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METABOLIC ALTERATIONS FOLLOWING ADMINISTRATION OF 2,3,7,8 - TETRACHLORODIBENZO - PARA - DIOXIN TO RATS

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METABOLIC ALTERATIONS FOLLOWING ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TO RATS

by

Carl Lynn Potter

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

In Partial Fulfillment of the Requirements
for the Degreee of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Carl Lynn Potter

entitled METABOLIC ALTERATIONS FOLLOWING ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TO RATS

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

J Glenn Lyons	December 6, 1982
	Date
Klaus Bracher	December 6, 1982
	Date
A Jay Knoblich Jr	December 6, 1982
	Date
Richard L. Stouffer	December 6, 1982
	Date
Diane H. Russell	December 6, 1982
	Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Diane H. Russell	December 6, 1982
Dissertation Director	Date

STATEMENT BY AUTHOR

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SIGNED: Carl Sattler

To my parents,

for their unfailing encouragement and assistance

through the years leading to the realization

of this dissertation

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ABSTRACT

The effects of TCDD on hepatic ornithine decarboxylase (ODC) acitivity and endocrine function in rats were investigated. Sixteen hours after partial hepatecomy, rats which had been pretreated with TCDD for one week exhibited a 3- to 4-fold increase in ODC activity, while vehicle controls exhibited an 8- to 10-fold increase. ODC induction after either aminophylline or dexamethasone administration, agents which act via cAMP-mediated and direct nuclear events, respectively, also was inhibited by pretreatment with TCDD. RNA polymerase I activity, which positively correlates to ODC activity in growth and development, decreased concomitant with decreased induction of ODC. In unstimulated liver, RNA polymerase I activity, as well as protein, DNA and RNA levels, remained unchanged one week after TCDD. However, TCDD administration resulted in decreased liver concentrations of putrescine and spermidine, but not spermine. Within 2 days following administration of TCDD (45 or 90 μg/kg), rats exhibited hypothermia, hypothyroidism and decreased growth rate compared to pair-fed controls and rats fed ad libitum. Within 2 weeks of the administration of 90 ug TCDD/kg, body temperature had fallen to below 35°C with a low mean value 34.5°C recorded on day 16. Mean body temperatures for control rats ranged from 36.8°C to 37.5°C. One week after the adminstration of TCDD (45 μg/kg) to rats, serum thyroxine (T_4) and triiodothyronine (T_3) levels declined to 42% and 82% of control, respectively. Mild hypoglycemia occurred subsequent to hypothyroidism and hypothermia. At 1 week after

administration of 45 μg TCDD/kg to rats, serum and pancreatic insulin levels were reduced to 25% and 76% of control, respectively. Hypophagia was determined to be responsible for decreased growth rate and hypoinsulinemia, but it could not account for hypothyroidism, hypothermia or hypoglycemia following administration of TCDD. No changes in glucagon or pancreatic, hepatic or serum somatostatin levels were found. Decreased somatostatin in the gastric antrum coincided with a 29% increase in stomach weight. The delayed toxicity of TCDD may be related to these striking hormonal alterations.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) represent an environmental health problem in the industrialized world (Kimbrough, 1980). Of particular concern are the halogenated PAHs since many of them exhibit extraordinary stability in the biosphere.

The polychlorinated biphenyls (PCBs) represent a class of halogenated PAHs which have received much attention as environmental contaminants over the last decade (Kimbrough, 1974, 1980). Due to their great stability and dielectric properties, PCBs were recognized for their commercial value as early as 1930 (Broadhurst, 1972; Kimbrough, 1974). When the biphenyl molecule contains 4 or more chlorine atoms, the parent and its decomposition products remain flame resistant at temperatures as high as 700^{o} F (Broadhurst, 1972). Therefore, PCBs have acquired widespread industrial use as filling agents in electrical transformers and capacitors, and as cooling and arc quenching agents. This in turn has lead to widespread environmental contamination (Risebrough et al., 1968; Dustman et al., 1971; Kimbrough, 1974). Furthermore, the remarkable stability of these compounds generates concern about the duration of their environmental sojourn.

The chlorinated dibenzofurans and dibenzo-p-dioxins, although of no commercial import, have drawn attention as contaminants in PCB mixtures and in 2,4,5-trichlorophenol, respectively (IARC, 1977, 1978). Although the spectra of toxic effects are similar among many of the

chlorinated PAHs, the 2,3,7,8-tetrachlorodibenzo-p-dioxin congener possesses inordinate toxicological potency (Poland and Glover, 1973a, 1973b; IARC, 1977). Therefore, in addition to environmental health concerns, this congener has attracted much academic interest and was the compound investigated in this study.

Chemistry of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

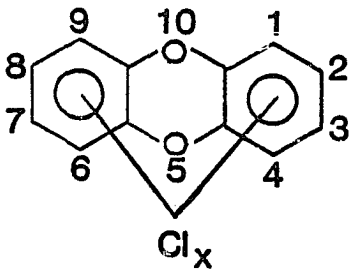
Since the basic dioxin structure has 8 potential points of chemical addition (Fig. 1a), 76 possible congeners of chlorinated-p-dioxin exist. Although there are 22 possible isomers of tetrachlorodibenzop-dioxin, the abbreviation TCDD will refer to the 2,3,7,8-tetrachlorodibenzo-p-dioxin isomer (Fig. 1b).

Physical Properties

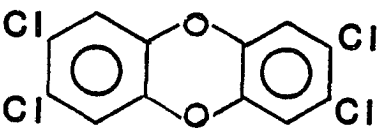
Due to its extreme toxicity, extensive chemical analysis of TCDD is incomplete. However, some important chemical concepts pertaining to formation and environmental stability have been elicidated and are briefly reviewed here.

TCDD is a solid substance with a molecular weight of 322 and a melting point of 305-306^{o}C. It is insoluble in water and slightly soluble in fats, dioxane, acetone and chlorinated solvents (Crummett and Stehl, 1973; Rappe and Buser, 1980). TCDD undergoes rapid photochemical dechlorination (Crosby and Wong, 1977), but is quite heat stable, requiring temperatures greater than 750^{o}C for thermal decomposition to occur (Stehl et al., 1973).

a)



b)



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

Empirical Formula: $C_{12}H_4Cl_4O_2$

Molecular Weight: 322

Figure 1: Structure of dibenzo-p-dioxin: a) Basic dioxin structure showing 8 possible points available for chlorine substitution. b) Structure of the 2,3,7,8-tetrachloro-dibenzo-p-dioxin isomer (TCDD).

Formation and Distribution

Although laboratory synthesis of TCDD was reported by Sanderman and coworkers (1957) as the chlorination of unsubstituted dioxin, TCDD is most commonly produced as an unwanted contaminant during the synthesis of trichlorophenol, a precursor of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and related herbicides (IARC, 1977). 2,4,5-Trichlorophenol is synthesized from 1,2,4,5-tetrachlorobenzene at a temperature near 180°C. When temperature and pressure are carefully controlled, the trichlorophenol product will contain 1-5 ppm TCDD. However, as temperature increases, the amount of TCDD formed increases significantly (Reggiani, 1981); the principle of this reaction is shown in Fig. 2.

The herbicide Agent Orange is formulated as a 1:1 misture of 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D). Prior to 1970, Agent Orange contained between 0.1 and 47 μg TCDD/g, and the levels of TCDD in Agent Orange used during the 1960s is estimated to have been as high as 100 μg/g (Rappe and Buser, 1980). Current procedures for purification of 2,4,5-trichlorophenol can reduce the TCDD content to less than 0.01 ppm in 2,4,5-T (Reggiani, 1981).

Recently, TCDD has been reported to exist in fly ash and flue gases from municipal incinerators (Olie et al., 1977; Buser and Boshardt, 1978; Buser et al., 1978a, 1978b; Dow Chemicals, 1978; Eiceman et al., 1979; Rawls, 1979; Bumb et al., 1980). Up to 30 polychlorinated dibenzo-p-dioxin and over 60 polychlorinated dibenzofuran isomers have been detected in municipal fly ash (Buser et al., 1978a, 1978b).

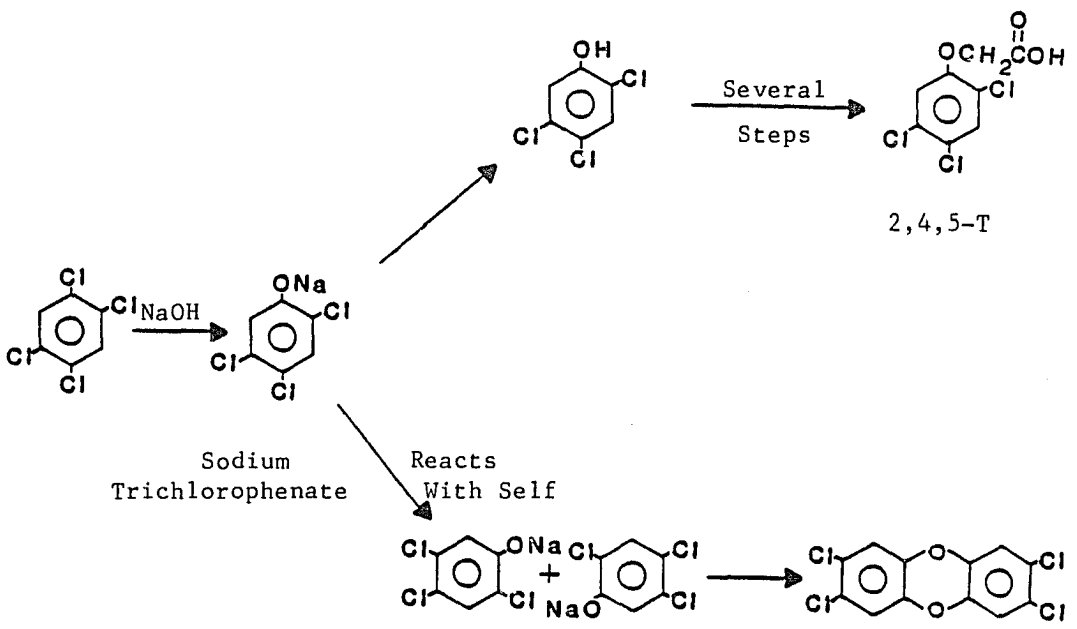


Figure 2: Possible route of formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Under conditions of high temperature and pressure, sodium trichlorophenate reacts with itself to form TCDD (Kriebel, 1981).

The highly toxic polychlorinated dibenzo-p-dioxins (2,3,7,8-tetra-, 1,2,3,7,8-penta- and 1,2,3,7,8-hexachloro isomers) were only minor constituents (Buser et al., 1978a) whereas the toxic dibenzofuran isomers (2,3,7,8-tetra- and 2,3,4,7,8-pentachloro) were major constituents in the fly ash (Buser et al., 1978b). In addition, TCDD, hexachlorodibenzo-p-dioxin and octachlorodibenzo-p-dioxin have also been identified in several commercial fatty acids which had been prepared by extraction with trichlorophenol and pentachlorophenol (Firestone, 1973).

Toxicology of TCDD

TCDD toxicity was first reported by Kimmig and Schulz (1957) when they linked TCDD exposure to the development of chloracne in laboratory workers. They were also the first to associate chloracne with exposure to TCDD in industrial accidents. Since then, numerous reports of TCDD toxicity in humans and animals have appeared (IARC, 1977).

In vitro studies, especially in cell culture, have been of limited value thus far in elucidating the mechanism of toxicity of TCDD (Vos and Moore, 1974; Knutson and Poland, 1980; Beatty et al., 1975). This may be partly due to poor solubility of TCDD in physiological buffers (Matsumura and Benezet, 1973), or it may reflect a TCDD effect on some regulatory process(es), which would require exposure of the whole animal to TCDD in order to manifest toxicity. Thus, the most useful TCDD-toxicology studies have been in vivo.

Animal Toxicity

A spectrum of effects can be produced by exposing animals to TCDD and other halogenated aromatic hydrocarbons such as dibenzofurans, certain polychlorinated biphenyl isomers and certain azo- and azoxybenzenes (Kimbrough, 1974; McConnell and Moore, 1979; McConnell, 1980). Also, large species differences exist with respect to sensitivity to the lethal effects of TCDD (Table 1). The guinea pig is the most sensitive species (LD_{50} = 0.6 μg/kg), whereas the hamster is the least sensitive species in which the LD_{50} values have been determined [LD_{50} = 3000-5000 μg/kg] (Henck et al., 1981; Olson et al, 1980a). Despite its extraordinary potency, TCDD exerts its toxicity slowly, requiring 2-8 weeks to produce death in experimental animals (Allen et al., 1977; McConnell et al., 1978a 1978b).

The mechanism(s) of TCDD action remains perplexing. Also, the long-term effects due to chronic low level exposure to the chlorinated aromatic hydrocarbons in general have been only partially characterized. Some of these compounds, particularly TCDD, have been reported to be teratogens (Courtney and Moore, 1971; Neubert et al., 1973; Smith et al., 1976), reproductive toxins (Murry et al., 1979) and suspected carcinogens (Kociba et al., 1978) in various animal species.

Gross and histopathologic observations have been largely ineffectual in discerning the mechanism of TCDD toxicity. Gross pathology has revealed chronic weight loss as great as 50% with mobilization of adipose tissue (McConnell, 1980), development of a fatty liver (McConnell, 1980) and severe thymic atrophy and immune suppression (Vos et

Table 1: LD_{50} values reported for animal exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Species	Strain	Sex	Route of Administration	Time of Last Death	LD_{50} ug/kg	Reference
Guinea Pig	Hartley	Male	P.O.	34 d	0.6	Schwetz et al., 1973
Rat	Sherman (Spartan)	Male	P.O.	27 d	22	" "
"	" "	Female	P.O.	43 d	45	" "
Monkey	Rhesus	Female	P.O.	34 d	70	McConnell et al., 1978
Rabbit	New Zealand Albino	Mixed	P.O.	39 d	115	Schwetz et al., 1973
" "	" "	"	Dermal	22 d	275	" "
Mouse	C57BL/10	Male	P.O.	38 d	146	Smith et al., 1981
" "	" "	Female	P.O.	--	450	" "
Hamster	Golden Syrian	Male	P.O.	55 d	5051	Henck et al., 1981
" "	" "	"	P.O.	50 d	1157	Olson et al., 1980a
" "	" "	"	I.P.	50 d	3000	"

al., 1973; Vos and Moore, 1974; Vos, 1977), but has failed to evince the lesion(s) responsible for death.

Hepatotoxicity

Sublethal doses of TCDD have been reported to produce hepatomegaly in all species studied (Kociba, 1976; McConnell 1980). Hepatomegaly results in part from proliferation of the smooth endoplasmic reticulum (Fowler, 1973; Kociba, et al., 1979) and lipid accumulation (Vos et al., 1974). Hepatic porphyrin accumulation has also been observed in mice, rats and chickens (IARC, 1978). In addition, the rate of synthesis of DNA and total hepatic DNA content increase following TCDD administration (Gasiewics et al., 1980; Dickens et al., 1981).

