TUMOUR PROMOTING ACTIVITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) AND RELATED COMPOUNDS IN RAT LIVER

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ABSTRACT

This presentation deals with liver tumour promoting activity of TCDD and polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners. Results from a comparative dose-response study of liver tumour promoting activity of TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) will be presented. In addition, some studies of modulation of TCDD-induced promotion of hepatocarcinogenesis will be summarized. Finally, some implications of positive tumour promotion data in risk assessment of TCDD and related agents will be considered.

INTRODUCTION

The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds are exogenous agents capable of sustaining carcinogenesis in experimental animals. TCDD has been shown to induce hepatic, pulmonary and skin tumours in long term feeding experiments in rodents. The recognition of chemical carcinogenesis as a multistage, convergent process with definable steps of initiation, promotion and progression has prompted studies on the action of various agents in these separate stages. An obvious application of results from such mechanistical studies is in risk assessment of carcinogenic agents with their main action in any particular stage of carcinogenesis.

The liver is by far the most common target organ of chemical carcinogens in rodents (Haseman *et al.*, 1984) and this organ has also proved very suitable for studies of multistage carcinogenesis. The development and use of medium-term *in vivo* assays with sepatate initiation- and promotion-treatments and using preneoplastic, altered hepatic foci (AHF) as the main histopathological endpoint, has been of great value in these achievements (reviewed by Goldsworthy *et al.* 1986). Such mechanistical studies have revealed that TCDD is a remarkably potent promoter of hepatocarcinogenesis in female rats (Pitot *et al.*, 1980, Flodström and Ahlborg, 1989, Flodström *et al.*, 1990a, Flodström *et al.*, 1990b). It may thus be argued that the carcinogenic effects of TCDD and related compounds in rat liver are due to a prominent promoting activity. However, the mode of action of TCDD and dioxin-like agents as tumour promoters is still not elucidated and such knowledge is needed to further substantiate a risk assessment of dioxins based on promoting activity as one critical effect.

PROMOTING ACTIVITY OF TCDD AND RELATED COMPOUNDS

Apart from TCDD, only a few polychlorinated dibenzo-*p*-dioxins and no dibenzofurans have been studied for carcinogenicity in long term rodent experiments. Studies of promotional activity of dioxins and furans in the liver are even more scarce. Meanwhile, a multitude of studies of other biological effects *in vitro* and *in vivo* have shown that many chlorinated dibenzo-*p*-dioxins and dibenzofurans (particularly 2,3,7,8-substituted congeners) exhibit dioxin-like activity more or less potently and they are believed to share a common mechanism of action with TCDD (Safe, 1986).

Both 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF) have earlier been shown to act as promoters of hepatocarcinogenesis in nitrosamine-initiated male Wistar rats (Nishizumi and Masuda, 1986). In this study the polychlorinated dibenzofurans (PCDFs) were administered by four subcutaneous injections during four weeks and tumour yield was evaluated at 8, 12 and 16 weeks after the last PCDF-injection. Both dose levels of PeCDF and the high dose level of HxCDF used in the study (10 and 100 µg/kg/week, respectively) significantly enhanced the development of benignant and malignant liver cell tumours.

In a comparative study in our laboratory we recently evaluated the liver tumour promoting potency of 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD and TCDD administered by subcutaneous injections. In this study we used enhancement of gamma-glutamyltranspeptidase (GGT) positive AHF induced by the PCDD/PCDFs in nitrosmine-initiated female SD rats as the histopathological endpoint. An outline of our experimental protocol is depicted in Fig. 1. below.

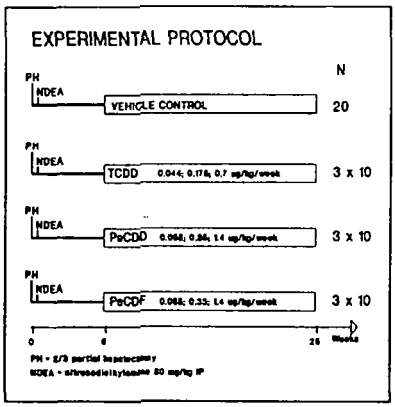

Figure 1. Experimental protocol for the AHF-study with PCDD/PCDF-congeners. The first PCDD/PCDF dose was administered as a loading dose (5 x maintenance doses)

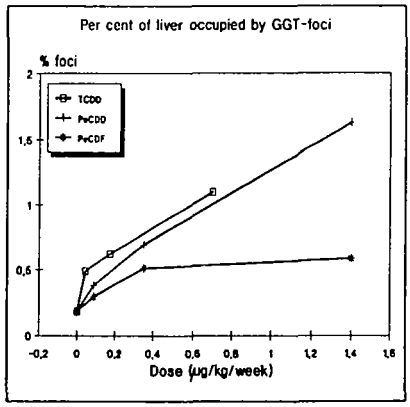

Figure 2. Results from the AHF-study with PCDD/PCDF-congeners. The graph shows dose-response curves for the respective congeners after 20 weeks of promotion.

