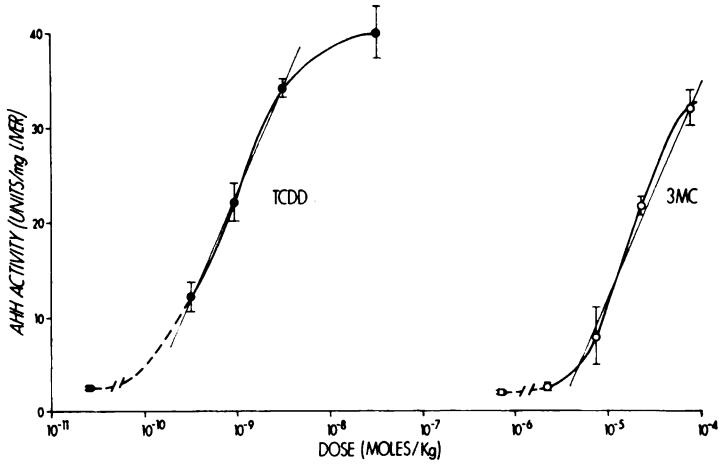

FIG. 1. *Log dose-response curve for induction of hepatic aryl hydrocarbon hydroxylase in rats*

Animals were dosed with TCDD in *p*-dioxane, 3-methylcholanthrene (3MC) in corn oil, or the solvents alone, and killed 24 hr later. Their livers were assayed for aryl hydrocarbon hydroxylase (AAH). The initial point on each curve is the value for control animals treated only with the solvent (■, *p*-dioxane; □, corn oil), and this point is connected to the log dose-response curvd by a dashed line. Each value represents the mean ± standard error of four or five rats.

TABLE 1

Effects of TCDD, MC, and combined treatment on hepatic aryl hydrocarbon hydroxylase, microsomal CO-binding cytochrome, and ethyl isocyanide difference spectra

Rats received an injection of the drug and were killed 24 hr later. Aryl hydrocarbon hydroxylase, microsomal CO-binding cytochrome, and ethyl isocyanide difference spectra were analyzed as described in METHODS. Values are mean ± standard errors.

Treatment	Dose	*n*	Aryl hydrocarbon hydroxylase activity	Microsomal CO-binding cytochrome	Ratio of absorption maxima of ethyl isocyanide difference spectra[a]
	μmoles/kg		*units/mg liver*	*nmoles/mg protein*	
Control (*p*-dioxane)		4	2.0 ± 0.4	0.82 ± 0.13	0.75 ± 0.03
TCDD	0.031	5	25.6 ± 2.6	1.24 ± 0.14	1.44 ± 0.03
MC	298.0	4	26.5 ± 1.9	1.04 ± 0.06	1.38 ± 0.05
TCDD + MC	0.031 + 298.0	5	30.0 ± 2.2	1.03 ± 0.13	1.37 ± 0.02

[a] $\Delta A_{455\text{-}490} : \Delta A_{430\text{-}490}$.

differed from MC not only in potency but also in duration of action (6). To examine this question further, rats were divided into four treatment groups: *p*-dioxane (0.3 ml/kg); corn oil (2.0 ml/kg); TCDD (0.31 nmole/kg in *p*-dioxane); and 3-methylcholanthrene (0.75 μmole/kg in corn oil). The rats were given a single intraperitoneal dose and killed 1–35 days later (Figs. 2–4). Since there was no significance difference between the corn oil- and *p*-dioxane-treated groups, the data from these control groups were used interchangeably.

Following a single dose of TCDD or MC, hepatic aryl hydrocarbon hydroxylase activity rose equally at days 1 and 4 (Fig. 2). Thereafter, hydroxylase activity in the MC-treated rats fell rapidly and returned to the enzyme activity in control rats by 8 days. However, the animals treated with TCDD continued to have elevated enzyme activity at 21 days, with a slight decrease at 35 days.