TCDD administration produces hepatic damage in a dose dependent manner, but the severity and distribution of hepatic changes varies among species (Schwetz et al.,1973; Gupta et al., 1973; Kociba et al., 1976; Henck et al., 1981). Although hepatic necrosis produced by TCDD may contribute to the death of rats and rabbits, hepatic damage is less extensive in mice and is minimal in guinea pigs and monkeys. Therefore it appears that the lethal effects of TCDD involve a factor(s) besides hepatic damage (Buu-Hoi et al., 1972; McConnell et al., 1978; Schulz, 1968; Schwetz et al., 1973; Vos et al., 1973, 1974; Gupta et al., 1973).

Lymphoid Toxicity

Thymic atrophy is a commonly reported effect of TCDD administration, and has been the most sensitive index of TCDD toxicity (Gupta et

al., 1973; Harris et al., 1973). In rats, guinea pigs and mice, thymic involution occurred even after doses of TCDD which did not alter body weight (Harris et al., 1973). Particularly involved are the cortical cells of the thymus. This hypoplasia has been observed in mice, rats, guinea pigs and monkeys. The most significant findings in both mice and guinea pigs treated with sublethal doses of TCDD were in the lymphoid system resulting in suppression of cell-mediated immunity, particularly in young animals (Vos et al., 1973, 1977, 1980). However, lymphoid involution should not be lethal as evidenced by the viability of athymic animals (Pantelouris, 1973). Furthermore, immunosuppression could not account for the lethal effects of TCDD when treated animals were maintained in a germ-free environment (Greig, 1973).

It remains unclear whether the effect of TCDD on the thymus is direct or indirect (Vos et al., 1980) and in vitro studies have been hampered by limited solubility of TCDD (Vos et al., 1980). Although a direct effect of TCDD on the thymus may be argued for since the thymus possesses high concentrations of the cytosolic receptor for TCDD (Poland et al., 1979), TCDD has produced little cytotoxicity when applied to lymphocytes in culture (Vos and Moore, 1974).

Hematological changes in mice, rats and guinea pigs treated with TCDD included lymphopenia and thrombocytopenia; manifest also was an increased susceptibility to infection concomitant with the suppression of cell-mediated immunity (Weisberg and Zinkl, 1973; Zinkl et al., 1973). Barsotti et al., (1979) reported anemia and severe pancytopenia in rhesus monkeys receiving a diet containing 500 ppt TCDD for 9

months. It was calculated that these animals had consumed approximately 3 μg total TCDD per kg of body weight. Death was attributed to complications arising from the severe pancytopenia.

Endocrine Effects

Although some endocrine changes have been noted, previous investigations into the effects of TCDD on the endocrine system have failed to satisfactorily elucidate the mechanism of TCDD toxicity. Since exposure to TCDD and polychlorinated biphenyls strongly induces several drug metabolizing enzymes (see "Microsomal Monooxygenase System" below), subsequent alterations in hormone metabolism may be expected, and indeed, changes in the metabolism of some steroid and thyroid hormones have been reported in animals treated with these compounds (Hook et al., 1975; Gustafsson and Ingelman-Sunberg, 1979; Nienstedt et al., 1979; Bastomsky, 1974, 1977a 1977b). Tofilon and coworkers (1980) reported decreased guinea pig testicular cytochrome P-450 following administration of TCDD, and alterations in testosterone metabolism have been reported by Hook et al. (1975), Gustafsson et al. (1979) and Nienstedt et al. (1980). TCDD has also been shown to produce decreased serum progesterone and estradiol levels in female rhesus monkeys (Barsotti et al., 1979).

Although TCDD has been shown to produce delayed increases in serum glucocorticoid levels, attempts to link TCDD toxicity to hyperglucocorticoidism have been unsuccessful (Neal et al., 1979; van Logten et al., 1980). Stress-induced release of glucocorticoids was not considered to be a factor in thymic involution (van Logten et al.,

1980). Also, hypophysectomy and adrenalectomy had no protective effect against TCDD toxicity (van Logten et al., 1980), and adrenalectomy may even increase the lethal effects of TCDD (Neal et al., 1979). Growth hormone administration had no effect on TCDD toxicity in hypophysectomized animals. Growth hormone restored thymus and body weight in hypophysectomized controls, but not after TCDD was administered (van Logten et al., 1980). Thymosin also failed to reverse TCDD effects on the thymus (Vos, 1978).

Bastomsky (1974, 1977a, 1977b) reported that diets containing PCBs or a single oral dose of TCDD produced goiters in rats. TCDD-treated rats exhibited 4-fold increases in biliary excretion of thyroxine (T_4) and 50% decreases in serum levels of T_4. In addition, serum thyrotropin (TSH) levels increased over 4 fold and thyroid weights per body weight were increased 42% in TCDD-treated rats. The goitrous effects were attributed to increased biliary excretion of glucuronidated T_4. Interestingly, the triiodothyronine (T_3) levels were not decreased in animals receiving either TCDD or PCBs.

Since thyroid hormones play an active role in metabolism, the results of Bastomsky (1977) suggested that the toxicity of TCDD and PCBs may result from thyroid dysfunction. However, when daily doses of up to 200 μg T_3 per kg body weight were given to rats following superlethal doses of TCDD, death of the animals was slightly delayed, but was not prevented (Neal et al., 1979). The slight increase in survival time was attributed to increased food intake in rats receiving the T_3 supplement. The effects of exogenous T_4 supplementation in TCDD-treated animals were not reported.

Metabolic Alterations

Biochemical studies suggest that the toxicity of TCDD may involve a number of metabolic alterations (Gasiewics et al., 1980; Swift et al., 1981). TCDD treatment has been shown to produce hypoglycemia (Gasiewics et al., 1980; Zinkl et al., 1973), hypercholesterolemia and hypertriglyceridemia when animals were provided food ad libitum (Gasiewics et al., 1980).

Although dehydration has been observed following TCDD administration, forced oral administration of water, electrolyte solution or balanced liquid diet did not alter or reverse the pattern of weight loss observed in rats treated with TCDD and fed ad libitum (Courtney et al., 1978). Despite hypophagia and weight loss, malnutrition cannot entirely account for the toxicity of TCDD since animals fed total parenteral nutrition died following TCDD administration without significant weight loss (Gasiewics et al., 1980).

Carcinogenicity

The association between TCDD and carcinogenesis remains ambiguous. Reports have indicated that chronic adminstration of low levels of TCDD results in increased incidence of liver carcinogenesis in rats (IARC, 1977; Kociba et al., 1978; van Miller et al., 1977) and Kouri et al. (1978) reported TCDD to be cocarcinogenic in the skin of mice when administered simultaneously with 3-methylcholanthrene without being carcinogenic itself. Conversely, 3-days topical pretreatment with TCDD inhibited the carcinogenic effects of 3-methylcholanthrene,

7,12-dimethylbenz(a)anthracene and (±)-trans-7-beta, 8-alpha-dihydroxy-9-alpha, 10-alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene in mice (Cohen et al., 1979; DiGiovanni et al., 1980).

In a two-year study, Kociba and coworkers (1979) discovered that continuous ingestion of a high dose of TCDD (0.1 ug/kg/day) in the diet resulted in toxicity to liver, lymphoid, respiratory and vascular tissues of rats. At that dose, the incidence of hepatocellular carcinomas of the liver and squamous cell carcinomas of the lung, hard palate/nasal turbinates and tongue was increased, while the incidence of spontaneous tumors in the pituitary, uterus, mammary gland, pancreas and adrenal medulla was decreased. Hopefully, further studies will reveal the carcinogenic potential of TCDD.

Other Effects

TCDD has been shown to be embryotoxic in rats (Sparschu et al., 1971; Courtney and Moore, 1971; Khera and Ruddick, 1973), mice (Courtney and Moore, 1971; Neubert and Dillman, 1972; Neubert et al., 1973; Moore et al., 1973; Smith et al., 1976; Courtney, 1976) and chickens (IARC, 1977; Cheung et al., 1981). In repeated or single doses, as little as 1-10 ug TCDD/kg body weight caused increased frequencies of cleft palate and kidney abnormalities in mice (Courtney and Moore, 1971; Neubert et al., 1973; Smith et al.,1976). Reproductive abnormalities were also found in rhesus monkeys fed a diet containing TCDD over a 9 month period (Barsotti et al., 1979).

Hyperplastic and hypertrophic lesions have been reported in the gastric mucosa of monkeys and cows (Allen, 1973; Barsotti et al., 1979;

McConnell, 1980) and marked hyperplasia, necrosis and hemorrhaging of mucosal epithelium have been reported in orally dosed monkeys and hamsters (Olson et al., 1980; McConnell, 1980). Shoaf and coworkers (1981) noted decreased ^{3}H-leucine incorporation into duodenal cells in rats receiving oral lethal doses of TCDD. These authors concluded that decreased lipoprotein synthesis may account for lipid accumulation and decreased nutrient absorption following TCDD exposure. In one or more species of laboratory animals, bone marrow hypoplasia, testicular degeneration, renal pelvis and urinary bladder hyperplasia and hemorrhage in the intestines and adrenals have been observed (IARC, 1978).

Biochemical Pathways Not Affected

TCDD administration to rats was found not to alter liver ATP content, redox state as determined by NAD:NADH, or state 3 and 4 mitochondrial respiration (Neal et al., 1979;). No changes have been noted in the absorption and metabolism of ^{14}C-Ala, ^{14}C-glucose or ^{14}C-oleate to $^{14}CO_2$ in the guinea pig, suggesting normal intermediary metabolism. Nucleotide metabolism, enzymes containing riboflavin coenzymes and enzymes of glycolysis and the TCA cycle also appeared to function normally in this study (Neal et al., 1979). Also, Manis and Apap (1979) reported no change in intestinal glutathione S-transferase and organic anion transport in rats 2 days after receiving oral or intraperitoneal doses of TCDD.

Pharmacokinectics and Metabolism

Orally administered doses of TCDD are distributed to the liver and to a lesser extent to fat as parent compound (Rose, 1976). TCDD is

predominately eliminated as the parent compound in the feces and to a lesser extent in the urine (Rose et al., 1976). The whole body $t_{\frac{1}{2}}$ has been reported to be 23.7 to 31 days in the rat (Rose, 1976), 22 to 43 days in the guinea pig (Nolan, 1979; Gasiewics et al., 1979) and 10.8 to 12 days (i.p.) and 15 days (p.o.) in the hamster (Olson et al., 1980b). Orally administered TCDD is more lethal than i.p. administered TCDD in hamsters (1157 μg vs 3000 μg, respectively, for LD_{50}), probably as a result of intestinal damage (Olson et al., 1980a).

Distribution appears similar among guinea pigs, rats and hamsters (Piper et al., 1973; Allen et al., 1975; Rose et al., 1976; Gasiewics and Neal, 1979; Olson et al., 1980b), with highest [^{3}H]TCDD activity found in liver, adipose tissue and adrenals. Hamsters excrete a large amount of TCDD in urine as metabolites, probably as conjugates. The increased metabolism probably contributes to, but does not totally explain, decreased TCDD-toxicity in the hamster (Olson et al., 1980b).

Human Studies

Table 2 shows possible routes of human exposure to TCDD and other polycyclic aromatic hydrocarbon compounds. Twenty two industrial accidents resulting in human exposure to TCDD were reported in 10 different countries between 1949 and 1976. The earliest reported incident of human exposure to TCDD occurred in Nitro, West Virginia in 1949. The involvment of TCDD in this industrial accident was concluded in retrospect in 1957 by Kimmig and Schulz. Probably the most famous industrial accident involving TCDD occurred in 1976 at the ICMESA

Table 2: Routes of human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[a]

Occupational Exposure

- Chemical plants producing 2,4,5-trichlorophenol
- Factories using 2,4,5-trichlorophenol
- Spraying 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)
- Using hexachlorophene or other chemicals containing TCDD as an impurity
- Incineration of dioxin precursors
- handling, transport and storage of TCDD used for experimentation and chemical analysis or handling of wastes

Public Exposure

- Herbicide spraying which may lead to skin contact, inhalation or ingestion of edible materials (e.g. wild berries) contaminated with TCDD
- Via food chain (bioaccumulation)
- Inhalation of flue gases or fly ash from incinerators and industrial heating facilities
- Unearthing of chemical wastes
- contact with workmen exposed to TCDD
- Environmental formation of TCDD

---

[a] Reggiani (1981).

2,4,5-trichlorophenol factory in Seveso, Milan, Italy (Firestone, 1977). In this incident an estimated 2-3 kg of TCDD were spread over an area of 1.8-3.5 km^2 (IARC, 1977).

Chloracne

Table 3 lists some of the common signs and symptoms which may occur at various times following exposure of humans to TCDD. The most common response to TCDD exposure in humans is chloracne (Crow, 1970; Taylor, 1974). Besides induction of acneform lesions in the skin of rabbit ears (Kimmig and Schulz, 1957a), TCDD exposure produces an analogous hyperkeratosis and modulation of subaceous structures to keratin cysts in monkeys, rabbits and hairless mice.

Chloracne and acneform dermatitis characterized by comedones, keratin cysts, pustules, papules and abscesses, may appear weeks or months after the initial exposure to TCDD. In 1957(b), Kimmig and Schulz found that TCDD was the agent responsible for causing occupational chloracne in employees of chlorophenol producing factories. Furthermore, in 1971 TCDD was implicated in causing chloracne in male workers in a plant producing 2,4,-D and 2,4,5-T (Poland et al., 1971).

Other Effects

In addition to chloracne, hepatic dysfunction, hypercholesterolemia, peripheral neuropathies (numbness and tingling), altered fat metabolism and porphyria cutanea tarda commonly occur in humans who are believed to have been exposed to TCDD (IARC, 1977; Huff et al., 1980; Kriebel, 1981). Emotional disturbances, neuropsychiatric syndromes and

Table 3: Signs and symptoms of human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[a]

Early
- Burning sensation in eyes, nose and throat
- Headache, dizziness, nausea and vomiting
Days Later
- Severe itching, redness and swelling of the face
- Develop more marked symptoms over the eyelids, nose and lips
Within Initial Weeks
- Inflamed nodules as well as pustules appear on the face, neck, shoulders, trunk and forearms
- Development of comedones
Within Initial Months
- Acneform eruptions
- Hyperpigmentation of skin
- Aching muscles, mainly in chest and thighs (aggravated by exertion)
- Insomnia, extreme irritability and loss of libido

[a] Huff et al. (1980)

neuromuscular weakness and pain with nerve conduction abnormalities have also been reported (Huff et al., 1980).

