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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Putting the 'ox' in toxin

by

Katrina S.M. Pedersen

Department of Radiation Oncology
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

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AhR	Aryl hydrocarbon receptor
GSH	Glutathione
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
ROS	Reactive oxygen species
$O_2^{\bullet-}$	Superoxide anion radical
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TBARS	Thiobarbituric acid reactive substances

Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most infamous member of the dioxin family because of its extreme toxicity to organisms. Notable victims of dioxin poisoning include those exposed to Agent Orange in Vietnam and Victor Yushchenko, the Ukrainian president. The molecule consists of halogen-substituted aromatic rings creating a highly stable, lipophilic molecule that may be retained within tissues for more than 10 years. Though the exact mechanism of toxicity remains unknown, an increase of reactive oxygen species (ROS), particularly $O_2^{\bullet -}$ and $HO^{\bullet}$, has been shown through TBARS analyses. Conversely, glutathione-mediated antioxidant activity is lowered within dioxin-treated systems. These observations suggest a key role for oxidative stress in TCDD-mediated cell damage. This paper will review the routes of ROS formation involved with TCDD toxicity.

Introduction

The term dioxin refers to a family of xenobiotic compounds that consist of aromatic hydrocarbons with halogen substitutions. These substances are byproducts of many industrial processes requiring chlorine and high heat and are otherwise not found in nature [1]. Organisms are exposed to dioxins that are present in plastics and packaging substances at low concentrations as well as that sequestered in soils[1]. Of the dioxins, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic due to its high binding affinity to the aromatic hydrocarbon receptor (AhR) [2,3] and has been directly linked to chloracne of the skin, such as noted in President Yushchenko's facial disfigurement. Dioxin is also thought to be a carcinogen and a causative agent in neuronal defects [4].

Though the exact mechanisms governing TCDD toxicity remain unknown, a growing body of evidence suggests that oxidative stress may play a key role. TCDD is a highly lipophilic compound and is, therefore, accumulated in the body [1]. As mentioned above, the molecule may bind to AhR leading to nuclear translocation and association with the AhR nuclear translator protein [5]. This complex may then bind the DNA composing the dioxin responsive element [6] and affect transcription of many genes. Many of these genes encode redox-active components, including cytochrome P450 [7], NADPH:quinone reductase [8], and glutathione S-transferase [9]. These biochemical actions have been correlated to increased levels of ROS in cells exposed to TCDD and will be discussed along with a review of antioxidant responses both *in vitro* and *in vivo*.

[1] from http://en.wikipedia.org/wiki/Dioxin (accessed on 2.25.2005).

Dioxin formation

Dioxins are byproducts of many industrial processes, including paper bleaching and plastic synthesis. The compound is entirely man-made and is a global pollutant. Most frequently, products like 2,3,7,8-TCDD are made during herbicide manufacture. 1,2,4,5-tetrachlorobenzene is hydroxylated to an alcohol 2,4,5-trichlorophenol in a two-step process that requires the input of at least 160°C (**Figure 1**).

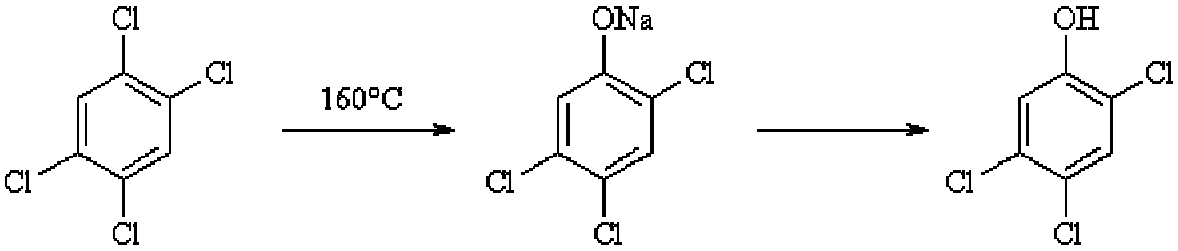

Figure 1. 2,4,5 trichlorophenol is formed during an endothermic process from 1,2,4,5 tetrachlorobenzene. From http://chimie.scola.ac-paris.fr (accessed on 2.27.2005).