The time course of the induction of the total CO-binding microsomal cytochrome by TCDD and MC is shown in Fig. 3. TCDD produced a doubling of the total CO-binding cytochrome by 4 days, and the elevation persisted for over 35 days. A more transient response was produced by the single dose of MC, and the total CO-binding cytochrome returned to control levels by 8 days. In the control rat there was an increase in the cytochrome P-450 concentration between 14 and

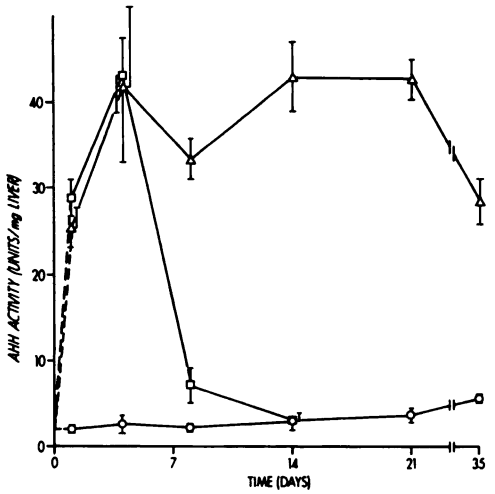

FIG. 2. *Time course of induction of hepatic aryl hydrocarbon hydroxylase in rats*

Following a single administration of TCDD (31 nmoles/kg in *p*-dioxane), MC (75 μmoles/kg in corn oil), or the solvents alone, the animals were killed at, 1, 4, 8, 14, 21, and 35 days, and their livers were assayed for aryl hydrocarbon hydroxylase (AHH). Each point represents the mean ± standard error of four rats. ○, controls (*p*-dioxane or corn oil); □, MC; △, TCDD.

35 days, which probably was due to the physiological increase in microsomal oxygenase activity reported to accompany puberty in male rats (7).

The induction of microsomal oxygenase by

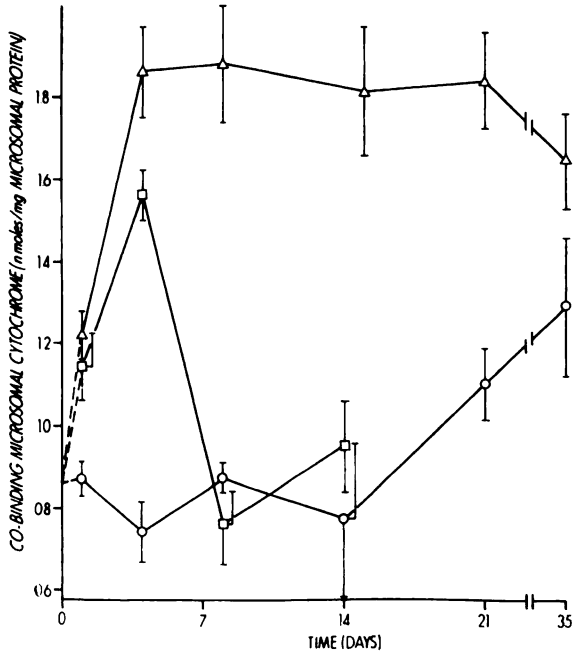

FIG. 3. *Time course of induction of hepatic CO-binding microsomal cytochrome*

Hepatic microsomes were prepared from rats treated as described in the legend for Fig. 2, and the reduced CO difference spectra were recorded. The control animals (○) had an absorption maximum at 450 nm, and the MC-treated (□) and TCDD-treated (△) rats had maxima at 448 nm, which reverted to 450 nm when the amount of cytochrome returned to control levels. Each point is the mean ± standard error of four rats.