A 30-year follow-up study of mortality in 121 workers exposed to TCDD showed no increase in general mortality or deaths due to neoplasms. Subjects were selected for the study on the basis of having developed chloracne after exposure to TCDD in a trichlorophenol process accident at the Monsanto Company plant in Nitro, West Virginia on March 8, 1949 (Zack and Suskind, 1980). Other epidemiologic studies of humans exposed to TCDD are in progress, especially people from geographic areas which experienced large scale spraying of Agent Orange.

Microsomal Monooxygenase System

Along with proliferation of the endoplasmic reticulum of renal and hepatic cells (Fowler et al., 1973, 1975), TCDD stimulates a number of enzymes in the liver and kidney (IARC, 1977; Beatty and Neal, 1976; Buu-Hoi et al., 1972; Fowler, 1975; Greig, 1972; Greig and DeMatteis, 1973; Hook et al., 1975a, 1975b; Lucier et al., 1973, 1975a, 1975b; Woods, 1973; Kitchin and Woods, 1978; Poland and Glover, 1973a, 1973b, 1975). The most notable and extensively studied among the enzyme activities affected by chlorinated PAHs in general are those associated with the microsomal monooxygenase (or mixed-function oxidase) system. This system consists of a battery of heme-containing membrane-bound enzymes which incorporate single oxygen atoms into a large variety of substrates (Mannering, 1971). The formation of polar products by monooxygenation of lipid soluble substrates offers an important mechanism for bioelimination of toxins. The monooxygenase system also plays

an important role in steroid hormone metabolism.

The most extensively studied microsomal monooxygenases are those associated with the cytochromes P-450 (Klingenberg, 1958; Garfinkel, 1958; Omura and Sato, 1964a, 1964b) and cytochrome P-448 (sometimes called cytochrome P_1-450) (Sladek and Mannering, 1966; Shoeman et al., 1969; Thorgiersson and Nebert, 1977). The term cytochrome P-450 originates from spectroscopic data, in that maximum absorbance of the dithionite-reduced cytochrome occurs at a radiation wavelength of 450 nm when combined with carbon monoxide (Omura and Sato, 1964a). The cytochrome P-450 molecule, embedded in the endoplasmic reticulum, receives two reducing electrons from the cofactor NADPH and/or NADH via cytochrome P-450 reductase (Nebert, 1979a; Nebert et al., 1981; Nebert et al., 1982). The reduced (activated) heme then binds an atmospheric oxygen molecule and inserts one of the oxygen atoms into the substrate. The other oxygen atom dissolves into cellular water (Nebert, 1982).

Structure-Activity

An interesting characteristic of the cytochrome P-450-mediated monooxygenases is their activation (or induction) by exposure to certain substrates (reviewed by Nebert, 1979a). Microsomal monooxygenase inducers may be generally categorized as either phenobarbital (PB)-type (cytochrome P-450) or 3-methylcholanthrene (MC)-type (cytochrome P-448) inducers (Conney, 1967), and PCB congeners have been found to segregate into either categories (Goldstein et al., 1977; Poland and Glover, 1977). Simultaneous administration of PB and MC to animals results in

a mixed-type induction of both P-450 and P-448. The commercial PCB mixture (Aroclor 1254) induces both cytochromes P-450 and P-488 (Ryan et al., 1979) and, therefore, has been referred to as a mixed-type inducer (Alvares and Kappas, 1977). It has been proposed that the mixed-type induction by Aroclor 1254 results from the presence of both cytochrome P-450- and P-448-inducing PCB congeners (Goldstein, 1979; Safe et al., 1981).

From structure-activity studies, several rules have been proposed for categorizing microsomal monooxygenase inducers (Safe et al., 1981).

Rule 1. Biphenyl chlorinated in ortho and para positions of both rings is a PB-type inducer of cytochrome P-450.

Rule 2. Biphenyl chlorinated in only the meta and para positions of both rings is an MC-type inducer of cytochrome P-488.

Rule 3. Most of the lower chlorinated biphenyls and those PCBs substituted in only one ring possess little inducing capacity.

It has been proposed that the type of planar conformation assumed by the biphenyl structure determines the type of induction which a PCB congener will exhibit. According to this hypothesis, noncoplanar conformation of the biphenyl is required for PB-type induction and coplanar conformation gives rise to MC-type induction (Goldstein, 1979; Poland and Glover, 1977). Chlorination in the ortho position of the biphenyl rings produces an obligatory noncoplanar conformation of the biphenyl structure as a result of steric hinderance. Substitution in meta or para positions relieves that constraint and the biphenyl structure remains free to assume a nearly planar

conformation (reviewed by Goldstein, 1979).

These rules then predict that chlorinated congeners that are fixed in the planar conformation will be the most potent MC-type inducers. Experimentally, TCDD is 10^3 times more potent than PCB isomers in inducing cytochrome P-448. TCDD, 2,3,6,7-tetrachlorodibenzofuran, 3,4,3',4'-tetrachloroazo- and 3,4,3',4'-tetrachloroazoxybenzene and 3,4,3',4'-tetrachlorobiphenyl are all planar, or can assume nearly planar configurations. They each fit a 3 X 10 A^{o} rectangle, have chlorine atoms at the four corners of the rectangle and all induce cytchrome P-448-associated AHH activity (Poland and Knutson, 1982).

Poland and Glover (1973a) reported the structure activity relationship for enzyme induction by TCDD and polychlorinated biphenyl congeners in the chick embryo. Certain structural characteristics were requisite for AHH inducing activity. At least 3 of the lateral ring positons must be occupied by halogen atoms in order for the compound to exhibit AHH inducing capacity. Maximum potency is obtained when 4 of the lateral ring positions are occuppied by halogens (2,3,7 and 8 positions for TCDD) and at least one ring position must remain unsubstituted (i.e. octachlorodibenzo-p-dioxin is inactive, while the heptachloro isomer retains some activity). Identical structure-activity relationships were found for delta-aminolevulinic acid synthetase and AHH induction in the chick embryo (Poland and Glover, 1973a). The significance of these structure-activity relationships lies in the fact that the same structure-activity relationships hold for the toxic potency of the halogenated aromatic hydrocarbon congeners (Goldstein et

al., 1976; McKinney et al., 1976; Kawanishi et al., 1978; Kohli et al., 1979; Poland et al., 1979; Yoshimura et al., 1979).

In addition to AHH activity, TCDD administration has been reported to induce the following enzymes in various species and tissues: delta-aminolevulinic acid synthetase (Poland and Glover, 1973a, 1973b); UDP-glucuronyl transferase (Hook et al., 1975a); DT-diaphorase (Beatty et al., 1976; Beatty and Neal, 1978); uroporphyrinogen decarboxylase (Smith et al., 1981); glutathione S-transferase (Kirsch et al., 1975); and ornithine decarboxylase (Nebert and Oka, 1976; Nebert et al., 1980).

Role of Ornithine Decarboxylase in Monooxygenase Induction

Ornithine decarboxylase (ODC, EC 4.1.1.17), the initial and rate-limiting enzyme in polyamine biosynthesis, has been shown to increase in activity in response to various hormones, drugs and hypertrophic stimuli (Russell and Durie, 1978; Russell, 1980). Increased ODC activity is also characteristic of rapidly proliferating tissues and cells in culture (Russell and Snyder, 1968).

A close temporal relationship between ODC induction and increased RNA polymerase I activity has been documented (Manen and Russell, 1975, 1977a, 1977b; Russell and Durie, 1978; Haddox and Russell, 1981). Hepatic RNA polymerase I, the enzyme responsible for synthesis of ribosomal RNA, increases in activity as much as 2 fold within 5 hours after partial hepatectomy. The RNA polymerase I peak is preceded by peak ODC activity, which occurs at 4-4½ hours after partial hepatectomy

(Russell and Durie, 1978). Therefore, the increased ODC activity appears to be involved in the production of new ribosomal RNA as the cell prepares for increased protein synthesis.

Since microsomal monooxygenase induction involves de novo protein sythesis (Alvares et al., 1968; Nebert and Gielen, 1971; Hook et al., 1975a; Niwa et al., 1975; Haugen et al., 1976; Kitchin and Woods, 1978), ODC activity has been evaluated prior to microsomal monooxygenase induction and has been shown to increase prior to induction of the cytochrome P-450 system in rats (Costa et al., 1976; Byus et al., 1976) and inbred strains of mice (Nebert et al., 1980). Additionally, pretreatment of rats and mice with the ODC inhibitor 1,3-diamino-2-propanol inhibited induction of cytochrome P-450, AHH and 7-ethoxycoumarin activities by subsequent administration of phenobarbital, beta-napthoflavone or the PCB mixture Clophen C (Raunio and Pelkonen, 1979). These results further suggested that ODC induction is a necessary step in induction of the microsomal monooxygenase system.

Ah Locus

Nebert and Gielen (1972), Nebert et al. (1972) and Thomas (1972) have reported differences in the sensitivity of 2 strains of mice to AHH induction. C57BL/J6 (C57) mice were found to be highly sensitive to AHH induction by TCDD, whereas DBA/2J (DBA) mice proved to be relatively refractory to AHH induction by TCDD and other AHH-inducing compounds. The DBA mice were totally insensitive to AHH induction by 3-methylcholanthrene and 10 times less sensitive than the C57 mice to AHH induction by TCDD (Poland and Glover, 1975). Niwa et

al. (1975) reported that cultured cells from DBA fetal mice were about 10 times less sensitive to AHH induction than cells from C57 fetal mice. As a result of these and other findings, existence of the genetic locus, Ah (Aryl hydroxylase), was postulated. Using this classification system, animals are said to be genetically responsive or nonresponsive at the Ah locus, according to their sensitivity to AHH induction by the known AHH-inducing compounds.

Induction Receptor

A high-affinity binding species was found in the 105,000 X g cytosol from C57 mice (Poland et al., 1976). Binding activity was insensitive to nucleases, but was heat labile and was eliminated by trypsin, suggesting that the binding species was a protein. The binding protein exhibited high affinity for TCDD (k_D = 0.27 X 10^{-9}M) which corresponded well with the potency of TCDD for inducing AHH (ED_{50} = 1 X 10^{-9} mol/kg) in vivo. Furthermore, a correlation was found between k_D and ED_{50} values for the various dibenzo-p-dioxin, dibenzofuran and polychlorinated biphenyl congeners. This further suggested that the receptor was directly involved in AHH induction and toxicity (Poland et al., 1975).

The receptor protein was found to have a stokes radius of 6.6 nm (Carlstedt-Duke et al., 1981), a sedimentation coefficient of 5.0 S and a calculated molecular weight of 136,000 with a functional ratio of 1.79. The receptor has been found in rat liver, lung, kidney, thymus and, with much lower concentration, in testis, brain and skeletal

muscle (Carlstedt-Duke et al., 1979a, 1979b). Recently, its presence has been demonstrated in the palatal region of fetal mice, arguing for its possible involvement in the production of cleft palate in fetal mice (Dencker and Pratt, 1981). Furthermore, the receptor has been shown to mediate nuclear uptake and binding of TCDD in mouse liver (Greenlee and Poland, 1979; Okey et al., 1980).

Role of Receptor in Toxicity

The correlation between the ability of a congener to bind the cytosolic receptor (induce AHH activity) and evoke toxicity suggests that the parent compounds, rather than metabolites, are responsible for toxicity and that the toxicity is mediated by receptor binding. It was concluded that the ability to elicit a single response indicates the potential to produce the entire toxic syndrome. However, a difficulty arises when attempting to extrapolate receptor affinity to toxicity because receptor affinity for TCDD is similar across species, but toxicity differs greatly (Poland and Knutson, 1982).

Phenobarbital, pregnenolone, 16-carbonitrile and other inducers of the cytochrome P450 system (but not AHH activity) did not compete for receptor binding. Steroid hormones and thyroxine also failed to compete with TCDD for the receptor, raising some doubt as to whether the receptor evolved to accommodate an endogenous hormone. It has been considered that the receptor may have evolved to facilitate a hormone which is now extinct (Poland and Knutson, 1982).

Importance of AHH Induction to Toxicity

There is no evidence to date that AHH induction per se can produce the toxic syndrome noted following intoxication with TCDD and congeners. AHH induction could result in increased toxicity by metabolism of parent compounds to toxic intermediates. However, this does little to explain TCDD toxicity since TCDD undergoes little metabolism (Rose et al., 1976), and the structure-activity relationships hold for parent compounds rather than their metabolites. Furthermore, reactive intermediates would be expected to evoke toxicity via covalent binding to macromolecules. It is difficult then, to imagine how postulated reactive intermediates could produce the "wasting" syndrome concurrent with hypertrophy and hyperplasia in certain tissues and organs in the absence of significant morphological tissue damage (Poland and Knutson, 1982). It would also be difficult to explain the toxicity of TCDD on the basis of increased steroid hormone metabolism alone. Therefore, it has been suggested that AHH induction be used as a signal response, but not implicated directly in toxicity of TCDD and related compounds (Poland and Knutson, 1982).

STATEMENT OF THE PROBLEM

The purpose of this study was to characterize metabolic and endocrine changes induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) administration in rats.

TCDD represents a problem as an industrial and environmental contaminant, and numerous reports of human exposure to TCDD have appeared. TCDD forms as an unavoidable contaminant during the synthesis of trichlorophenol and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). TCDD has become environmentally distributed via industrial accidents and spraying of the 2,4,5-T-containing herbicide, Agent Orange.

TCDD is one of the most toxic small molecules yet discovered. But, despite it extraordinary potency, the mechanism by which TCDD causes death in animals remains unknown. TCDD toxicity in laboratory animals is manifest as a "wasting" syndrome in which the animal exhibits lethargy and progressive weight loss leading to death. Since previous investigations have suggested that TCDD toxicity may involve metabolic and endocrine disorders, we considered it important to evaluate the posttreatment status metabolic-regulatory factors relative to the toxicity of TCDD.

By characterizing metabolic and hormonal changes in rats following administration of TCDD, it was aniticipated that this study would contribute to the elucidation of the mechanism of toxicity of TCDD.

MATERIALS AND METHODS

Chemicals

L-[1^{14}C]Ornithine hydrochloride (54 mCi/mmol) was purchased from Amersham/Searle Corporation. Dithiothreitol (DTT), pyridoxal phosphate, phenylmethylsulfonylfluoride, EDTA, bovine serum albumin, dexamethasone acetate, aminophylline, NADH, NADPH, benzamidine, benzo(a)pyrene, and all buffers and metals employed were obtained from Sigma, St. Louis, MO. Sodium borate was purchased from J.T. Baker Chemical Co., Phillipsburg, NJ. Acetone (pesticide grade) was purchased from Fisher Scientific Co., Fairlawn, NJ. TCDD was a generous gift from Dr. H.B. Matthews, NIEHS, Research Triangle Park. 3-hydroxybenzo(a)pyrene was generously provided by Dr. D.W. Nebert, National Institutes of Health. 2,2',4,4',5,5'-Hexachlorobiphenyl was a generous gift from Dr. Joyce Goldstein, NIEHS, Research Triangle Park and 3,3',4,4',5,5'-hexachlorobiphenyl was generously provided by Dr. Mary Jo Vodicnik, Medical College of Wisconsin, Milwaukee, WI.

