# Dose- and time-response of TCDD in Tg.AC mice after dermal and oral exposure

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## Introduction

Dioxin-like compounds have been shown to be good tumor promoters in hairless mice[1,2]. In addition, topical application of TCDD in female Swiss-Webster mice for 2 years resulted in an increased incidence of fibrosarcoma in the integumentary system[3]. Dermal exposure for two years of a binary mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (ratio 1:2) to female Swiss-Webster mice resulted in an increased incidence of fibrosarcoma of the skin[4].

A promising tool in the screening of carcinogens has been the development of transgenic mouse models[5]. For example, the Tg.AC transgenic model, carrying a v-Ha-*ras*-construct, has shown to result in papillomas and malignant tumors after 26 weeks of dermal exposure to a number of mutagenic and nonmutagenic carcinogens[5]. The p53 (+/-) line responds rapidly to genotoxic carcinogens[5]. A pilot experiment with TCDD in male and female Tg.AC transgenic mice resulted in an increased incidence of keratoacanthomas (females only) and squamous cell papillomas in the skin after dermal exposure[6]. In addition, increased incidences of non-neoplastic lesions in the liver were observed for hepatocyte vacuolization (males only) and focal necrosis. In the p53 (+/-) line only hepatocyte vacuolization was observed in both males and females[6].

In order to evaluate the development of papillomas over time in the Tg.AC transgenic mouse model, a dose-response study with TCDD was performed after topical application or gavage. This is the first study describing the comparison of the formation of skin papillomas after oral and dermal exposure in the Tg.AC mouse model.

## Materials and Methods

*Chemical*: TCDD was purchased from Accu Standard (New Haven, CT) and was identified by proton NMR. Gas chromatography indicated a purity of 99%.

*Animals and treatment*: Female hemizygous Tg.AC mice were purchased from Taconic Laboratory Animals and Services (Germantown, NY). Water and food were available *ad libitum*. Animals were randomly assigned to treatment groups and were housed individually in polycarbonate cages. In the dermal study, groups of 20 mice received 0, 5, 17, 36, 76, 121, 166, 355, or 760 ng/kg of TCDD in acetone three times a week for 26 weeks. The dosing volume was 3.3 mL/kg body wt. In the gavage study, groups of 20 mice received 0, 105, 450, or 1250 ng/kg of TCDD in corn oil containing 1% acetone five times a week for 26 weeks. The dosing volume was 10 mL/kg body wt. Animals were observed twice a day for clinical signs of toxicity. On a weekly basis, the number of skin papillomas of each animal (maximum of 20) and individual body weights were recorded. At the end of the 26-

week studies, animals were killed by $CO_2$ asphyxiation. The forestomach, kidney, liver, lung, skin, adrenals, pituitary gland, thyroid gland, lymph nodes, ovary, uterus, spleen, and thymus were taken for microscopic evaluations. Liver and lung pieces were collected and stored at –70°C for cytochrome P4501A analysis.

*Cytochrome P450 analyses*: Microsomal fractions of the liver and lung were prepared according to Pearce and co-workers[7]. Hepatic and pulmonary CYP1A1 activity measurements were based on a method by Kennedy and co-workers[8], whereas hepatic CYP1A2 activity measurements were based on methods of DeVito and colleagues[9].

**Results**

No TCDD-induced alterations in body weight gain or mortality were observed in either study.

In both the dermal and gavage studies TCDD-related neoplastic lesions were confined to the skin and included squamous cell carcinomas and squamous cell papillomas. Non-neoplastic lesions in the liver in both studies were typical as those observed for TCDD.

The average number of skin papillomas per animal based on all animals in the dermal and gavage studies is presented in Figs. 1 and 2. A dose-dependent increase in the average number of papillomas per animal was observed. In addition, at higher doses these papillomas were observed earlier. A statistically significant increase in the number animals with papillomas was observed at a dermal dose of 17 ng TCDD/kg and higher (5/20 vs. 0/18 in controls) or an oral dose of 1250 ng TCDD/kg (8/20 vs. 0/16 in controls) at the end of the study ($p < 0.05$).

A dose-dependent increase in hepatic and pulmonary cytochrome P4501A activities was observed (Fig. 3A-C). TCDD increased significantly hepatic CYP1A1 and CYP1A2 activities in all dose groups (dermal and gavage). Pulmonary CYP1A1 activities were increased significantly at 36 ng TCDD/kg and higher in the dermal study and at all dose levels in the gavage study. At similar mean daily dose levels, hepatic and pulmonary CYP1A1 activities were higher after oral exposure in comparison to dermal exposure.

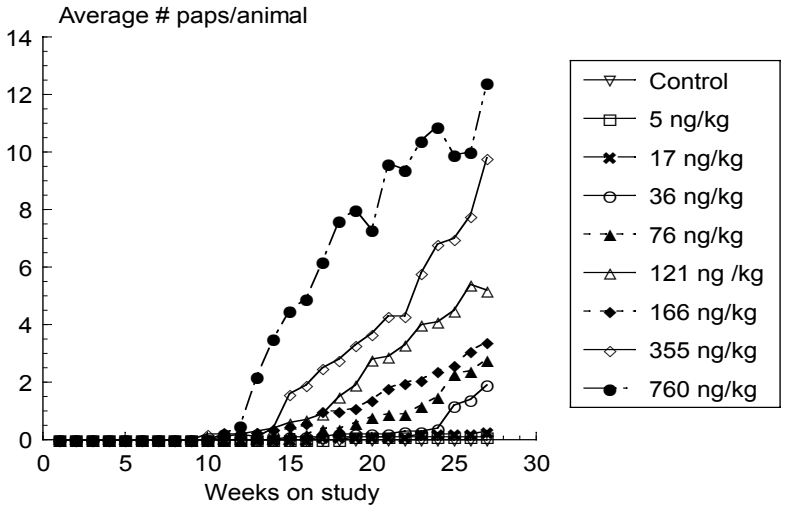


Fig. 1. The average number of papillomas per mouse following dermal exposure to TCDD.

