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ANALYSIS OF 2,3,7,8-TCDD TUMOR PROMOTION ACTIVITY AND ITS RELATIONSHIP TO CANCER

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ABSTRACT

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (hereafter referred to as TCDD) has a high estimated cancer potency in animals which has been reasoned to imply that TCDD might be carcinogenic to man. The animal cancer data show that TCDD can act in a solitary manner causing tumors without the participation of other known factors. However, there exists animal cancer data indicating that TCDD can act as a tumor promoting compound. This analysis examines which type of carcinogen and which mechanism best characterizes TCDD cancer activity. It is suggested that TCDD acts by a hormonal mechanism to cause cancer in a solitary manner, at low doses, in two species, and in a number of different organs, including rare sites. These observations *in toto* characterize TCDD as a complete carcinogen, which by definition encompasses both initiation and promotion carcinogenic activities.

KEYWORDS

Total carcinogen; tumor promoter; tumor initiator; hormonal carcinogen.

INTRODUCTION

In order to best assess the hazards to human life from chemical carcinogens, it is necessary to incorporate as much understanding of the mechanism of action of animal and human carcinogens into the hazard assessment process as possible. The carcinogenic response to TCDD in mice and rats seems to indicate a dichotomous mechanism: one mechanism proceeding by complete carcinogenesis and the other by tumor promotion. The first mechanism indicates that TCDD can act as a solitary carcinogen needing no other factors to cause cancer. The second mechanism refers to a type of incomplete carcinogenesis where the compound completes the initial, subcarcinogenic insult of a total carcinogen, *e.g.* from another source, so as to cause tumorigenesis.

We consider, in the delineation of the cancer mechanism, that it is important to qualitatively model what type of mechanism is likely to be operative in TCDD-exposed man. Quantitative models used to explain cancer dosimetry must of necessity be based on the best available realistic qualitative mechanistic model.

This analysis shall proceed in turn through the extant TCDD tumor promotion data, complete carcinogenesis data, hormonal-related mechanisms to TCDD, and finally through a summary analysis of the mechanism which best characterizes TCDD carcinogenicity.

EVIDENCE FOR THE TUMOR PROMOTION EFFECTS OF TCDD

a. *in vivo* Support for TCDD-Related Tumor Promotion (Direct Bioassays)

Pitot *et al.* (1980) have presented data on the promotion characteristics of TCDD in female Charles River rats (Fig.1). The primary purpose of this study was to demonstrate tumor promoter-related quantitative liver enzyme focus formation. Positively staining foci for γ -glutamyl transpeptidase (GGT) was used as a liver tumor-promotion marker for liver cells disposed to preneoplasia by TCDD. These foci were markedly increased compared to controls (see protocol, Fig. 1).

The Pitot study also enumerated the number of female rats with hepatocellular carcinoma among the dose groups (4-7 rats/TCDD group, Fig.1). No liver cancers were found in any control group, none in the low-dose TCDD group (10 ng/kg/day given s.c. in corn oil), but 5/7 rats had liver cancers at the high-dose TCDD group (100 ng

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TCDD/kg b.w./day). A positive control group was tested also with phenobarbital (0.05% in diet) which showed 8/10 rats had liver cancers. Although few animals were tested giving a lowered statistical inference of the tumor results (compared to a standard bioassay with 50 animals/sex/dose group), it is clear that sufficient fields of cells were scanned to determine the TCDD-related histochemical effects of TCDD and phenobarbital in the rat liver. Pitot *et al.*(1980) have suggested that, because of the positive tumor promotion results, which are associated with particular enzyme foci formation in the liver, it is reasonable to hypothesize that all the tumors associated with persistent TCDD exposure arise from the tumor promoting effects of TCDD on "already initiated" cells in the test animal. This further implies that there is no primary initiating effect of TCDD on these female rat cells (cf. DISCUSSION section).

A study in HRS/J mouse skin is presented in **Fig. 2** (Poland *et al.*,1982). When hairless mice (hr/hr) were first exposed to 0.2 nmol of DMBA (initiation) and then exposed to repetitive exposures of either 50 ng TCDD/mouse or 2000 ng TPA/mouse (a known skin tumor promoter), papillomas of the epithelium were formed in both cases. It is notable that TCDD alone in this experiment caused no increases in skin papillomas. But when a subthreshold dose of a complete carcinogen was applied to skin, then subsequent TCDD or TPA exposure caused tumor promotion in hairless mice. TCDD is about 40 times more potent as a tumor promoter in this system than is TPA. In congeneic mice which are genetically (hr/+), no tumors can be promoted by repetitive exposures to TCDD, thereby suggesting that a single recessive trait (hr) may be involved in the TCDD-mediated tumor promotion in HRS/J mice. However, the possibility of other genetic loci involvement is not ruled out.