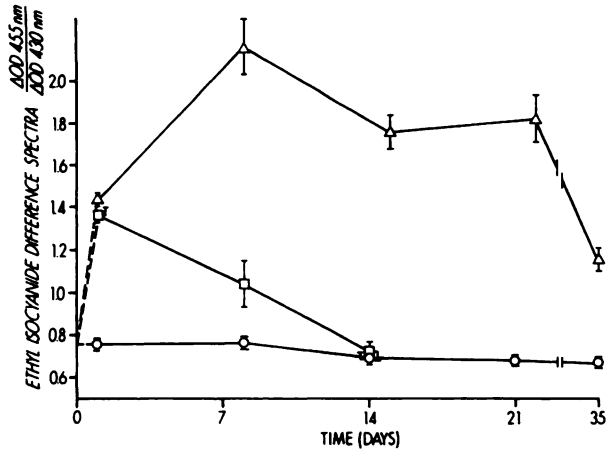

FIG. 4. *Time course of changes in hepatic microsomal ethyl isocyanide difference spectra following administration of TCDD and MC*

Hepatic microsomes were prepared from rats treated as described in the legend for Fig. 2, and the ethyl isocyanide difference spectra were measured. The results are expressed as ratios of the 455 nm and 430 nm peak heights (see METHODS). Each point is the mean ± standard error of four rats. ○, controls; □, MC; △, TCDD.

polycyclic hydrocarbons is accompanied not only by an increase in the total CO-binding cytochrome, but by a qualitative change in the type of cytochrome present, as reflected by a shift in the absorption maximum in the carbon monoxide difference spectrum (18), differences in the electron spin resonance signal (19), and differences in ligand binding (20–22). Both TCDD and MC produced a shift in the absorption maximum of the carbon monoxide difference spectrum of the reduced cytochrome from 450 nm to 448 nm. As the total amount of cytochrome and the aryl hydrocarbon hydroxylase activity returned to control levels, the spectral maximum shifted back to 450 nm. In TCDD-treated animals, the absorption maximum was 448 nm from days 1 to 21 and 449 nm at day 35.

Ethyl isocyanide binds to reduced cytochrome P-450 to give a difference spectrum with two maxima, one at 430 nm and the other peak around 455 nm (13). The distinct type of microsomal CO-binding cytochrome (so-called P-448 or P_1-450) that is produced after administration of polycyclic hydrocarbons (18) is reflected by an altered ethyl isocyanide difference spectrum with an increase in the maximum at 455 nm, and thus an altered ratio of the 455 nm and 430 nm peaks (21). The time course of the effect of TCDD and MC administration on the ethyl isocyanide difference spectra of the microsomal cytochrome is shown in Fig. 4. In control rats the ratio of the 455 nm and 430 nm peaks was about 0.7 (at pH 7.4), in reasonable agreement with the findings of others (20, 23). The administration of TCDD and MC increased this ratio, primarily by increasing the maximum at 455 nm, with little change in the 430 nm peak. A single dose of TCDD produced an altered peak ratio of approximately 1.8, which persisted for 3 weeks before gradually returning toward the spectral peak ratio found in the control animals. Again, the response produced by MC is more transient.

Extrahepatic induction. Aryl hydrocarbon hydroxylase differs from most other microsomal oxygenase activities in that it is both detectable and inducible in a number of extrahepatic tissues (24). Both TCDD and MC induce hydroxylase activity in the kidney, lung, and intestines, but fail to induce activity in the testicle (Table 2) and spleen, thymus, and adrenal.[3] It is noteworthy that the doses of TCDD and MC that produce the same maximal hydroxylase induction in the liver differ in extrahepatic tissue. TCDD consistently produced a greater response in extrahepatic tissue, which may reflect differences between the two drugs in distribution, metabolism, or binding affinity.

Effects of TCDD and 3-methylcholanthrene on aminopyrine N-demethylase and NADPH–cytochrome c reductase. The polycyclic hydrocarbons produce a more selective response than phenobarbital on microsomal oxygenase activity and reportedly do not significantly increase aminopyrine *N*-demethylase or NADPH–cytochrome *c* reductase activity (7). As shown in Table 3, phenobarbital treatment produced a 5-fold increase in the rate of *N*-demethylation of aminopyrine and a 4-fold increase in NADPH–cytochrome *c* reductase activity. TCDD and MC each produced about a 50% stimulation of aminopyrine *N*-demethylase activity and about a 30% increase in the NADPH–cytochrome *c* reductase activity.