Safe Handling Procedures for TCDD

Due to the extreme toxicity of TCDD, certain handling precautions are in order. The following summarizes the safety precautions established for the handling of TCDD during this study.

Containment

Crystalline stock TCDD was stored in a tightly capped glass bottle in a plastic bag inside a locked drawer. Work with open

containers of TCDD was kept to a minimum. TCDD was prepared for injection by weighing and depositing 0.5 mg quantities into 5 ml vials with caps and septa (rubber with Teflon^{R} coating). Acetone (4.5 ml/0.5 mg TCDD) was added to the closed vial by injection through the septum to yield a saturated solution of 0.09 mg TCDD/ml acetone (DiGiovanni et al., 1979). Diluted solutions of TCDD were prepared for animal dosing by mixing aliquots of the TCDD-acetone solution with corn oil in closed vials. In some experiments, the acetone was evaporated under vacuum to leave TCDD suspended in corn oil. All TCDD containers were stored in well-marked small desicator jars.

Protective Equipment

Disposable rubber gloves (2 pairs) were worn at all times when handling any TCDD container. The outer pair of gloves was replaced frequently to minimize the possibility of spreading contamination. If the gloves were believed to have come into contact with any of the TCDD solution, both pairs were quickly removed and placed in the hazardous waste bag (see "Handling of Contaminated Wastes", below). When weighing crystalline TCDD, disposable isolation gowns and masks were also worn. Hands and forearms were thoroughly washed after each handling of TCDD.

Handling of Contaminated Wastes

Attempts were made to generate as little contaminated waste as possible. However, when protective equipment and other materials were contaminated they were discarded immediately into small plastic bags,

which were then placed in large red hazardous waste bags. Bagged waste was stored in a well-labeled waste can with a plastic bag liner. When sufficient waste had been generated to warrant disposal, the liner bag, along with contents was delivered to be incinerated at 1500°C.

Animal Wastes

Rats were housed in plastic pans with wood-shaving bedding. Twice the normal amount of bedding was used in rat pans to absorb excrement. The pans were labeled such that animal-care workers were informed not to change the bedding; bedding was changed weekly by the investigator and was incinerated with animal carcasses in the Department of Animal Resources.

Animals

Male Sprague-Dawley rats (80-120 g) were obtained from the University of Arizona breeding colony or from Hilltop, (Scottsdale, PA). Unless specifically stated in each experiment, the animals were provided tap water and food (Wayne Lab Blox) ad libitum. The rats were maintained on an 0800h -2000h light cycle in the Department of Animal Resources at the Arizona Health Sciences Center throughout all studies.

Paired Feeding

In the paired feeding studies, rats were housed in suspended stainless steel cages, 2 rats per cage. Powdered food was provided to the rats in 100 ml beakers which were positioned in the corners of the cages.

Since TCDD-treated rats spill more powdered food than control rats do (Seefeld et al., 1982), spillage must be accounted for in order to avoid overestimating food consumption by the TCDD-treated rats. Therefore, polystyrene weigh boats (3-3/16 x 3-3/16 inches, Curtin Matheson Scientific, Inc., Houston, TX) were suspended below the corners of the cages in which the food beakers were positioned.

Rats received TCDD (45 ug/kg, i.p.) at 1000 h. The pair-fed rats received the corn oil/acetone vehicle at the same time on the following day, and were allowed the amount of food per gram of body that the TCDD-treated rats had consumed on the previous day. Ad-libitum-fed control rats were treated and handled on time schedules as TCDD-treated or pair-fed rats.

Partial Hepatectomies

Partial (70%) hepatectomies were performed under light ether anesthesia on both TCDD-treated and vehicle control animals according to the procedure of Higgins and Anderson (1931). Animals were killed by cervical dislocation. Tissues were removed and homogenized in 4 parts of the buffer required for the individual assays. Homogenates were centrifuged for 5 min at 50,000 x g. The supernatant fraction was used for all cytosolic enzyme assays.

Body Temperature Determinations

Rectal temperatures were determined with a Model Bat 8 digital thermometer (Bailey Instruments, Saddle Brook, NJ). The rectal probe was left in place for approximately 10 sec to obtain a measurement.

Enzyme Assays

Ornithine Decarboxylase Assay

Ornithine decarboxylase (ODC) activity was determined in tissue supernatant (50,000 x g) fraction obtained from a 5 min centrifugation. ODC activity was measured as the liberation of $^{14}CO_2$ from L-[1-^{14}C]-ornithine (Russell and Snyder, 1968). Incubations were conducted at 37°C in a medium containing 50 mM sodium-potassium phosphate buffer, pH 7.2, 0.1 mM EDTA, 2 mM dithiothreitol, 0.012 mM pyridoxal phosphate, 0.04 mM phenylmethylsulfonylfluoride, 5 mM NaF, 1.0 μCi L-[^{14}C]ornithine (specific activity = 20 mCi/mmol) and 0.5 mM L-ornithine in a final volume of 0.2 ml.

The reaction was initiated by the addition of 0.05 ml of tissue supernatant fraction to the reaction mixture. After 30 min, the reaction was terminated by the addition of 0.5 ml of 1 M citric acid. The samples were allowed to stand another 20 min and the CO_2 evolved was trapped by 0.02 ml of 2 N NaOH on a 3 MM filter paper (Whatman, Clifton, NJ) suspended above the reaction mixture in a plastic well (Kontes, Vineland, NJ). The filters were counted in toluene-Omnifluor scintillation cocktail (New England Nuclear, Boston, MA). Activity was expressed as pmoles of CO_2 evolved per min per mg of cytosolic protein at 37°C. The enzyme activity was linear with respect to incubation time and enzyme concentration.

Aryl Hydrocarbon Hydroxylase Assay

Aryl hydrocarbon hydroxylase (AHH) activity was determined by a modification of the procedure of Nebert and Gelboin (1968). In a total reaction volume of 1.0 ml, 0.2 ml of tissue supernatant fraction was incubated in the presence of 0.01 M Trizma buffer, pH 7.4, 5 mM $MgCl_2$, 0.3 mg of NADPH and 0.3 mg of NADH. The reaction was initiated by adding 0.08 mmole of benzo(a)pyrene (in 0.04 ml of methanol). The mixture was shaken at 37°C for 10 min. The reaction was stopped by the addition of 4.25 ml of cold hexane:acetone solution (3.25 ml hexane:1 ml acetone). Samples were then vortexed and incubated with shaking at 37°C for 10 min. A 1.0-ml sample of the organic phase was extracted with 3.0 ml of 1 N NaOH. The concentration of hydroxylated benzo(a)-pyrene extracted in the alkaline phase was determined spectrophoto-fluorometrically with activation at 396 nm and fluorescence at 522 nm. Activity was expressed as pmoles of 3-hydroxybenzo(a)pyrene formed per min per g of tissue or mg of protein.

RNA Polymerase I Assay

Nuclei were isolated by the procedures of Blobel and Potter (1962) and Busch et al. (1967). Nuclei were adjusted to approximately equal amounts of protein (0.6 mg/0.15 ml) before use in the RNA polymerase assay. RNA polymerase I activity was determined by measuring the incorporation of [^{3}H]UTP (37.5 mCi/mmol, New England Nuclear, Boston, MA) into RNA in the presence of α-amanitin. The RNA polymerase assay mixture contained in a volume of 0.375 ml: 7.5 ug pyruvate phosphoenol pyruvate; 0.6 µmol mercaptoethanol; 3.0 µmol KCl;

0.6 μmol $MnCl_2$; 2.25 μmol NaF; 21.75 μmol $(NH_4)_2SO_4$; 8 x 10^{-5} μmol [^{3}H]UTP; 0.0375 μmol unlabeled UTP; and 0.225 μmol each of GTP, ATP and CTP. Activity was expressed as trichloroacetic acid precipitable cpm.

Radioimmunoassays

Preparation of Serum for Radioimmunoassay

Rats were laparotomized under ether anesthesia. Blood was drawn from the portal vein, placed in plastic tubes with caps, allowed to coagulate on ice and was centrifuged at 1000 x g for 20 min. Serum was removed and stored at -70°C until the various determinations were performed. Blood which was collected for glucagon or somatostatin determinations was collected in tubes containing 0.05 ml of 1.0 M benzamidine per ml of blood. Approximately 7 mg of EDTA was added to each tube prior to collection of blood for somatostatin determinations.

Preparation of Tissues for Radioimmunoassay

Frozen tissues were weighed and homogenized in hot (82°C) 2 N acetic acid in the following volumes: pancreas, 2.5 ml; gastric antrum, 1.0 ml; 1 g liver, 2.5 ml. The tissues were then placed in a water bath (82°C) for 30 min and centrifuged at 17,000 x g for 15 min. The precipitate was rehomogenized in hot 2 N acetic acid and the procedure was repeated. The supernatant fractions from the two centrifugations were combined in glass scintillation vials with screwcaps, frozen (-70°C) and lyophilized. Lyophilized extracts were stored at

-20°C until hormone assays were performed, at which time the extracts were reconstituted with 5.0 ml of 0.4% BSA-borate (0.1 M) buffer, pH 8.4.

Triiodothyronine (T_3) Assay

Serum triiodothyronine (T_3) levels were determined by radioimmunoassay using the Tri-Tab RIA Kit (Nuclear-Medical Laboratories, INC., Dallas, TX). The assay employed a single antibody technique in which the T_3 not bound by the antibody was bound to charcoal and removed by centrifugation for 10 min at 1500 x g. The T_3 concentration in the sample was determined as the amount of ^{125}I-T_3 displaced from the antibody in the charcoal pellet.

Thyroxine (T_4) Assay

Serum thyroxine (T_4) assays were performed either by the Clinical Laboratories at the University of Arizona Health Sciences Center, using Kit No. CA-535 (Clinical Assays, Cambridge, MA) or by the investigator using the Tetra-Tab RIA Kit (Nuclear-Medical Laboratories, Inc., Dallas, TX). The Tetra-Tab RIA Kit employed a single antibody technique in which the antibody-T_4 complex was precipitated with ammonium sulfate. T_4 concentrations in the samples were determined as the amount of ^{125}I-T_4 displaced from the antibody in the precipitate.

T_3 Uptake and Free Thyroxine Index

T_3 uptake was determined by the Clinical Laboratories at the University of Arizona Health Sciences Center using the Tri-Tab T_3

Uptake Diagnostic Kit (Nuclear-Medical Laboratories, Inc., Dallas, TX).

The free thyroxine index (FTI) was determined as:

$$FTI = \frac{T_3 \text{ uptake } (\%) \times [T_4] \text{ (ug/ml)}}{NS}$$

where NS represents a normalization value (around 30) derived from a standard each time T_3 uptake was determined.

Insulin Assay

Serum and tissue insulin levels were determined by double-antibody radioimmunoassay using rat insulin as the standard (Immunonuclear, Stillwater, MN). Tissue insulin levels were determined in a 100-fold dilution of reconstituted lyophylized tissue extracts.

Somatostatin Assay

Serum and tissue somatostatin levels were determined by double-antibody radioimmunoassay (Immunonuclear, Stillwater, MN). Somatostatin levels were determined in serum samples diluted 1:2, reconstituted lyophilized tissue extracts of pancreas and gastric antrum diluted 1:100 or reconstituted lyophilized liver extracts diluted 1:4.

Glucagon Assay

Serum glucagon determinations were kindly provided by Dr. David Johnson, Department of Internal Medicine, University of Arizona. The procedure of Ensinck et al. (1972) was used, which employs porcine glucagon as the standard.

Glucose Assay

Glucose was determined in nondeproteinized serum by the glucose oxidase method with Sigma (St. Louis, MO) glucose Kit No. 510 UV.

RNA and DNA Determinations

Tissue RNA and DNA contents were determined by the methods of Schneider (1957). An aliquot (1.0 ml) of a 20% tissue homogenate in 0.05 M Tris-HCl, pH 7.9, was extracted sequentially with 10% trichloroacetic acid and 95% ethanol to remove acid-soluble compounds and lipids. Remaining precipitates were dissolved in hot trichloroacetic acid, and supernatant aliquots were taken separately for the determination of total RNA and DNA.

Polyamine determinations

Polyamine analysis was performed with a Durram D-500 amino acid analyzer (Dionex Corporation, Sunnyvale, CA) as described by Dunzendorfer and Russell (1978).

ODC Induction Kinetics

To investigate the effects of TCDD on the turn-over rate of ODC, the half life of ODC activity in the livers of rats treated with TCDD was compared to that of control animals. The time required for the ODC activity to decline by one-half was determined from a semi-logarithmic plot of activity vs time.

The induction kinetic studies consisted of 2 parts. Experiment #1 involved measurement of the decline of ODC activity in the presence

of ongoing induction. In experiment # 2, the activity half life was determined in the presence of a protein synthesis inhibitor.

Experiment #1

The half life of ODC activity was determined during the natural decline in activity after hormonal induction. This allowed comparison of the half lives of ODC in TCDD-treated and control animals in the presence of any short-lived enzymes which may be responsible for a posttranslational modification (inactivation) of the ODC-enzyme protein.

Rats were pretreated with .20 μg TCDD/kg body weight one week prior to receiving dexamethasone-acetate (0.5 mg/kg, i.p.) in 0.9% saline and 5 x 10^{-4} N NaOH. Rats were dosed with dexamethasone-acetate 7.0, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5 or 0 h before termination. Dosing was scheduled such that all terminations were performed within ½ h.

Experiment #2

In this experiment, the rats received cycloheximide (50 mg/kg, i.p.) to block further de novo enzyme synthesis. This allowed measurement of the decline of ODC activity in the absence of further ODC induction or induction of any enzymes responsible for a post-translational modification of ODC.

Dexamethasone-acetate was administered to the rats 4 h prior to the administration of cycloheximide. The rats were killed 0, 7, 12, 20 or 30 min after receiving cycloheximide.

Statistics

Student's t-test was used to determine the significance of difference between two means. One-way analysis of variance was used to determine differences among means in the time-course and paired-feeding experiments. Following analysis of variance, significance of difference between two means in the time course experiments was determined by the least-significant difference (LSD) method of multiple comparisons t-test. Values of $p < 0.05$ were considered significant.

RESULTS

General Toxicity

Lethality

To investigate the TCDD-sensitivity of University of Arizona breeding-colony rats, an LD_{50} value was determined (Table 4). Male Sprague-Dawley rats received i.p. injections of 20, 30, 45 and 60 µg TCDD/kg body weight and the number of deaths was determined. The 30-day LD_{50} value was calculated to be 46.0 µg/kg for TCDD in these animals (Litchfield and Wilcoxon, 1949). These results agree with reported LD_{50} values for rats, which have ranged from 22-45 µg/kg (Rowe et al., 1971; Schwetz et al., 1973).