Because this endothermic process is generally not well controlled, when temperatures exceed 200°C, a small number of the products or intermediates may fuse to create 2,3,7,8-TCDD and either two molecules of hydrochloric acid or sodium chloride, respectively (**Figure 2**).

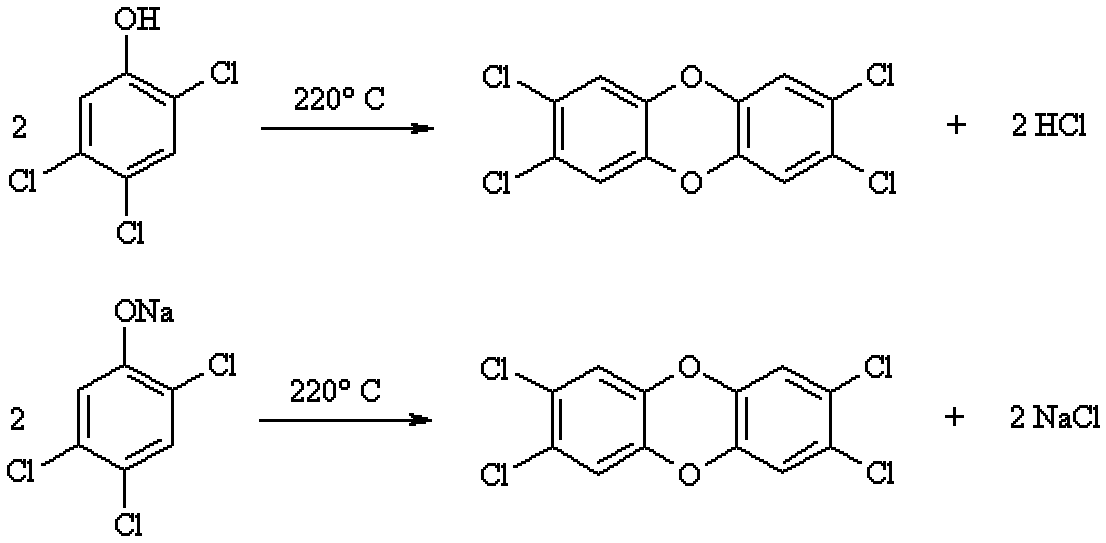

Figure 2. Formation of 2,3,7,8-TCDD during the synthesis of 2,3,4-trichlorophenol (TCP) requires high heat for two molecules of either 2,3,4-TCP (above) or its intermediate (below) to fuse, causing two chloride atoms to leave. From http://chimie.scola.ac-paris.fr (accessed 2.27.05).

Dioxin toxicity and the formation of radicals

The stability of TCDD is believed to contribute to the extremely deleterious nature of the molecule. Dioxin is a very stable species that may persist with a half-life of 1.5 years upon release into water [10], recalling that, due to its hydrophobicity, TCDD is likely associated with a nonpolar sediment. In soil dioxin has been reported to have half-lives ranging from 1-12 years [10]. Airborne TCDD, despite having a very low vapor pressure of 7.4×10^{-10} mm Hg at 25°C that would suggest the molecule is unreactive, readily interacts with atmospheric hydroxyl radicals and, therefore, has a half-life of only 8.3 days [10]. In humans exposed to TCDD, the half-life in tissues and blood has been recorded at an average of 11.3 years, though this number varies depending on the size of the individual [11]. These data suggest that dioxin persist long enough within an environment to cause damage.

The relative toxicities of the various dioxins appear to be directly influenced by the strength of interaction with the AhR [12], of which TCDD has the smallest dissociation value ($K_D=10^{-10}$) [13]. Conversely, this means that TCDD has the greatest binding affinity and thereby induces the strongest response. Kwok *et al*. have suggested that the greater the halogen (chloride) substitution on the benzene rings, the greater the electron-withdrawing nature of the groups thereby allowing for stronger interactions with the receptor and decreased reaction rates [14]. This chemistry may explain why TCDD is more reactive than other less halogenated dioxins.