Comparison of induction of hepatic aryl hydrocarbon hydroxylase by TCDD in various species. It is useful to compare the log dose-response curves for induction of hepatic aryl hydrocarbon hydroxylase by TCDD in various species. The results obtained in chick embryos, C3H/HeN female mice, and Sprague-Dawley male rats are shown in Fig. 5*A–C*, respectively. In the chick embryo, as previously reported (6), the dose of TCDD that produced half-maximal induction (ED_{50}) was 10 pmoles/egg. Conversion to a weight basis[4] gives an ED_{50} of 0.4 nmole/kg. The dose of TCDD which produced half-maximal induction in the C3H/HeN female

[3] A. Poland and E. Glover, unpublished observations.

[4] A Leghorn chicken egg weighs approximately 50 g, and an embryo of 18 days' gestation weighs approximately 25 g (25). The ED_{50} was calculated by assuming that the drug is distributed only in the embryo; if one assumes distribution throughout the entire egg, the ED_{50} is 0.2 nmole/kg.

TABLE 2

Induction of aryl hydrocarbon hydroxylase activity in extrahepatic tissues of rats

Male Sprague-Dawley rats (65–75 g) received a single intraperitoneal dose of *p*-dioxane, 0.3 ml/kg (control rats); TCDD, 0.031 μmole/kg in *p*-dioxane; or MC, 74.5 μmoles/kg in corn oil, and were killed 24 hr later. Enzyme activity was measured in the 10,000 × *g* supernatant fraction; the results are expressed as means ± standard errors of four determinations.

Treatment	Activity			
	Kidney	Intestine	Lung	Testicle
	units/mg tissue, wet wt			
Control	0.018 ± 0.005	0.006 ± 0.002	0.022 ± 0.005	<0.01
MC	2.00 ± 0.260	0.203 ± 0.077	0.165 ± 0.014	<0.01
TCDD	3.25 ± 0.300	0.773 ± 0.132	0.243 ± 0.047	<0.01

TABLE 3

Comparison of effects of TCDD, MC, and phenobarbital on induction of aminopyrine N-demethylase and NADPH-cytochrome c reductase

Rats were treated with *p*-dioxane, 0.3 ml/kg (control); TCDD, 0.031 μmole/kg in *p*-dioxane; or MC, 298 μmoles/kg in corn oil, 48 hr before death. Phenobarbital sodium, 148 μmoles/kg in NaCl, was given twice a day for 5 days. Data are means ± standard errors.

Treatment	*n*	Aminopyrine *N*-demethylase	NADPH–cytochrome *c* reductase
		pmoles HCHO/mg wet wt liver/min	*nmoles/mg microsomal protein/min*
Control	5	66.8 ± 14.7	35.5 ± 4.0
TCDD	5	110.3 ± 13.5	49.6 ± 1.9
MC	5	105.7 ± 3.0	47.6 ± 4.6
Phenobarbital	4	381.8 ± 23.5	138.0 ± 10.3

mouse was 0.42 nmole/kg (Fig. 5*B*). The ED_{50} in female mice of other inbred strains was found to be slightly higher: C57BL/6J, ED_{50} = 1.2 nmoles/kg; and BALB/cJ, ED_{50} = 1.0 nmoles/kg.[3] The dose of TCDD which produced half-maximal induction in the Sprague-Dawley male rat was 0.85 nmole/kg (Fig. 5). It is impossible to compare directly the log dose-response curves obtained on different species, because the basal enzyme activity and the responsiveness (degree of induction) vary. However, this comparison can easily be achieved by transforming the absolute enzyme activities to fractional responses (control, 0%; maximal response, 100%) (not shown). Statistical analysis of the transformed data showed that the slopes of all the curves were not significantly different. Thus we are dealing with a series of parallel log dose-response curves whose midpoints (ED_{50} values) all fall in a 3-fold dose range.