Body Weight and Temperature

Following administration of a single 90 µg/kg dose of TCDD, rats exhibited progressive weight loss accompanied by declining body temperature (Fig. 3). Both body weight and body temperature were significantly decreased by 2 days after TCDD treatment. By day 18, the rats had lost 15% of their body weight from day 0. By 2 weeks after TCDD administration, the body temperatures of the rats had fallen below 35°C with a low mean value of 34.5°C occurring on day 16. During the observation period, mean body temperatures for controls ranged from 36.8°C to 37.5°C.

To investigate the possible involvement of hypophagia in decreased body temperature and weight gain following administration of

Table 4: Lethality of TCDD in rats[a]

Dose[b]	Deaths[c]
20	0/5
30	1/5
45	2/5
60	3/4
LD_{50} = 46.0 μg/kg[d]	

[a] Male Sprague-Dawley rats, weighing 110-150 g, were obtained from the animal colony at the University of Arizona Department of Animal Resources

[b] μg TCDD/kg body weight in acetone/corn oil

[c] Number of deaths/number treated

[d] As determined by the method of Litchfield and Wilcoxon (1949)

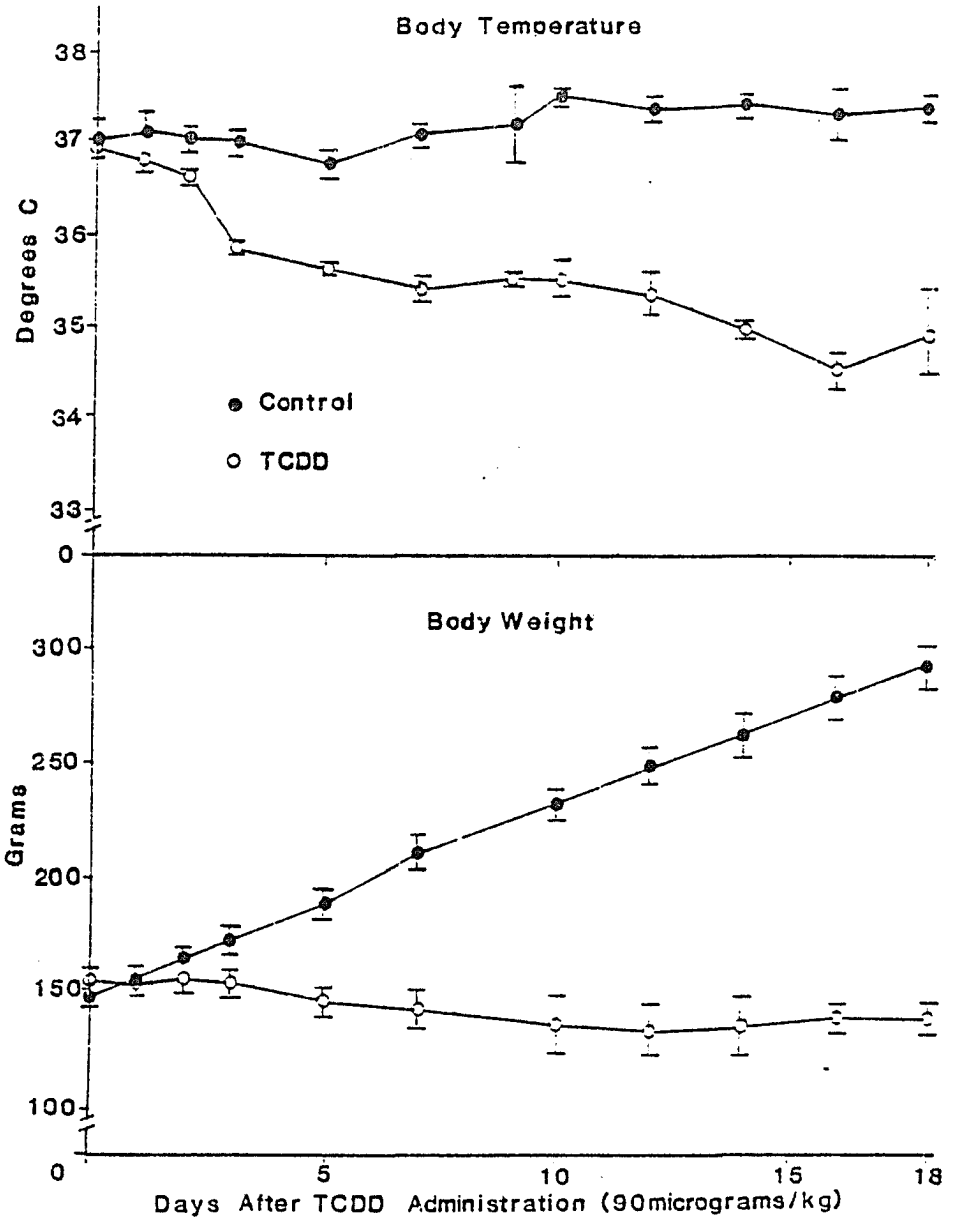


Figure 3: Temporal comparison of body temperature and body weight between control and TCDD-treated rats. * Significantly different from control ($P < 0.05$).

TCDD, these parameters were measured in pair-fed rats, as well as in rats fed ad libitum. The pair-fed rats were allowed only the amount of food consumed by the TCDD-treated rats. Hypophagia in rats treated with 45 μg TCDD/kg appeared to account for the lower body-weight gain since the pair-fed rats gained no more weight than the TCDD-treated rats. The pair-fed rats appeared capable of maintaining their body temperature while the TCDD-treated rats exhibited hypothermia. The mean body temperature of the TCDD-treated rats remained significantly below control values starting 2 days after treatment. Although the mean body temperature of the pair-fed control rats fell below the mean value of the ad-libitum controls on day 7, the mean temperature still remained greater than 37°C and appeared slightly higher than the day-7 values for TCDD-treated rats ($P = 0.067$). The effects of TCDD on body temperature appeared to be dose dependent since the values remained above 36°C for 7 days following 45 μg TCDD/kg (Table 5), whereas rats treated with 90 μg TCDD/kg exhibited temperatures which remained below 36°C commencing 3 days after treatment (Fig. 3).

Organ Weights

Administration of 45 μg TCDD/kg body weight produced differential effects on the weights of various organs (Table 6). Of the 9 organs shown in Table 6, 3 increased in dry weight, 3 decreased, and 3 were not altered in dry weight per 100 g body weight. Increased organ dry weights were noted in the liver (14%), stomach (29%) and adrenals (40%) of rats treated with TCDD. Decreased dry weights were noted in

Table 5: Effects of TCDD and paired feeding on body weight and temperature[a]

Day	Ad Lib Control	Pair-Fed Control	TCDD
0	36.94 ± 0.28	37.10 ± 0.23	36.92 ± 0.48
1	37.63 ± 0.13	37.20 ± 0.16	37.13 ± 0.20
2	37.24 ± 0.06	37.53 ± 0.12	36.60 ± 0.13[b]
3	37.74 ± 0.18	37.87 ± 0.09	36.52 ± 0.13[b]
4	37.75 ± 0.08	37.87 ± 0.14	36.80 ± 0.13[b]
5	37.80 ± 0.17	37.63 ± 0.08	37.08 ± 0.22[c]
6	37.86 ± 0.14	37.92 ± 0.18	36.57 ± 0.24[b]
7	37.88 ± 0.14	37.16 ± 0.10[d]	36.55 ± 0.35[b]
Body Weight[e]	+49.9 ± 1.7	+15.7 ± 0.9	+14.8 ± 5.6

Rats received TCDD (45 µg/kg, i.p.) on day 0. Values represent mean ± S.E.M. for 6 to 8 rats.

[a] Degrees Celsius

[b] Statistically different from ad libitum control as determined by analysis of variance and least significant difference test ($P < 0.001$)

[c] $P < 0.01$

[d] $P < 0.05$

[e] Body weight gained between days 0 and 7 given as percent of starting weight.

Table 6: Organ weights per 100 gm body weight one week after TCDD[a] in male Sprague-Dawley rats

	Wet Weight		Dry Weight		% Water Weight	
Tissue	Control	TCDD	Control	TCDD	Control	TCDD
Pancreas	0.47 ±0.01	0.54 ±0.04	0.15 ±0.01	0.16 ±0.01	67.9 ±0.8	70.9[b] ±0.8
Spleen	0.50 ±0.02	0.37[b] ±0.01	0.11 ±0.005	0.09[b] ±0.003	77.2 ±0.2	76.9 ±0.2
Liver	4.57 ±0.22	5.16[c] ±0.15	1.25 ±0.06	1.42[c] ±0.05	72.6 ±0.3	72.6 ±0.4
Stomach	0.65 ±0.02	0.87[b] ±0.04	0.14 ±0.004	0.18[b] ±0.01	79.1 ±0.3	79.0 ±0.2
Intestine	4.42 ±0.34	4.34 ±0.17	0.88 ±0.04	0.80[c] ±0.02	80.1 ±0.2	81.5 ±0.8
Kidneys	1.11 ±0.05	1.06 ±0.07	0.29 ±0.02	0.28 ±0.02	74.2 ±0.5	73.9 ±0.4
Thymus	0.38 ±0.03	0.14[b] ±0.01	0.08 ±0.01	0.03[b] ±0.003	78.1 ±0.3	77.7 ±0.8
Heart	0.44 ±0.01	0.42 ±0.01	0.10 ±0.004	0.10 ±0.003	77.6 ±0.6	76.3 ±0.4
Adrenals	---	---	0.005 ±0.0002	0.007[b] ±0.0004	---	---

	Control	TCDD
Body weight at dosing	108.4 ± 1.5	103.8 ± 3.3
Body weight at termination	166.8 ± 2.0	111.8 ± 6.3[b]

[a] TCDD dose = 45 μg/kg, i.p.

[b] Significantly different from control ($p < 0.05$)

[c] ($p < 0.07$)

the spleen (18%), intestine (9%), and thymus (55%). No changes were found in the dry weights of the pancreas, kidney or heart.

The pancreas showed a slight increase in wet weight per 100 g body weight. This was accomplished without a change in dry weight. The increased wet weight may be accounted for by a slight (3%) but statistically significant ($p = 0.034$) increase in water content of the pancreas in TCDD-treated rats. No other organs that were studied exhibited altered water content. No gross morphological changes were noted in any tissues at one week after TCDD other than a slight grayish coloration of the liver.

Adrenal and thymus weights were observed over time following administration of 90 μg TCDD/kg body weight (Table 7, 8). Thymic involution appeared as an early sign of toxicity with significant ($P < 0.05$) decreases occurring 1 day after TCDD treatment (Table 7). By day 5 after TCDD, thymus weight per body weight had decreased to 47% of the day-0 value (Table 8). By day 19, TCDD-treated rats exhibited thymus weight per body weight values of 29% of controls. TCDD administration produced little change in adrenal dry weight per body weight. In control animals, a 35% decrease in adrenal weight occurred by 19 days (Table 8), but TCDD-treated rats exhibited an adrenal weight per body weight ratio that was 135% of controls. No difference in water content of the adrenals appeared between TCDD-treated and control rats at day 19.

Table 7: Temporal effects of TCDD on thymus weights

Day	Thymus Wt/Body Wt[a]
0	0.074 ± 0.003
1	0.060 ± 0.005[b]
2	0.063 ± 0.005[b]
3	0.050 ± 0.005[c]
4	0.040 ± 0.002[c]

Rats received TCDD (90 μg/kg, i.p.) on day 0.

[a] Dry weight/100 g body weight

[b] $P < 0.01$ as determined by analysis of variance and the least significant difference test for multiple comparisons.

[c] $P < 0.001$

Table 8: Adrenal and thymus weights per 100 gm body weight following administration of TCDD

Day	Adrenal Weight[a]	Thymus Weight[a]
0	0.0054 ± 0.0002	0.070 ± 0.002
5	0.0051 ± 0.0006	0.033 ± 0.003
10	0.0054 ± 0.0003	0.018 ± 0.004
19	0.0059 ± 0.0009[b]	0.014 ± 0.003[b]
Control[c]	0.0035 ± 0.0001	0.040 ± 0.002

Rats were killed 0,5,10 or 19 days after administration of TCDD (90 µg/kg) body weight, i.p.). Values represent the mean ± S.E.M. for 4 rats.

[a] Dry weight per 100 g body weight

[b] N = 3, one rat died on day 18.

[c] Controls were killed on day 19 after receiving corn oil (1 ml/kg body weight, i.p.).

Effects of TCDD on Enzyme Induction

TCDD is recognized as a potent inducer of numerous enzymes, including the cytochrome P-448-associated aryl hydrocarbon hydroxylase (AHH). Since ornithine decarboxylase induction has been implicated as an early step in induction of cytochrome P-450-associated enzymes (Costa et al., 1976; Byus et al., 1976; Raunio and Pelkonen, 1979), ornithine decarboxylase activity was evaluated with respect to induction of the cytochrome P-448-associated AHH activity following administration of TCDD to rats.

Inhibition of Ornithine Decarboxylase Induction

No increase in ODC activity in rat liver was observed at 2, 4, 6, 12, 16, 24 or 72 h after i.p. administration of TCDD at doses ranging from 5-135 μg/kg. However, these same doses of TCDD produced large increases in renal and hepatic AHH activity, which may serve as a positive control for TCDD-biological activity in the rodent (Poland et al.,1974). Table 9 summarizes the effects of a single dose of TCDD (135 μg/kg) administered i.p. 16 h before termination on the activities of hepatic and renal ODC and AHH. The kidney exhibited higher basal ODC activity than did the liver. Interestingly, ODC activity in the kidney appeared to be inhibited at 16 h following TCDD administration, although a large S.E.M. precludes a definitive statement. Based on this observation, it was considered that TCDD may actually decrease ODC activity in the liver, but that such a decrease would be difficult to substantiate because of the low basal ODC activity.

Table 9: Ornithine decarboxylase and aryl hydrocarbon hydroxylase activities 16 h after TCDD

	Control	TCDD
Liver		
ODC[a]	1.0 ± 0.2	0.9 ± 0.3
AHH[b]	2360 ± 740	27,119 ± 3974[c]
Kidney		
ODC	10.1 ± 2.0	6.2 ± 2.2
AHH	38 ± 15	4665 ± 1492[c]

Rats received TCDD (135 μg/kg, i.p.) in acetone (1.5 ml/kg). Values represent the mean ± S.E.M. for 5 rats.

[a] pmol/min/mg protein

[b] pmol/min/g tissue

[c] Data significantly different from control ($p < 0.05$).