Upon the binding of TCDD to the AhR, the complex translocates to the cellular nucleus where it may heterodimerize and enhance transcription of redox responsive genes, such as cytochrome P450 and others mentioned above [15]. It is believed that

some of these genes may play a role in an oxidative stress mediated pathway of TCDD toxicity and carcinogenesis; however, the relationship between the two remains unclear. Upon application of TCDD to cells or animals, the levels of ROS, some antioxidant enzyme activities, as well as oxidative damages are induced. These mechanisms will be discussed in greater detail below.

ROS induction

Though not as accurate as EPR and other physicochemical modalities for measuring reactive oxygen species, biochemical means of determining the presence of ROS have been used extensively in the literature to observe the oxidative effects of TCDD on *in vitro* and *in vivo* model systems. Thiobarbituric acid reactive substances (TBARS) are formed when thiobarbituric acid is oxidized and may therefore serve as an indicator of ROS and lipid peroxidation within cells and organisms.

TBARS increase in response to TCDD application. This increase is reversible following the addition of catalase, indicating that H_2O_2 is being produced during toxic interaction with dioxins [4]. The presence of H_2O_2 suggests that $O_2^{\bullet -}$ is also increased during the TCDD reaction [16]. Indeed, addition of superoxide dismutase led to reduced TBARS levels [4]. Hydroxyl radical, not surprisingly, appears to be produced because $HO^{\bullet}$ retarders decrease the TBARS produced within the system [4]. TCDD-induced oxidative stress is upregulated in a dose-dependent manner [2].

Free iron may participate in redox chemistry in cells. Adding an iron chelator, such as desferrioximine, to a TCDD-treated model reduces the amount of TBARS product formed [17]. This finding further supports the involvement of ROS in TCDD-mediated toxicity. TCDD has been shown to be associated with increased levels of free

catalytic iron in a system [17]. The increased levels of iron may participate in Fenton (**Equation 1**) and Haber-Weiss chemistry (**Equation 2**) to help generate the ROS used to peroxidize lipids in cells exposed to dioxin [4].

$$Fe(II) + H_2O_2 \longrightarrow HO^{\bullet} + OH^- + Fe(III) \quad (1)$$

$$O_2^{\bullet -} + H_2O_2 \longrightarrow HO^{\bullet} + OH^- + O_2 \quad (2)$$

Interestingly, the addition of antioxidants, such as ascorbic acid (vitamin C), has had a pro-oxidant effect in the presence of TCDD seen in the enhanced generation of ROS and lipid damage [4]. This enhanced oxidation is likely the result of reactions between the ascorbic acid and the free iron released in response to TCDD application. Conversely, vitamins A and E have maintained their antioxidant action against TCDD-induced toxicity [4,15].

Preventive antioxidants also play a role in TCDD-induced oxidative stress. Glutathione (GSH) is a key electron donor in the glutathione peroxidase-catalyzed conversion of H_2O_2 to water (**Equation 3**).

$$2\ GSH + H_2O_2 \longrightarrow GSSG + 2\ H_2O \quad (3)$$

The reaction precludes the formation of $HO^{\bullet}$, one of the most oxidizing species known, thereby preventing cellular damage. Researchers have shown that acute exposure of TCDD leads to suppression of GSH production *in vivo* [18,19,20]. Because dioxin inhibition of GSH is NADPH-dependent [16,21], TCDD may interfere with glutathione reductase activity (**Equation 4**).

$$GSSG + NADPH \longrightarrow 2\ GSH + NADP^+ \quad (4)$$

By doing so, glutathione peroxidase may no longer properly modify the peroxide substrate and oxidative stress increases as a direct result of xenobiotic application [22].

Taken together, these data provide a strong body of evidence showing that TCDD exposure not only induces the formation of ROS but also may suppress antioxidant activity.

Summary

Though the exact mechanism governing the link between TCDD exposure and oxidative stress remains unclear, it is well established that oxidation is significantly increased in a dose-dependent manner. This effect may be due not only to the rapid production of ROS following dioxin-mediated expression of certain genes but also due to the downregulation of antioxidant activity. Until the mechanism is more fully understood, the toxin will continue to claim both accidental and targeted victims.

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