As shown below, while the dose of TCDD that produced half-maximal induction is nearly the same in the species examined, the mean lethal dose (LD_{50}) varies more than 100-fold.

	ED_{50}[5]	LD_{50}[6] (ref.)
	nmoles/kg	
Chick embryo	0.4	<3 (26)
Rat, male	0.85	68 (4)
Mouse, female	0.4–1.2	>400 (4)

It is apparent that in these species there is no relationship between their sensitivity to induction of aryl hydrocarbon hydroxylase by TCDD and their susceptibility to the toxic effects of this compound.

DISCUSSION

TCDD was found to be a potent inducer of microsomal oxygenase activity. TCDD

[5] ED_{50} refers to the dose of TCDD that produced half-maximal induction of hepatic aryl hydrocarbon hydroxylase. In all cases the drug was administered parenternally.

[6] LD_{50} denotes the mean lethal dose of TCDD after a single administration. These acute lethality studies were terminated when it was evident that the survivors were not showing signs of toxicity (2–8 weeks) (4). In the rat and mouse, the drug was given orally; the chick embryo was dosed through a small hole punched into the eggshell.

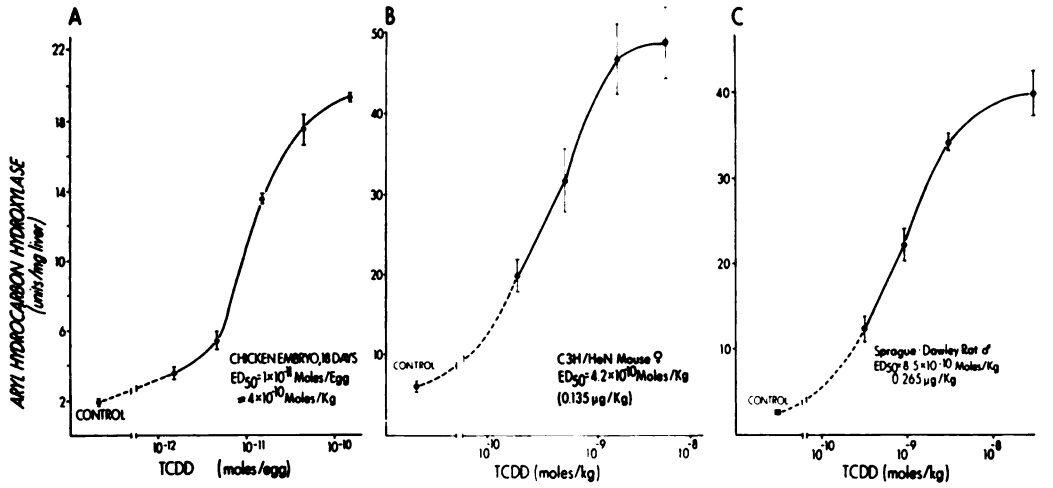

FIG. 5. *Comparison of log dose-response curves for induction of hepatic aryl hydrocarbon hydroxylase by TCDD in various species*

All animals received TCDD dissolved in *p*-dioxane, and 24 hr later their livers were assayed for aryl hydrocarbon hydroxylase. The optimal conditions for the enzyme assay in each species have been reported previously (chick embryo) or can be found under METHODS (rats and mice). The value for the control animals (solvent-treated) is connected to the dose-response curve by a dashed line. Each point is the mean ± standard error of four or five animals (B and C), and of four groups of three pooled livers each in the chick (A).

and the polycyclic hydrocarbon MC produced very similar effects on microsomal oxygenase in the rat. Both drugs induced hepatic aryl hydrocarbon hydroxylase to the same maximal response, simultaneous administration of both drugs at maximally inducing doses produced no greater effect, and the log dose-response curves of hydroxylase induction produced by each drug were parallel. TCDD and MC produced little change in aminopyrine *N*-demethylase activity or NADPH–cytochrome *c*-reductase activity. Both drugs induced the formation of a distinct microsomal hemoprotein, cytochrome P_1-450, as measured by the shift in the absorption maximum of the CO difference spectrum of the reduced cytochrome from 450 nm to 448 nm, and the altered ratio of the absorption maxima of the ethyl isocyanide difference spectrum. TCDD and MC induced aryl hydrocarbon hydroxylase activity in extrahepatic tissues. Finally, the initial time courses of induction of hydroxylase activity and microsomal CO-binding cytochrome and the change in the ethyl isocyanide difference spectra of this hemoprotein were similar after administration of TCDD and MC.