Therefore, the effects of TCDD pretreatment on ODC activity following administration of hormones or partial hepatectomy, regimens known to enhance hepatic ODC activity (Russell and Snyder, 1968; Raina and Janne, 1968; Russell and Durie, 1978; Russell and Snyder, 1969; Holtta, 1975), were investigated. Following partial hepatectomy, hepatic ODC activity increases in a biphasic manner with peak activities occurring at 4 and 16 h postsurgery (Russell and Snyder, 1968; Russell and Durie, 1978). In these experiments, similar elevations of ODC activity were found at both 4 h and 16 h. Therefore, we monitored TCDD effects at 16 h posthepatectomy. Rats pretreated with TCDD for 7 or 14 days and then hepatectomized exhibited 3- to 4-fold increases in ODC activity (Table 10) compared to 8- to 10-fold elevations by 16 h posthepatectomy in those not pretreated with TCDD. Thus, TCDD pretreatment partially inhibited the ODC activity induced by partial hepatectomy.

Since ODC is known to be induced either by direct nuclear action, as with certain steroid hormones, or via the activation of cAMP-dependent protein kinase(s) in response to increased intracellular levels of cAMP (Russell and Durie, 1978; Byus et al.,1976a; Richards, 1978; Gehring and Coffino, 1977; Panko and Kenney, 1971; Byus et al., 1976; Jungmann and Russell, 1977; Costa et al., 1975; Beck et al., 1972), the effects of TCDD on ODC activity induced by either pathway were investigated. The inducing agents were dexamethasone, which is believed to act directly at a nuclear site (Gehring and Coffino, 1977), and aminophylline, a phosphodiesterase inhibitor which acts by increas-

Table 10: Effects of TCDD on ornithine decarboxylase and aryl hydrocarbon hydroxylase activities 16 h after partial hepatectomy

TCDD Treatment	ODC[a]	AHH[b]
At hepatectomy		
Control	1.0 ± 0.2	2,360 ± 740
Hepatectomy alone	12.5 ± 2.2	1,200 ± 140
TCDD plus hepatectomy[c]	10.7 ± 1.6	12,000 ± 220[d]
7 days pretreatment		
Control	1.2 ± 0.1	2,400 ± 350
Hepatectomy alone	12.5 ± 2.1	3,500 ± 165
TCDD plus hepatectomy[e]	4.3 ± 1.0[d]	29,000 ± 1600[d]
14 days pretreatment		
Control	0.9 ± 0.1	3,480 ± 490
Hepatectomy alone	8.5 ± 0.9	6,700 ± 470
TCDD plus hepatectomy[e]	3.4 ± 1.0[d]	34,000 ± 6000[d]

Rats received TCDD at the times indicated prior to partial hepatectomy. Values represent the mean ± S.E.M. for 5 rats.

[a] pmol/min/mg protein

[b] pmol/min/g liver

[c] 30 μg/kg body weight, i.p.

[d] data significantly different from hepatectomy alone ($p < 0.05$).

[e] 5 μg/kg body weight, i.p.

ing intracellular levels of cAMP. Both agents produced substantial increases in ODC activity in control rats (Table 11). However, TCDD pretreatment totally abolished the ability of aminophylline to induce ODC (Table 11) and substantially reduced the induction of ODC in repsonse to dexamethasone. Administration of 90 μg TCDD/kg produced a 72% inhibition of ODC induction by dexamethasone within 24 h with greater than 80% inhibtion appearing on days 2 and 3 (Table 12).

When TCDD was administered at the time of partial hepatectomy, no difference in the 16-h ODC activity appeared between TCDD-treated and control animals (Table 10). This suggested that TCDD required a certain amount of time, following administration of 5 μg TCDD/kg, to produce inhibitory effects on hepatic ODC induction. In all cases, TCDD-treated animals exhibited AHH activity 5- to 10-fold higher than controls (Table 10).

Half Life of Ornithine Decarboxylase

To investigate whether increased degradation of ODC was involved in the decreased ODC response to growth simulation, the half life of hepatic ODC activity was evaluated in rats 1 week after treatment with TCDD (20 μg/kg). Figure 4 shows the rate of decline from peak activity 4 h after administration of dexamethasone. The semi-logarithmic plots show not only lower peak ODC activity, but a more rapid decline from peak activity in TCDD-treated rats relative to controls. The half-life values for hepatic ODC activities were calculated to be 40.8 min and 27.6 min for control and TCDD-treated rats, respectively.

Table 11: Effects of TCDD pretreatment on hepatic ornithine decarboxylase activity[a] induced by aminophylline or dexamethasone

	Control	TCDD
Saline	0.9 ± 0.05	---
Aminophylline	13.1 ± 1.2	1.4 ± 0.3
Dexamethasone	11.0 ± 1.5	4.8 ± 1.2[b]

Rats received TCDD (5 μg/kg, i.p.) in acetone:corn oil (1:17) one week prior to i.p. challenge with saline (1 ml/kg), aminophylline (0.2 mmole/kg) or dexamethasone (0.5 mg/kg). Values represent the mean ± S.E.M. for 5 rats.

[a] pmol/min/mg protein

[b] Significantly different from control ($p < 0.05$).

Table 12: Temporal effects of TCDD pretreatment on hepatic ornithine decarboxylase activity induced by dexamethasone

Days after TCDD Treatment	ODC Activity[a]
0	30.7 ± 2.3
1	8.6 ± 1.9[b]
2	4.4 ± 0.4[b]
3	5.4 ± 0.5[b]
Saline Control[c]	0.6 ± 0.1

Rats received a single i.p. dose of TCDD (90 μg/kg) 0, 1, 2 or 3 days prior to dexamethasone challenge (0.5 mg/kg, i.p.). Values represent the mean ± S.E.M. for 4 rats.

[a] picomoles/min/mg cytosolic protein

[b] Significantly different from controls ($P < 0.05$).

[c] Rats received 0.9% saline (1 ml/kg, i.p.) 4 h before termination.

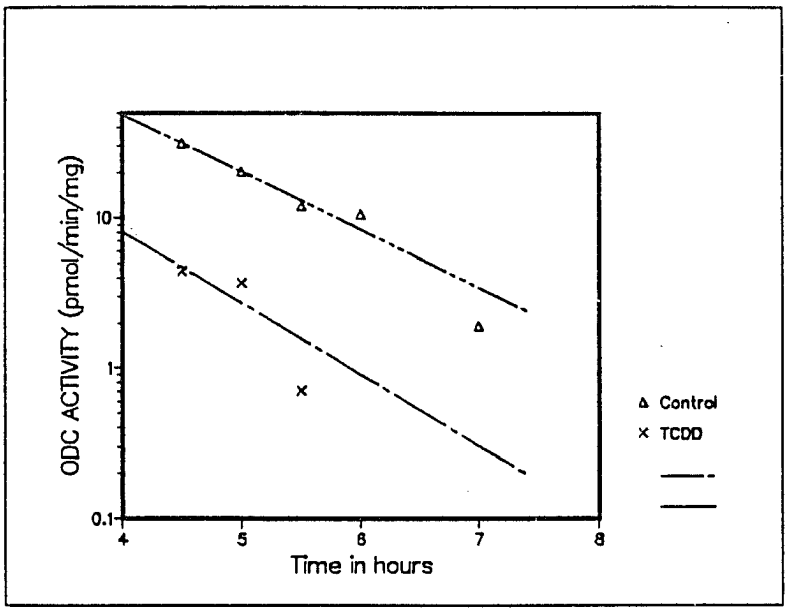


Figure 4: Effects of TCDD on the natural decline of ODC activity following adminstration of dexamethasone. The half-life values were calculated to be 40.8 min and 27.6 min for control and TCDD-treated rats, respectively.

Since a more rapid decline of ODC activity in TCDD-treated rats could result from decreased enzyme synthesis, the half life of ODC activity was determined after administration of cycloheximide, a protein synthesis inhibitor (Fig. 5). Three days after receiving 90 μg TCDD/kg, a regimen shown to inhibit ODC stimulation (Table 12), rats were challenged with dexamethasone. Cycloheximide was administered 4 h after dexamethasone treatment and the rate of decline of hepatic ODC activity was measured (Fig. 5). The half life of ODC was calculated to be 15.6 ± 5.3 min and 4.2 ± 2.8 min in control and TCDD-treated rats, respectively.

Ah-Receptor Binding and Inhibition of Ornithine Decarboxylase

Since specific physiological and toxicological responses to TCDD have been shown to segregate with Ah-receptor binding, it was of interest to investigate whether inhibition of ODC induction was receptor dependent. Two hexachlorobiphenyl isomers were selected for this experiment based on their ability to compete for the Ah receptor. 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-HCB), an efficient inducer of cytochrome P-450-associated enzymes such as aminopyrine N-demethylase, exhibits little AHH-inducing capacity and low binding affinity for the Ah receptor. 3,3',4,4',5,5'-hexachlorobiphenyl (3,4,5-HCB) has been shown to compete strongly for the Ah receptor and to induce AHH activity with little effect on cytochrome P-450-associated enzymes (Poland and Glover, 1977).

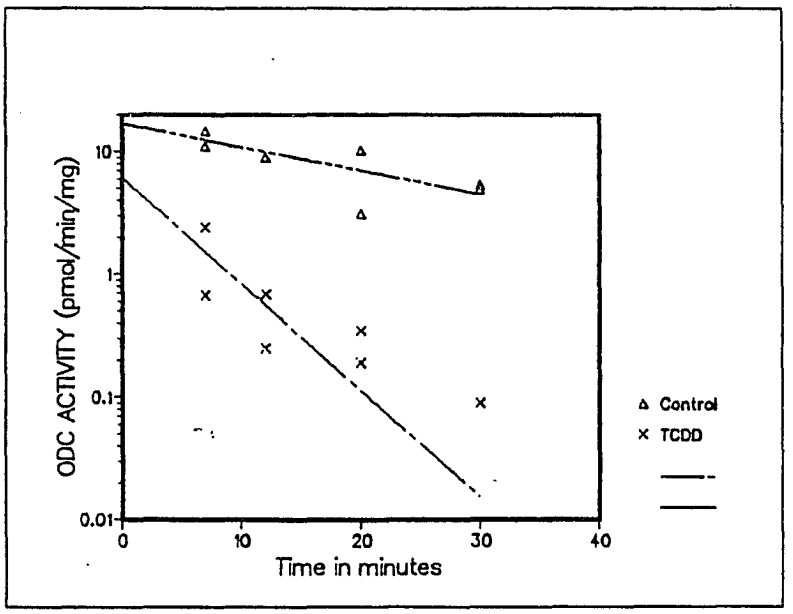


Figure 5: Effects of TCDD on the decline of ODC activity after administration of cycloheximide, 4 hours after dexamethasone. Half-life values were calculated to be 15.6 min and 4.2 min for control and TCDD-treated rats, respectively.

Table 13 shows the effects of pretreatment with 2,4,5-HCB and 3,4,5-HCB on AHH and aminopyrine N-demethylase activities and on the ODC response to dexamethasone. A clear segregation of effects on enzyme activities resulted from pretreatment with the different PCB isomers. Rats pretreated with 2,4,5-HCB exhibited significant stimulation of the N-demethylase activity with little effect on AHH activity, while rats pretreated with 3,4,5-HCB exhibited a significant increase in AHH activity with decreased N-demethylase activities. Differential effects were also noted in the ODC response to dexamethasone challenge. Animals pretreated with corn oil or 2,4,5-HCB responded with large increases in ODC activity, while pretreatment with 3,4,5-HCB resulted in an 83% inhibition of the ODC response when compared to control animals.

Inhibition of RNA Polymerase I Induction

Because increased ODC activity has been correlated to a subsequent increase in RNA polymerase I activity (Manen and Russell, 1975, 1977a, 1977b; Haddox and Russell, 1981), the effect of TCDD administration on hepatic RNA polymerase I activity was determined at times when ODC activity was inhibited. RNA polymerase I activities in unstimulated rat liver or following partial hepatectomy (Fig. 6) or dexamethasone (Fig. 7) were evaluated one week after TCDD. When hepatic ODC activity was found to be inhibited by TCDD, 20-50% decreases in RNA polymerase I activity also were detected. RNA polymerase I activities in unstimulated rat liver were essentially the same for TCDD-treated and control animals (Fig. 6A, 7A).

Table 13: Effects of two hexachlorobiphenyl isomers on ornithine decarboxylase, aryl hydrocarbon hydroxylase and aminopyrine N-demethylase activities

	ODC[a]	AHH[a]	N-Demethylase[b]	%Body weight Gain
Corn Oil and Saline	0.26 ± 0.02	53.4 ± 5.4	0.87 ± 0.04	---
Corn Oil and Dexamethasone	12.4 ± 2.3	40.0 ± 5.6	0.75 ± 0.05	35.0 ± 1.0
2,4,5-HCB[c] and Dexamethasone	16.5 ± 3.9	79.7 ± 9.9	1.84 ± 0.03[d]	28.5 ± 1.7
3,4,5-HCB[e] and Dexamethasone	2.1 ± 0.8[f]	256.9 ± 44.2[d]	0.62 ± 0.04[g]	4.6 ± 5.8[d]

Rats received 2,4,5-HCB (50 mg/kg), 3,4,5-HCB (30 mg/kg) or corn oil (4ml/kg) i.p. 1 week before dexamethasone challenge (0.5 mg/kg, i.p.). The rats were killed 4 h after receiving dexamethasone. Values represent the mean ± S.E.M. for 5 rats.

[a] pmoles/min/mg protein

[b] nmoles/min/mg protein

[c] 2,2',4,4',5,5'-Hexachlorobiphenyl

[d] Significantly different from corn oil and dexamethasone control ($p < 0.001$) as determined by analysis of variance and the least significant difference test for multiple comparisons.

[e] 3,3',4,4',5,5'-Hexachlorobiphenyl

[f] $P < 0.005$

[g] $P < 0.05$

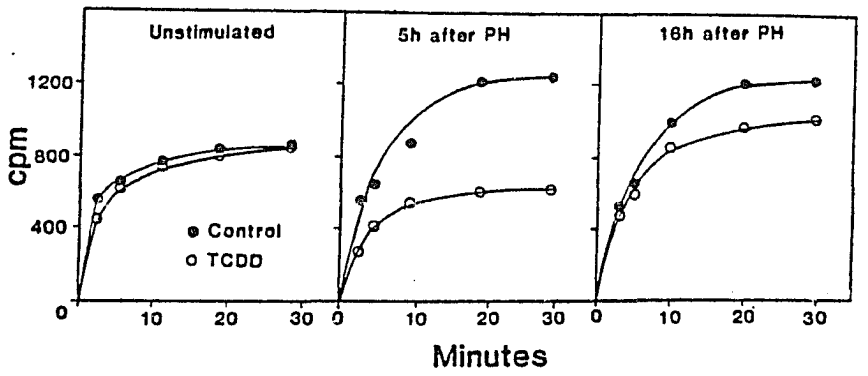


Figure 6: RNA polymerase I activities 1 week after TCDD administration (5 ug/kg). Values represent the time-dependent incorporation of [^{3}H]uridine into rRNA in rat liver (A) without stimulation, (B) 5 h after partial hepatectomy (PH), and (C) 16 h after partial hepatectomy.