A tumor promotion bioassay was done for the U.S. National Toxicology Program (U.S.NTP, 1982a) in mice. TCDD was applied dermally onto the dorsal region of skin (**Fig. 3**). Swiss-Webster mice showed marginal increases in fibrosarcomas upon TCDD exposure in either the male TCDD or DMBA/TCDD dose groups which are not viewed as statistically increased cancer responses; however, definite increases in fibrosarcomas were observed in the female TCDD and DMBA/TCDD dose groups. These increases, which were about the same (30% and 28%, Fig. 3), showed that TCDD alone increased the fibrosarcomas, but prior initiation with DMBA was not further enhanced by repetitive exposure to TCDD. This suggests that TCDD is acting as a total carcinogen. It is interesting that fibrosarcomas were caused by TCDD and not cancers of the mouse epithelium, onto which the three times/week applications in 0.1 ml. acetone were applied. It is known, for instance, that TPA and certain other phorbol esters do promote tumors in that same epithelium (Pelling and Slaga, 1985, and references therein).

Other results (**Fig. 4**) found in "normal" wild-type mice (presumably +/+ at the hr locus) are also negative for TCDD skin tumor promotion (Berry *et al.*, 1978). The results in all of the mice strains tested for tumor promotion taken together suggest that HRS/J hairless mice may be unique in showing skin tumor promotion responses as a result of repetitive TCDD exposures to the shaved and depilatated mouse dorsal skin epithelium.

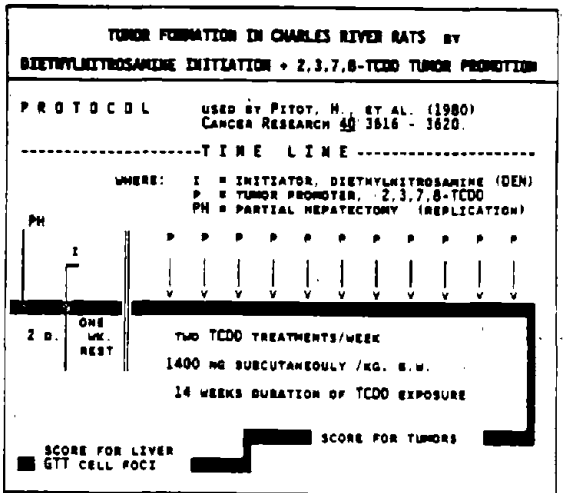
b.*in vitro* Support for TCDD-Related Tumor Promotion Effects

TCDD has little transforming ability in 10 T 1/2 cells alone, but was a promoter of transformation following MNNG ((N-Methyl-N'-nitro-N-nitrosoguanidine), a known tumor initiator (Abernethy *et al.*, 1985; Abernethy and Boreiko, 1987). Following initiating (low) levels of MNNG in this assay, TCDD was a very potent transforming agent, since it has a high transformation efficiency: 29%-39% @ 4 picomolar.

Fig. 1 Tumor Promotion in Charles River Rats.

Top Panel. Protocol is presented as a time line. Initiation is by intragastric intubation (1X) of diethylnitrosamine; subcarcinogenic dose of DEN = 10 mg/kg. After a rest period, tumor promotion was effected by persistent subcutaneous injections of TCDD at either 140 ng or 1400 ng TCDD per kg body weight per dose. A promotion dose was given twice/week. Dosing periods: DEN, TCDD, or phenobarbital alone, 28 wks; as tumor promoters, TCDD for 14 wks or phenobarbital for 28 wks.

Bottom Panel. Indicates the incidence of cancer in the rat liver identified as hepatocellular carcinomas. Note the short period of TCDD treatment for TCDD as a promoter while causing about the same incidence as for phenobarbital as a promoter at twice the treatment TCDD period (14 versus 28 wks). Hence, at doses given by Pitot and his collaborators, TCDD tumors in the liver develop faster.



TREATMENT	RATS	No. FOCI/cm²	LIVER CANCER INCIDENCE
PH + DEN	4	309	0/4 = 0%
PH + TCDD	5	25	0/5 = 0%
PH + PHENOBARBITAL	4	56	0/5 = 0%
PH + DEN + TCDD	7	871	5/7 = 71%
PH + DEN + PHENOBARBITAL	10	533	8/10 = 80%

Fig. 2

TUMOR PROMOTION BY 2,3,7,8-TCDD IN HAIRLESS MICE

INITIATOR	PROMOTER	TUMOR INCIDENCE	TUMOR MULTIPLICITY
DMBA (0.2 NMOL)	ACETONE	1 %	0.1
DMBA (0.2)	TPA (2000 NG/MSE.)	68 %	1.9
DMBA (0.2)	TCDD (50 NG/MSE.)	98 %	2.0
MNNG (5 MOL)	TCDD (3.8 NG/MSE.)	5 %	0.05
MNNG	TCDD (7.5 NG/MSE.)	55 %	0.7
MNNG	TCDD (15 NG/MSE.)	77 %	1.5
MNNG	TCDD (30 NG/MSE.)	100 %	4.0
ACETONE	TCDD (30 NG/MSE.)	0 %	0