TCDD appears to differ from MC primarily in its potency and duration of action. The sustained induction observed in rat liver following TCDD administration probably resulted from the long biological half-life of the drug, reported to be 17 days in the rat (27).

Potency of TCDD. The unique property of TCDD is its potency as a toxin (4) and a teratogen (28, 29), in producing acne (30), and as an inducer of hepatic δ-aminolevulinic acid synthetase (5) and microsomal oxygenase.

A dose of 0.85 nmole/kg of TCDD produced half-maximal induction of hepatic aryl hydrocarbon hydroxylase in the rat liver. From the number of cells per gram of liver (31) and experiments on the rate of hepatic accumulation of [^{14}C]TCDD,[3] we estimate that at this dose (ED_{50}) there were about 2.5×10^4 molecules of TCDD per cell.

Several chracteristics of this drug effect suggest the existence of a primary binding site, an "induction receptor" to which TCDD binds. This drug-receptor interaction initiates a series of events that ultimately result in the induction of hydroxylase activity. The potency of TCDD, its ability to produce a graded biological response which is

dose-related, its chemical specificity [other halogenated dibenzo-*p*-dioxins vary greatly in their ability to induce hydroxylase activity (6)], and the biological specificity of the response [a specific type of microsomal oxygenase activity is induced in some, but not all, tissues (Table 2)] all suggest the existence of a specific receptor for hydroxylase induction.

On a molar basis, TCDD is 30,000 times as potent as MC as an inducer of hepatic aryl hydrocarbon hydroxylase. The observations that both drugs induce the enzyme activity to the same maximum level, that administration of both drugs at maximally effective doses evokes no further increase in enzyme activity, and that the drugs produce parallel dose-response curves suggest that TCDD and MC either share the same receptor or compete at some later step(s) in the series of events leading to the induction of hydroxylase activity (32).

It is not readily apparent why TCDD is so much more potent than MC. The potency of TCDD suggests a high apparent affinity for the "induction receptor." The lower potency of MC might be attributable to a lower affinity for the receptor, diminished hepatic uptake, or faster metabolic inactivation. Both drugs are rapidly concentrated by the liver (27, 33, 34), and differences in hepatic localization should not contribute significantly to the greater potency of TCDD. MC is much more rapidly metabolized in the liver than TCDD, and 30% of MC is excreted in the bile in 1 hr (34, 35). TCDD has a half-life of 17 days in the rat (27). No metabolites were detected in the tissues and excreta of mice given tritiated TCDD, nor were metabolites found after incubation *in vitro* with the microsomal fraction of mouse liver (36).

Similarity of dose-response curves in different species. The log dose-response curves for the induction of hepatic aryl hydrocarbon hydroxylase by TCDD are remarkably similar in the rat, chick embryo, and several inbred strains of mice: their slopes do not differ significantly, and their midpoints, i.e., the doses of TCDD producing half-maximal induction, vary over a relatively narrow range (0.4–1.2 nmoles/kg). These observations suggest that the apparent binding affinities of the "induction receptor" for TCDD are similar among these species. The resistance of TCDD to metabolism, and its concentration in the liver tend to minimize the effect of species differences in drug metabolism and tissue distribution.

In summary, the effects of TCDD on microsomal oxygenase are similar to those produced by MC in all respects, except for the greater potency and duration of action of TCDD. The resistance of TCDD to metabolic conversion and its high apparent affinity for the "induction receptor" suggest that one might use radioactively labeled TCDD to investigate the nature and localization of such a receptor.

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