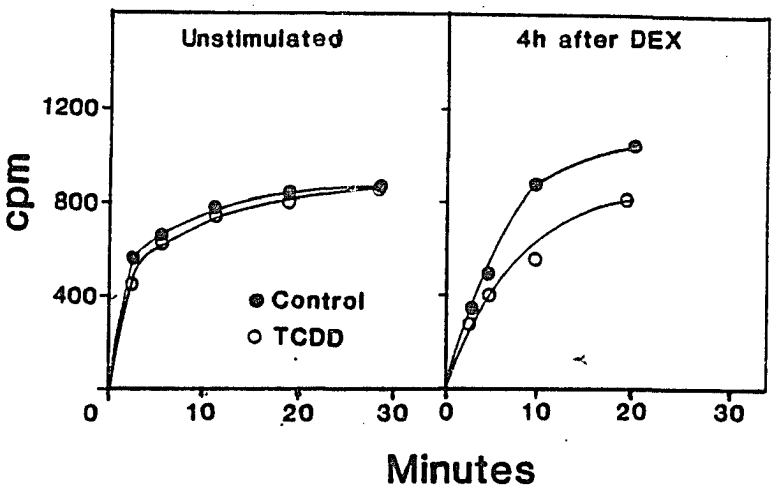


Figure 7: RNA polymerase I activities 1 week after TCDD administration (5 ug/kg). Values represent the time-dependent incorporation of [^{3}H]uridine into rRNA in rat liver (A) without stimulation and (B) 4 h after dexamethasone (DEX) [0.5 mg/kg, i.p.].

Growth Factors and Liver Regeneration

One week after treatment with 5 μg TCDD/kg, the liver contained lower concentrations of the growth related polyamines, putrescine and spermidine (Table 14). However, no significant differences in liver protein, DNA, RNA or spermine were found between TCDD-treated and control animals at one week. Since TCDD pretreatment decreased the basal levels of putrescine and spermidine and inhibited the induction of the growth related enzymes, ODC and RNA polymerase I, the effects of TCDD pretreatment on the rate of liver regeneration were investigated. No difference in the rate of liver regeneration appeared between TCDD-treated and control animals during the 9-day observation period following 2/3 hepatectomy (Table 15).

Hypothyroidism in TCDD-Treated Rats

TCDD-treated rats had decreased serum levels of T_3 and T_4 and a decreased free thyroxine index (Table 16). Following administration of 45 μg TCDD/kg, the plasma level of T_4 was decreased to 33% of control. Although the decrease in plasma T_3 level was less pronounced (65% of control), the difference approached statistical significance ($p = 0.057$). The decreased free thyroxine index indicated that the decreased plasma T_3 and T_4 values were due to decreased serum levels of the hormones, as opposed to an alteration in protein binding of the hormones (Clark and Horn, 1965).

Following administration of 90 μg TCDD/kg, serum levels of T_4 were significantly decreased by 5 days and remained depressed through-

Table 14: Liver content of protein, DNA, RNA and polyamines one week after TCDD

	Control	TCDD
Liver weight	4.6 ± 0.2	5.8 ± 0.1[d]
Total protein[b]	223 ± 3.2	210 ± 6.5
Total DNA[b]	2.6 ± 0.2	2.7 ± 0.1
Total RNA[b]	9.2 ± 0.6	8.7 ± 0.6
Total polyamines[c]		
Putrescine	11.0 ± 2.5	5.7 ± 1.0[d]
Spermidine	942 ± 130	644 ± 76[d]
Spermine	612 ± 73	687 ± 101

Rats received TCDD (5 μg/kg, i.p.) in acetone:corn oil (1:17). Values represent the mean ± S.E.M. for 5 rats.

[a] g/100 g body weight

[b] mg/g wet weight of liver

[c] nmol/g wet weight of liver

[d] Significantly different from controls ($P < 0.05$).

Table 15: Effects of TCDD pretreatment on liver regeneration following partial hepatectomy

	Liver Wt/Body Wt[a]		Thymus Wt/Body Wt[a]	
Day	Control	TCDD	Control	TCDD
2	1.15 ± 0.01	1.26 ± 0.07	0.31 ± 0.01	0.15 ± 0.09
4	1.69 ± 0.03	1.75 ± 0.11	0.23 ± 0.01	0.17 ± 0.06
9	1.97 ± 0.14	2.18 ± 0.07	0.29 ± 0.05	0.17 ± 0.01

Rats were partially hepatectomized one week after receiving a single injection of TCDD (5 μg/kg, i.p.). The rats were killed 2, 4 or 9 days after hepatectomy. Values represent the mean ± S.E.M. for 5 rats.

[a] Organ weight per 100 g body weight

Table 16: Effects of TCDD administration on the serum levels of thyroid hormones

	Control	TCDD
Triiodothyronine (T_3, ng/dl)	100.4 ± 10.0	65.3 ± 12.2[a]
Thyroxine (T_4, µg/dl)	4.3 ± 0.2	1.4 ± 0.2[b]
Free Thyroxine Index	7.2 ± 0.4	2.4 ± 0.3[b]

Rats received TCDD (45 µg/kg, i.p.) one week before termination. Values represent the mean ± S.E.M. for 5 rats.

[a] Data differ from controls, ($p = 0.057$)

[b] Data differ significantly from controls, ($p < 0.001$)

out the 19-day study (Table 17). Although control levels of serum T_4 were decreased by day 19, they were still significantly higher than values from TCDD-treated animals. Table 18 shows that serum T_4 levels were depressed nearly 50% by 2 days following administration of 90 μg TCDD/kg to the rats. Although serum glucose levels were significantly decreased by 5 days following administration of 90 μg TCDD/kg (Table 17), they were not decreased by TCDD treatment during the 4 day study (Table 18). Serum T_3 levels showed a slight, but not statistically significant, decrease during 19 days following TCDD treatment (Table 17).

Hypoglycemia In TCDD-Treated Rats

Table 19 shows serum levels of glucose and immunoreactive insulin and glucagon in rats one week after administration of 45 μg TCDD/kg. In TCDD-treated rats, serum glucose was decreased to 66% of control and the serum insulin concentration was decreased to 52% of control. Pancreatic insulin levels also were decreased to 76% of control following TCDD administration. There was no change in the serum glucagon levels, as determined by immunoreactivity.

Somatostatin In TCDD-Treated Rats

Since somatostatin has been shown to decrease serum levels of glucose, insulin and glucagon (Leblanc et al., 1975; Luft et al., 1978), serum and tissue concentrations of somatostatin were measured. No changes in somatostatin levels were found in the serum, pancreas or liver one week after TCDD treatment (Table 20). However, TCDD-treated

Table 17: Effects of TCDD administration on serum levels of thyroxine (T_4), triiodothyronine (T_3) and glucose

Day	T_4	T_3	Glucose
0	4.7 ± 0.2	128.9 ± 7.8	177.0 ± 12.4
5	1.1 ± 0.1[b]	128.2 ± 9.5	116.3 ± 4.6[b]
10	1.0 ± 0.2[b]	70.0 ± 21.7	106.4 ± 5.6[b]
19	1.7 ± 0.1[b]	80.6 ± 43.9	97.1 ± 13.7[b]
Control[c]	3.2 ± 0.2[b]	109.9 ± 14.3	156.1 ± 8.8

[a] Rats were killed 0, 5, 10 or 19 days after administration of a single i.p. dose of TCDD (90 µg/kg body weight).

[b] Data differ significantly from day-0 value as determined by 1-way analysis of variance and the least significant difference test ($P < 0.05$).

[c] Controls were killed 19 days after receiving corn oil (1 ml/kg, i.p.).

Table 18: Temporal effects of TCDD administration on serum levels of thyroxine (T_4) and glucose

Day	T_4[a]	Glucose[b]
0	4.0 ± 0.5	152.5 ± 11.3
1	3.5 ± 0.4	153.0 ± 7.3
2	2.1 ± 0.1[c]	154.6 ± 8.8
3	1.6 ± 0.1[c]	131.7 ± 6.1
4	1.8 ± 0.3[c]	143.0 ± 4.4

Rats were killed 0, 1, 2, 3 or 4 days after receiving a single i.p. dose of TCDD (90 µg/kg). Values represent the mean ± S.E.M. for 4 rats.

[a] µg T_4/dl serum

[b] mg glucose/100 ml serum

[c] Data differ significantly from day-0 value as determined by 1-way analysis of variance and the least significant difference test ($P < 0.05$).

Table 19: TCDD induced alteration in serum and pancreatic glucose, insulin and glucagon

	Control	TCDD
SERUM		
GLUCOSE[a]	150.0 ± 3.8	99.0 ± 8.1[e]
INSULIN[b]	8.3 ± 0.8	4.3 ± 1.4[e]
GLUCAGON[c]	110.0 ± 26.4	115.2 ± 46.0
PANCREAS		
INSULIN[d]	654.0 ± 20.0	500.0 ± 52.0[e]

Rats received TCDD (45 μg/kg, i.p.) one week before termination. Values represent the mean ± S.E.M. for 5 rats.

[a] mg/100 ml serum

[b] μUnits/ml

[c] pg/ml

[d] μUnits/mg protein

[e] Significantly different from control ($P < 0.05$)

Table 20: Effects of TCDD on somatostatin concentration in serum and tissues

	Control	TCDD
SERUM[a]	907.2 ± 31.8	875.0 ± 75.8
LIVER[b]	0.16 ± 0.01	0.17 ± 0.01
PANCREAS[c]	1.91 ± 0.28	1.65 ± 0.49
GASTRIC ANTRUM[c]	2.74 ± 0.47	0.81 ± 0.23[d]

Rats received TCDD (45 μg/kg, i.p.) one week before termination. Values represent the mean ± S.E.M. for 5 rats.

[a] pg somatostatin/ml serum

[b] ng somatostatin/gram liver

[c] ng somatostatin/mg protein

[d] Significantly different from control (P = 0.006).

rats exhibited somatostatin levels decreased to 30% of control in the gastric antrum, an area with the highest known extrahypothalamic concentration of somatostatin (Elde et al., 1978).

Paired Feeding Study

To determine whether hypophagia was involved in endocrine changes noted following TCDD treatment, a paired-feeding study was performed in which pair-fed control rats received only the amount of food eaten by TCDD-treated rats. Table 21 shows the effects of TCDD treatment and paired feeding on thyroid status in rats. Serum T_4 levels were sigificantly decreased 1 week after administration of TCDD at 45 μg/kg. Pair-fed rats exhibited no decrease in serum T_4 levels relative to ad libitum controls. No statistically significant differences appeared in the serum T_3 levels or in thyroid weight per body weight in either the TCDD-treated or pair-fed rats.

TCDD-treated rats exhibited small, but significant, decreases in serum glucose levels and strongly depressed insulin levels during the 1-week paired-feeding study (Table 22). Although the pair-fed rats did not exhibit decreased serum glucose levels, the insulin levels were greatly reduced relative to ad libitum controls. There was no significant difference in serum insulin levels between TCDD-treated and pair-fed rats at 1 week.

Table 21: Effects of TCDD and paired feeding on thyroid weight and serum thyroxine and triiodothyronine levels

	Thyroxine[a]	Triiodothyronine[b]	Thyroid Wt/Body Wt[c]
Ad Lib Control	5.46 ± 0.40	90.8 ± 4.8	1.49 ± 0.06
Pair-Fed Control	5.02 ± 0.18	89.7 ± 3.5	1.38 ± 0.04
TCDD	2.25 ± 0.10[d]	74.9 ± 8.7	1.62 ± 0.10

Rats received TCDD (45 μg/kg, i.p.) or corn oil (1 ml/kg, i.p.) 7 days before they were killed. Values represent the mean ± S.E.M. for 4 to 8 rats.

[a] μg/100 ml serum

[b] ng/100 ml serum

[c] Thyroid weight/100 g body weight

[d] Statistically different from ad libitum control as determined by analysis of variance and the least significant difference test ($P < 0.001$).

Table 22: Effects of TCDD and paired feeding on serum glucose and insulin levels

	Glucose[a]	Insulin[b]
Ad lib Control	165.4 ± 6.8	63.1 ± 6.3
Pair-Fed Control	155.7 ± 8.8	25.2 ± 4.3[c]
TCDD	130.0 ± 11.1[c]	16.3 ± 2.5[c]

Rats received TCDD (45 µg/kg, i.p.) or corn oil 7 days before they were killed. The pair-fed rats received the amount of food eaten by the TCDD-treated rats. Values represent the mean ± S.E.M. for 6 to 8 rats.

[a] mg glucose/100 ml serum

[b] µUnits insulin/100 ml serum

[c] Statistically different from ad lib control as determined by analysis of variance and the least significant difference test. ($P < 0.01$)

DISCUSSION

Perhaps the most troubling aspect of investigations into the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been the lack of a demonstrable effect in any organ which could account for the death of the animal. Although hepatic necrosis occurs in some species following administration of high doses of TCDD (Gupta et al., 1973; Schwetz et al., 1973; Kociba et al., 1976; Henck et al., 1981), this cannot constitute the total toxicity since in the guinea pig, the species most sensitive to TCDD [LD_{50} = 0.6 ug/kg] (Schwetz et al., 1973), there is little or no liver damage in response to TCDD (Gupta et al., 1973). Thymic atrophy is the most consistent organ effect reported in literature (Gupta et al., 1973). Similar effects were found in the present study. Although this atrophy leads to immunosuppression (Vos et al., 1973; Vos, 1977), it should not be lethal in itself as evidenced by the reported viability of athymic animals (Pantelouris, 1973).

The present study has elucidated several metabolic and endocrine alterations which may help to discern the mechanism(s) of the TCDD-induced "wasting" syndrome, which characterizes a lethal dose of TCDD in laboratory animals. A single i.p. injection of TCDD produced hypothermia and reduced growth rate which were accompanied by reduced responsivity of ODC and RNA polymerase I activity to hormonal stimulation. In addition, reduced levels of serum thyroid hormones and

serum and tissue levels of insulin and somatostatin were manifest in rats following administration of TCDD.

Since ornithine decarboxylase (ODC) induction has been implicated as a prerequisite for induction of the cytochrome P-450-associated monooxygenase system (Costa et al., 1976; Russell et al., 1976; Byus et al., 1976; Manen et al., 1978; Raunio and Pelkonen, 1979; Nebert et al., 1980), the effects of TCDD on ODC were investigated with respect to the cytochrome P-448-associated aryl hydrocarbon hydroxylase (AHH) activity. The lack of ODC induction prior to AHH induction suggests that ODC induction may not be required for in vivo AHH induction following administration of TCDD. Recently, it has been reported by Raunio et al. (1982) that benz(a)anthracene induced AHH activity in cultured cells was not affected by the presence of, or pretreatment with, difluoromethylornithine, a specific inhibitor of ODC activity. Kano and Nebert (1981) also failed to find a relationship in vitro between ODC and AHH induction. The authors concluded that AHH induction in vitro does not require ODC activity or ongoing polyamine synthesis.