The results from the study are summarized in Fig.2 showing per cent of liver tissue occupied by GGT-positive foci in the treatment groups shown in Fig. 1. As expected, TCDD acted as a tumour promoter and enhanced the development of AHF at all dose levels tested (0.044 – 0.7 µg/kg/week). Both PeCDD and PeCDF also showed prominent activity as enhancers of foci development and thus, acted as very potent liver tumour promoters. TCDD and PeCDD were virtually equipotent in this respect whereas PeCDF elicited a potency of about one tenth of the dioxins. No clinical signs of dioxin/furan-induced toxicity were recorded during the study. However, thymus weights were decreased and liver weights and plasma transaminase activities were increased at least at the medium and high dose levels.

MODULATION OF THE PROMOTING ACTIVITY OF TCDD

In some recent papers we have investigated the modulating effects on TCDD-induced promotion of hepatocarcinogenesis by the type of diet, vitamin A deficiency, exposure duration and administration schedule (Flodström and Ahlborg, 1989, Flodström *et al.*, 1990a, Flodström *et al.*, 1990b). In these studies it was noticed that rats fed on a purified, casein-based diet elicited slower foci development and less pronounced signs of hepatotoxicity after TCDD-promotion as compared to animals maintained on a conventional, cereal-based rat chow. Vitamin A deficiency was shown to enhance the TCDD-induced promotion of AHF-growth and to accentuate several TCDD-related toxic responses including degenerative changes in the liver. As opposed to the non-toxic, mitogenic liver tumour promoter phenobarbital, TCDD was ineffective or less effective as a promoter of foci development when shorter promotion periods (10 – 15 weeks) were used. This difference could be partially counterbalanced by beginning the TCDD-promotion with a loading dose (5 x maintenance dose) provided that the rats were fed the cereal diet but not the purified casein diet.

Thus, the type of diet, vitamin A deficiency, exposure duration and the administration schedule all modulated TCDD-induced promotion of hepatocarcinogenesis. In some of these cases retarded AHF-development was accompanied by a decreased degree of hepatotoxicity.

CONCLUSIONS

Several national and international working groups have suggested the use of so called Toxic Equivalency Factors (TEF) for the assessment of risk from exposure to PCDD/PCDF-mixtures. These factors relate the toxic potency of PCDD/PCDF-congeners to the potency of TCDD. In a Nordic risk assessment of PCDDs/PCDFs (NORD, 1988) such TEFs were assigned to a number of congeners including 2,3,4,7,8-PeCDD **(0.5)**, 1,2,3,7,8-PeCDF **(0.5)** and 2,3,7,8-substituated HxCDFs **(0.1)**.

From our study with TCDD, PeCDD and PeCDF and the PeCDF/HxCDF study of Nishizumi and Masuda Toxic Equivalency Factors for these congeners may be derived. In our study PeCDD and TCDD were virtually equipotent as promoters of hepatocarcinogenesis and consequently 1,2,3,7,8-PeCDD could be assigned a TEF of **1.0**. We recorded a potency of some 10% of the dioxins for PeCDF rendering 2,3,4,7,8-PeCDF a TEF around **0.1**. By comparisons between the potencies of TCDD and the PCDFs found in our and the Nishizumi-Masuda study, respectively, an estimated TEF of **0.01** could be assigned

to 1,2,3,4,7,8-HxCDF. However, this latter value suffers in reliability from the differencies between the two studies regarding animal strain and sex, length of promotion and the histopathological endpoint.

A scientifically unobjectionable risk assessment of TCDD and other agents with dioxin-like activity will only feasible when their molecular and cellular mode(s) of action have been finally elucidated. However, circumstantial evidence regarding genotoxicity, carcinogenicity and promotional activity supports a risk assessment for individual congeners based partly on carcinogenic/promotional activity. However, interactive effects must not be overlooked, and a strictly "TEF-based" risk assessment of mixtures of dioxin/furan congeners may prove to be an undue oversimplification. In our and other laboratories actual studies on interaction between PCDD/PCDF-congeners and related compounds have indicated antagonistic, additive as well as synergistic effects regarding biological endpoints such as liver tumour promotion, EROD-induction and effects on vitamin A homeostasis. (Jensen and Sleight, 1986, Evans and Sleight, 1989, Ahlborg et al., 1989, van Vliet, 1990), Thus, *in vivo* validation of tumour promotion-based TEFs by doing interaction experiments is urgently needed.

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