# EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON MOBILIZATION AND HEPATIC CATABOLISM OF LIPIDS

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## ABSTRACT

A single 20 μg/kg dosage of TCDD caused, within 3 days, a significant mobilization of depot fat into the plasma compartment resulting in 1.5-2.8 fold increase in plasma free-fatty acid concentrations. With respect to the fate of mobilized fatty acids, the same treatment of TCDD caused a 32% inhibition ($p<0.008$) of the rate of hepatic oleate oxidation without significantly affecting the rate of fatty acid esterification. In contrast, hepatic ketogenic rate was markedly stimulated by 85% ($p<0.001$). These results support the concept that although the beta-oxidation pathway of the fatty acids must be working normally, their complete oxidation to $CO_2$ via the TCA cycle is impaired. At the same time, TCDD seems to preferentially divert the acetyl CoA generated from the beta-oxidation of fatty acids to the ketogenic pathway.

## KEY WORDS:

TCDD; Fat mobilization; Fat oxidation; Fatty acid esterification; Ketogenesis.

## INTRODUCTION

TCDD is the most toxic contaminant of the herbicides used as defoliants. Lipid abnormalities have been reported in workers exposed to TCDD (Poland et al., 1971; Oliver, 1979; Walker and Martin, 1979; Martin, 1984). Elegant studies by Albro et al. (1978) and McConnell et. al. (1978) have clearly shown that a sublethal dose of TCDD causes a transient increase in serum triglycerides and free fatty acids and a decrease in sterol esters.

Our recent studies (Lakshman et.al., 1986) have established that the bulk of administered TCDD is deposited in the liver and adipose tissues with half lives of 5.3 and 7.6 weeks, respectively. Human studies (Gross et.al. 1984; Patterson et.al. 1986; Sampson & Houk, 1986) also indicate the adipose tissue as a major storage site of TCDD. Therefore, these two tissues are the major target organs for the toxic manifestations of TCDD. We further showed (Lakshman et al., 1988) a dose dependent progressive inhibition of hepatic fatty acid synthetic rate and cholesterol synthetic

rate with concomitant decreases in the key lipogenic enzyme activities. Significantly, the adipose tissue was found to be more sensitive than the liver with respect to inhibition of fatty acid synthesis by increasing dosage of TCDD. Nonetheless, the biochemical mechanism of loss of adipose mass caused by TCDD exposure remains to be clarified. It will be demonstrated in this study that even a single 20 μg/kg dose of TCDD causes within 3 days increased mobilization of depot fat, decreased hepatic oxidation to $CO_2$ and increased ketogenesis, whereas the fatty acid esterification in the liver was unaffected.

## MATERIAL AND METHODS

TCDD. TCDD was purchased from the IIT Research Institute *Chemical Carcinogen Standard* Reference Repository, Chicago, IL, and was found to be more than 98% pure based on high performance liquid chromatography (Lakshman et al., 1986). All other chemicals and reagents were of analytical grade.

Animals. Wistar male rats (body weight 250-270 g) were procured from Charles River, Wilmington, MA, and were maintained on the normal Wayne Lablox chow (4.4 % fat content) ad libitum for two weeks before experimentation.

TCDD Administration. 20 rats were divided into two groups of 10 each. Ten animals in the experimental group received an intraperitoneal injection of 20 μg/kg body weight of TCDD in corn oil, while the corresponding 10 control animals received an equivalent volume of corn oil vehicle only. All the animals were fed the normal Wayne Lablox chow ad libitum. Four animals from each group were killed at the end of 3 days of exposure by aortic exsanguination and the serum was analyzed for free-fatty acids as their methyl esters by gas-liquid chromatography. Hepatocytes were isolated from the other 6 animals from each group at 9 am as described by us previously (Lakshman et.al., 1977). They were incubated at 37° C for 30 min. with 1mM [1-$^{14}$C]oleate (Specific activity: 574 dpm/nmole). The reaction was stopped by 3N sulfuric acid and the liberated $^{14}CO_2$ was trapped in Hyamine and analyzed for radioactivity in Hydrofluor scintillation fluid. The reaction mixture was also analyzed for $^{14}$C incorporation into esterified lipids (Lakshman et.al., 1984) and into ketone bodies (Bates et.al., 1968).

## RESULTS & DISCUSSION

The effects of 3 days of exposure to a single 20 μg/kg dosage of TCDD upon body weights and food consumption revealed that although the average amount of the food consumed by the

TCDD treated group was 10% lower than that of the Control group, the mean body weight of the TCDD treated group did not change significantly from the Control group. Thus, at 20 μg/kg dosage, TCDD did not have any significant deleterious effects within three days.

The effects of the same single 20 μg/kg dosage of TCDD upon plasma free-fatty acids after 3 days of exposure are presented in Table 1.

TABLE 1. Effects of 3 days of TCDD Exposure on Plasma Free-Fatty Acids

| Treatment | Plasma Free-Fatty Acids (mg%) | | | |
|---|---|---|---|---|
| | C16:0 | C18:0 | C18:2 | C20:4 |
| Control | 13.5 ± 0.7 | 12.9 ± 0.3 | 5.3 ± 1.1 | 15.4 ± 2.1 |
| TCDD | 19.7 ± 4.2 | 18.5 ± 2.7 | 15.0 ± 0.9 | 31.5 ± 3.1 |
| Fold-increase | 1.46 | 1.44 | 2.84 | 2.05 |
| p value | (0.1<p<0.2) | (0.05<p<0.1) | (0.001<p<0.005) | (0.001<p<0.01) |

Each value is the MEAN + SE of 4 animals.

It is obvious from Table 1 that TCDD caused a 1.5-2.8 fold increases in plasma concentrations of palmitic, stearic, linoleic and arachidonic acids. These results clearly demonstrate a marked mobilization of depot fat into the plasma compartment.

In order to determine the fate of the mobilized fatty acids, the effects of TCDD on the oxidation, esterification and ketogenesis of labeled oleate were determined in hepatocytes isolated from Control and TCDD treated groups (Table 3).

TABLE 2. Effects of 3 days of TCDD Exposure on Hepatic Oleate Metabolism

| Oleate Metabolized μmoles $g^{-1}$ $h^{-1}$ | Control | TCDD | % Change |
|---|---|---|---|
| Oxidation to $CO_2$ | 3.49 ± 0.23 | 2.39 ± 0.26 | -32 (p<0.008) |
| Esterification | 2.11 ± 0.49 | 2.44 + 0.37 | N.S |
| To Ketone bodies | 0.27 ± 0.02 | 0.50 ± 0.02 | +85 (p<0.001) |

Each value is the MEAN + SE of 6 animals.

As can be seen in Table 2, TCDD caused a 32% inhibition (p<0.008) of hepatic oleate oxidation to $CO_2$. These results imply that the normal hepatic beta-oxidation and/or their further oxidation to $CO_2$ via the tricarboxylic acid (TCA) cycle of the mobilized fatty acids may be

impaired. However, their esterification to triglycerides was unaffected. In contrast, the rate of total ketone body production from the labelled oleate was increased by 85% ($p<0.001$). This shows that eventhough the beta-oxidation pathway of the fatty acids must be working normally, their complete oxidation to $CO_2$ via the TCA cycle must be impaired. At the same time, the acetyl CoA generated from the beta-oxidation of the fatty acids must be preferentially diverted through the ketogenic pathway as a result of TCDD exposure. Further studies are currently in progress to pin point the exact site/s of action of TCDD in affecting these various pathways of mobilized fat in the liver.

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