The ability of TCDD to inhibit hormonally induced ODC activity depended upon a pretreatment period. TCDD administration at the same time as dexamethasone, aminophylline, or partial hepatectomy did not result in the ODC inhibition in liver that was detected in animals pretreated for one week with a single dose of TCDD. The requirement for this pretreatment period suggests that ODC inhibition by TCDD was not the result of direct inhibition by TCDD, but resulted from an alteration in the responsiveness of the liver to hormonal challenge.

The fact that TCDD pretreatment inhibited ODC activity induced either by direct nuclear (dexamethasone) or cAMP-mediated (aminophylline) events, suggests that this phenomenon relates to marked alteration in hormone responsiveness of the animal rather than to a selective effect on a particular hormonal-ODC induction pathway.

The polyamine, putrescine has been shown to strongly inhibit ODC acitivity (Janne and Hölttа, 1974; Clark and Fuller, 1975; Pösо and Jänne, 1976). However, in the present study, putrescine levels were decreased in the liver at a time after TCDD treatment when ODC induction was inhibited. Therefore, feedback inhibition by increased intracellular levels of free putrescine may be ruled out as the mechanism of ODC inhibition by TCDD pretreatment.

TCDD did not significantly alter the basal levels of liver protein, DNA or RNA at 1 week posttreatment. These data support our findings that the basal RNA polymerase I activity was essentially unaltered in unstimulated rat liver at 1 week after treatment with 5 μg/kg TCDD. However, the decreased putrescine and spermidine levels reflect decreased polyamine biosynthesis or increased turnover of the polyamines following TCDD administration. These results suggest that TCDD had altered intracellular regulation prior to stimulation with hormones or partial hepatectomy.

Since TCDD treatment resulted in large increases in AHH activity, it may be suggested that the subsequent inhibitory effects on ODC and RNA polymerase I activities could be due to increased metabolism (inactivation) of aminophylline, dexamethasone or endogenous

hormones involved in ODC induction following partial hepatectomy. This seems unlikely, however, since administration of aminophylline 24 h after TCDD administration, a time when AHH activity is well induced, resulted in none of the inhibitory effects noted 1 week after TCDD (data not included).

Similar results have been reported by Bulger and Kupfer (1976) in the rat uterus. They reported that the treatment of ovariectomized female rats with 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane (o,p'DDT) resulted in an induction of ODC activity by 6-7 h. However, 48-h pretreatment with o,p'DDT resulted in an inhibition of rat uterine ODC induction by subsequent administration of estradiol, tamoxifen, or o,p'DDT. They also provided evidence that increased activity of the monooxygenase system was not responsible for the reported ODC inhibition. Pretreatment of rats with tamoxifen, which does not induce the monooxygenase system, resulted in an inhibition of ODC induction by subsequent treatment with o,p'DDT or estradiol (Bulger and Kupfer, 1976). Furthermore, p,p'DDE, a structural analog of o,p'DDT which induced the hepatic monooxygenase system, did not possess the inhibitory effects of o,p'DDT on ODC induction (Bulger and Kupfer, 1977). Therefore, it appears that a factor(s) other than increased metabolism (inactivation) of the inducing agent was responsible for the inhibition of ODC induction produced by o,p'DDT and TCDD pretreatment in rat uterus and liver, respectively.

To investigate whether increased degradation of ODC was involved in the inhibition of the ODC response to growth stimulation, the

half life of hepatic ODC activity was evaluated in rats stimulated with dexamethasone 1 week after pretreatment with TCDD. Following stimulation with dexamethasone, ODC activity declined from peak activity at 4 h with a half life of 40.8 min and 27.6 min in control and TCDD-treated rats, respectively. When cycloheximide, a protein synthesis inhibitor, was administered 4 h after dexamethasone, the half life of ODC was 15.6 $\pm$ 5.3 min and 4.2 $\pm$ 2.8 min in control and TCDD-treated rats, respectively. These data suggest that the inhibitory effects of TCDD on ODC induction may, at least in part, result from more rapid inactivation, possibly via posttranslational modification, of ODC

Since specific physiological and toxicological responses to TCDD have been shown to segregate with Ah-receptor binding (Thomas, 1972; Poland and Glover, 1975; Niwa et al., 1975; Poland and Glover, 1980; Poland and Knutson, 1982), it was of interest to investigate whether inhibition of ODC induction was receptor dependent. It was found that pretreatment with 3,3',4,4',5,5'-hexachlorobiphenyl (3,4,5-HCB), which competes effectively for the Ah receptor protein, strongly inhibited ODC induction by dexamethasone, whereas pretreatment with 2,2,'4,4,5,5'-hexachlorobiphenyl (2,4,5-HCB), which does not compete for the receptor (Poland and Glover, 1977), did not inhibit ODC. The segregation of the inhibitory effects with the ability to compete for the Ah receptor suggests that the decreased ODC response to hormonal stimulation may result from specific Ah-receptor-mediated gene expression following administration of TCDD or 3,4,5-HCB.

In spite of the alterations in growth factors noted above, TCDD treatment produced hepatic hypertrophy and permitted liver regeneration to proceed at a normal rate following partial hepatectomy. Future investigation of long-term effects of these changes will be important in understanding the delayed metabolic toxicity of TCDD.

In light of decreased hormonal responsivity in rats pretreated with TCDD, the question arises as to the effects this may have on circulating hormonal levels. Biochemical studies have suggested that the toxicity of TCDD may involve a number of metabolic alterations (Gasiewics et al., 1980; Swift et al., 1981), and since TCDD lacks toxicity in cell culture (Knuston and Poland, 1980), the involvement of the endocrine system in TCDD toxicity is strongly suggested. In the present study, a single i.p. injection of TCDD produced hypothermia and reduced growth rate along with reduced levels of serum thyroid hormones and serum and tissues levels of insulin and somatostatin following administration of TCDD to rats.

TCDD administration has been shown to result in hypophagia in laboratory animals (Gasiewics and Neal, 1979; Seefeld et al., 1982). Therefore, metabolic indices were evaluated in pair-fed rats, which were allowed only the amount of food eaten by the TCDD-treated rats. Since pair-fed rats gained only the amount of body weight gained by TCDD-treated rats (Table 5), this study supports recent findings by Seefeld et al. (1982) that body weight loss following administration of TCDD may be accounted for by hypophagia.

Body temperatures were significantly decreased within 2 days after administration of TCDD in a dose-related fashion. The temporal correlation between hypothermia and hypothyroidism suggests that TCDD-induced hypothermia may result from declining thyroid hormone levels following administration of TCDD. Although starvation has been shown to produce hypothyroidism (DeGroot et al., 1977), hypophagia cannot account solely for the TCDD-induced hypothyroidism or hypothermia since pair-fed rats appeared capable of maintaining both thyroid function and body temperature (Table 5, 21).

Bastomsky (1977) reported that rats exhibited decreased serum T_4 levels with increased biliary excretion of T_4 as a glucuronide conjugate 9 days after treatment with TCDD (25 μg/kg), but in his study, T_3 levels were significantly increased. Therefore, it was suggested that decreased serum T_4 levels may result from increased glucuronide formation following TCDD administration, but that T_3 may be transformed by a different pathway. In the present study, T_3 levels were not decreased as dramatically as T_4, but nevertheless, tended to fall below control values ($P = 0.07$). It remains unclear why our results differ from those of Bastomsky in this regard. One possibility is that our rats suffered an impairment of thyroid function not manifested in the rats used by Bastomsky. This could result from inherent differences in the strains of rats and/or doses of TCDD employed.

Decreased serum and pancreatic insulin levels rule out hyperinsulinemia as a cause of hypoglycemia. These results combined with normal glucagon levels suggest that TCDD-induced hypoglycemia is not

the result of altered pancreatic function. The pair-fed rats experienced hypoinsulinemia as did the TCDD-treated rats, implying that the hypoinsulinemia produced by TCDD administration resulted from hypophagia. Hypophagia cannot account for TCDD-induced hypoglycemia, however, since pair-fed rats maintained their serum glucose levels. Furthermore, Gasiewics et al. (1980) have reported that TCDD administration produced hypoglycemia in rats fed total parenteral nutrition as well as in rats fed ad libitum. Therefore, a factor(s) other than hypophagia must be considered when proposing a mechanism for TCDD-induced hypoglycemia. Since hypothyroidism preceded hypoglycemia (Table 18) and since thyroid hormone has stimulatory effects on gluconeogenesis, the involvement of hypothyroidism in the hypoglycemic response to TCDD cannot be ruled out.

Somatostatin has been shown to decrease serum levels of glucose and numerous hormones including glucagon, insulin and thyroid hormones (LeBlanc et al., 1975; Luft et al., 1978; Weeke et al., 1980). Therefore, we investigated the effects of TCDD on serum and tissue levels of somatostatin as a possible route for TCDD-induced hypoglycemia and endocrine alterations. However, somatostatin did not appear to be responsible for the glucoregulatory changes noted in this study, since no changes in serum or pancreatic somatostatin levels were detected following TCDD administration.

Although the effects of hypophagia on somatostatin were not elucidated in this study, other studies have shown starvation to result in decreased gastric and pancreatic secretion, and decreased

hepatic-portal venous levels, of somatostatin in rats (Shapiro et al., 1980; Kazumi et al., 1980; Seino et al., 1980; Tsuda et al., 1981). Although TCDD administration did not lower portal venous or pancreatic levels of somatostatin, it significantly decreased somatostatin in the gastric antrum. It remains unresolved whether the effects of TCDD on gastric-antral somatostatin were secondary to hypophagia or mediated through nonstarvation-related alterations in gut physiology.

Figure 8 shows a tentative model for the metabolic alterations induced by TCDD. According to this model, altered thyroid hormone levels resulting from TCDD-induced gene expression, along with decreased responses of ODC and RNA polymerase I activities to hormonal stimulation, impair the animal's ability to maintain homeostasis and properly respond to stress. T_3 has been shown to stimulate ODC and RNA polymerase I activities (Combest et al., 1978; Combest and Russell, 1980; Igarashi et al., 1980; Berti et al., 1981), and T_3 administration to rats has been shown to restore RNA polymerase I activity which had been reduced by thyroidectomy (Igarashi et al., 1980). However, the involvement of hypothyroidism in the decreased responsivity of ODC and RNA polymerase I activities in the present study is questionable, since T_3 was only slightly decreased following administration of TCDD. Also, T_3 administration to rats prior to dexamethasone challenge, in this study, failed to restore ODC and RNA polymerase I responsivity (Data not presented).

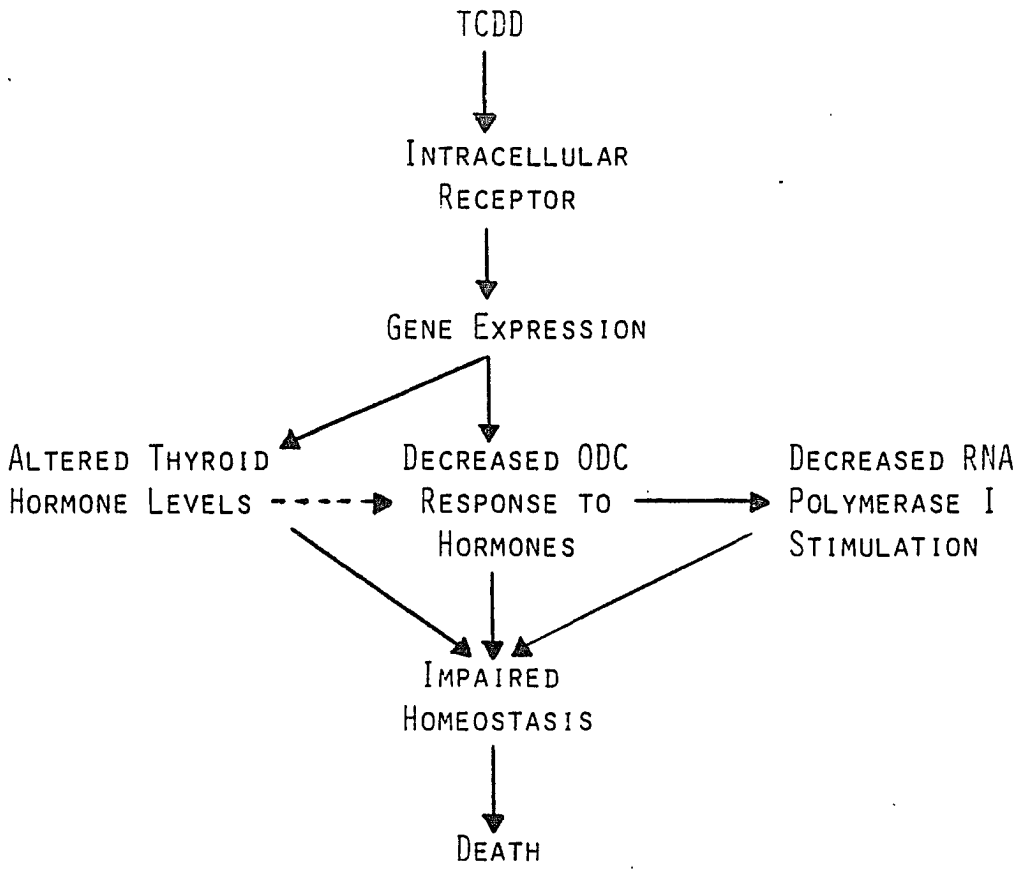


Figure 8. Hypothetical model for TCDD-induced alterations in metabolism and hormone levels.

Since thyroid hormones strongly influence the release and activity of numerous other hormones, including growth hormone, 5 alpha-hydroxytestosterone, cortisol, insulin, glucagon and catecholamines (Hoch, 1962; Caldwell and Fain, 1971; West et al., 1975; Utiger, 1979; Oppenheimer, 1979; Fain, 1981), TCDD-induced hypothyroidism may result in more generalized endocrine imbalances. Previously, van Logten et al. (1980) reported that growth hormone therapy restored body and thymus weights in hypophysectomized control rats but not in rats pretreated with TCDD. The above model then suggests that the lack of response to growth hormone may have resulted from hypothyroidism in TCDD-treated rats. Additionally, TCDD-induced hypothyroidism may alter the concentrations and activities of catecholamines. Seefeld et al. (1982a, 1982b) have reported that TCDD administration reduces energy expenditure and oxygen consumption which would be consistent with decreased catecholamine activity. Furthermore, the hypothermia and hypoglycemia noted in this study add further circumstantial evidence for decreased catecholamine activity following TCDD administration.

The results of this study indicate that TCDD is capable of producing marked thyroregulatory, thermoregulatory and glucoregulatory alterations in rats. Since TCDD pretreatment also resulted in decreased liver response to hormonal stimulation, as reflected by decreased ornithine decarboxylase and RNA polymerase I activities, these results strongly suggest that chronic TCDD toxicity involves severe endocrine alterations. Furthermore, characterization of the endocrine effects in response to TCDD will be important in the elucidation of the etiology of TCDD-induced metabolic alterations.

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