**Discussion**

The TCDD-induced increase in skin papillomas and non-neoplastic lesions in the liver after dermal exposure is consistent with a previous study in female Tg.AC transgenic mice[6]. By inclusion of low doses in the current study, the shape of the dose-response curve can be well characterized. The no-observable-adverse-effect level (NOAEL) for the development of visible papillomas was 5 ng TCDD/kg, equivalent to an average daily dose of 2.1 ng TCDD/kg (Fig. 1). The lowest-observable-effect level (LOAEL) was 17 ng/kg (equivalent to a daily dose of 7.3 ng TCDD/kg). This is in the same range as the NOAEL (1 ng/kg/day) and LOAEL (10 ng/kg/day) for an increased incidence of combined liver tumors (adenomas and carcinomas) and hepatic hyperplastic nodules in female Sprague Dawley rats exposed to TCDD by feed for 2 years[10,11]. This extended dose-response study with TCDD after dermal exposure facilitates the development of a mathematical model to describe the papilloma formation in Tg.AC mice[12].
The remarkable finding in the gavage study with TCDD was that the formation of neoplastic lesions was confined to the skin. No papillomas were observed in the forestomach in either study. This is the first study describing the comparison of the formation of papillomas in the Tg.AC model after oral and dermal exposure.

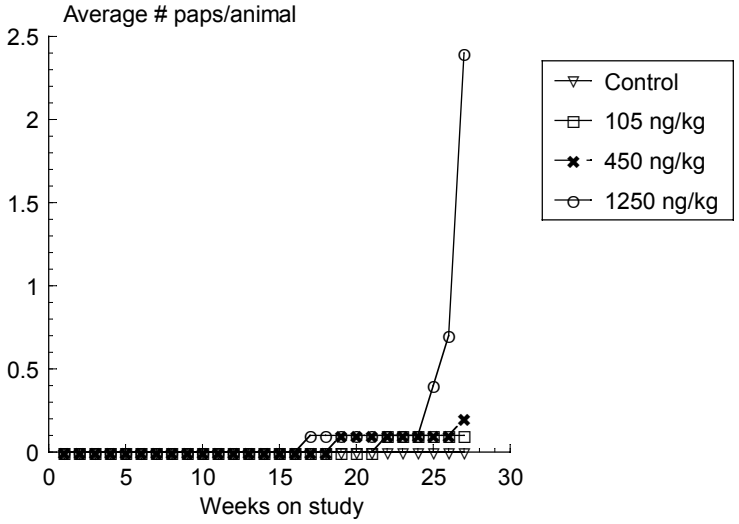


Fig. 2. The average number of papillomas per mouse following oral exposure to TCDD.

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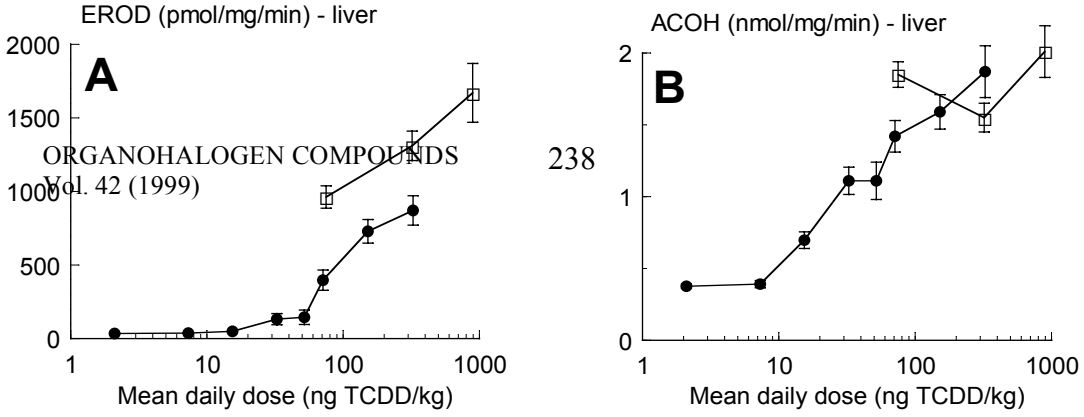

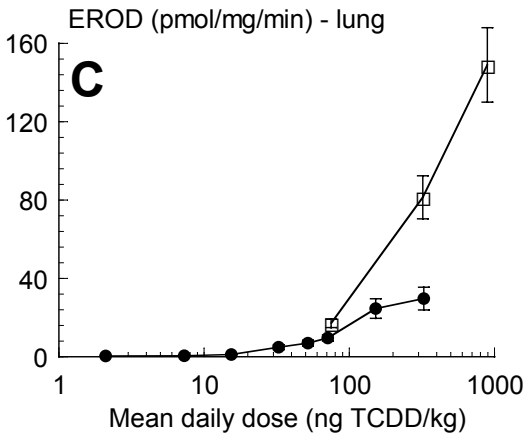


Fig. 3A-C. Hepatic cytochrome P4501A1 activities measured as ethoxyresorufin *O*-deethylase (EROD) activity after dermal (●) or oral (□) exposure (A). Hepatic cytochrome P4501A2 activities measured as 4-hydroxylation of acetanilide (ACOH) activity after dermal (●) or oral (□) exposure (B). Pulmonary cytochrome P4501A1 activities measured as ethoxyresorufin *O*-deethylase (EROD) activity after dermal (●) or oral (□) exposure (C). Data are presented as mean ± SE.