REFERENCE: POLAND, PALEN, & GLOVER (1982) 300 (NOV.) 271 - 273

Fig. 3

NATIONAL TOXICOLOGY PROGRAM - 1982 DERMAL EXPOSURE TO TCDD
(SWISS - WEBSTER MICE)

TREATMENT	MALE DOSE	FEMALE DOSE
50 UG. DMBA	ONE TIME SKIN APPLICATION (0.1ML)	ONE TIME SKIN APPLICATION (0.1ML)
TCDD DOSE RATE	REPETITIVE SKIN TREATMENTS (0.1ML) 1 NG/.025 KG B.W. 3X/WEEK	REPETITIVE SKIN TREATMENTS (0.1ML) 5 NG/.025 KG B.W. 3X/WEEK
TOTAL DOSE	17.1 NG/KG/DAY	85.7 NG/KG/DAY
DURATION	2 YEARS	2 YEARS

TREATMENT	FIBROSARCOMA INCIDENCE	PERCENT (%)	P-VALUE
	MALES		
CONTROL	3/42	7	--
TCDD	6/28	21	0.08
DMBA/TCDD	5/30	17	0.18
	FEMALES		
CONTROL	2/41	5	--
TCDD	8/27	30	0.007
DMBA/TCDD	8/29	28	0.01

STATISTICAL INFERENCE: [TCDD INCIDENCE] VERSUS [DMBA/TCDD INCIDENCE]

MALES, P = 0.50, FEMALES, P = 0.55 ---- NO DIFFERENCES

THEREFORE, TCDD TREATMENT APPARENTLY NOT DIFFERENT FROM THE DMBA/TCDD TREAMENT.

Fig. 4

TUMOR PROMOTING ABILITY IN CD-1 MICE BY 2,3,7,8-TCDD

PROTOCOL: IN CD-1 MOUSE SKIN (DORSAL); TREATED TWICE/WEEK FOR 30 WEEKS; 100 NG/APPLICATION IN 0.2 ML. ACETONE

INITIATOR	PROMOTER	DOSE	INCIDENCE	TUMORS/MOUSE
DMBA (200NMOL)	-	-	0	0
-	TPA	2,000 NG	3	0 .03
DMBA	TPA	10,000 NG	92	8.1
DMBA	TCDD	100 NG	0	0
-	TCDD	100 NG	0	0

REFERENCE:

BERRY, DIGIOVANNI, JUCHAU, BRACKEN, GLEASON, SLAGA RESEARCH COMMUNICATIONS IN CHEMICAL PATHOLOGY AND PHARMACOLOGY 20 (1) 101 - 108 (1978).

Fig. 5

KOCIBA [1978] GAVAGE STUDY

CANCER INCIDENCE IN SPRAGUE-DAWLEY RATS

[EVIDENCE FOR INDUCED CANCER FROM 2,3,7,8-TCDD]

MALIGNANT CANCER TYPE/SEX	2,3,7,8-TCDD DOSE-RATE (NG/KG/DAY) 0	1.0	10	100	RESULT
	(PERCENT INCIDENCE)				
HARD PALATE M	0/85 (0)	0/50 (0)	0/50 (0)	4/50* (8)	+ INCREASE (P =0.017)
HARD PALATE F	0/86 (0)	0/50 (0)	1/50 (2)	4/49* (8)	+ INCREASE (P =0.016)
TONGUE M	0/85 (0)	1/50 (2)	1/50 (2)	3/50* (6)	+ INCREASE (P = .048)
TONGUE F	1/86 (1.1)	0/50 (0)	0/50 (0)	2/49 (4)	NO EFFECT
LIVER M	2/85 (2.3)	0/50 (0)	0/50 (0)	1/50 (2)	NO EFFECT
LIVER F	1/86 (1.1)	0/50 (0)	2/50 (4)	11/49* (22)	+ INCREASE ($P = 10^{-6}$)
LUNG M	5/85 (5.8)	0/50 (0)	0/50 (0)	1/50 (2)	NO EFFECT
LUNG F	0/86 (0)	0/50 (0)	0/50 (0)	7/49* (14)	+ INCREASE ($P = 10^{-3}$)

The degree of cellular malignancy associated with the *in vitro* focus formation was likely significant because type III cells [defined originally by Reznikoff *et al.*, 1973] were scored. Since transformation is viewed to be an essential series of cellular conversions in the development of cancer, these observations in 10 T 1/2 cells indicate this subset of cellular transformations is caused by TCDD. The connection between *in vitro* transformation to tumor promotion is not well understood as of yet, however, and can only be surmised as being part of the process.

Although TCDD facilitates cell transformation like a tumor promoter, TCDD does not inhibit intercellular communication - as many tumor promoters have been observed to do (Williams, 1980;Trosko *et al.*,1981) measured by the ^{3}H-Uridine cellular exchange, or by movement of Lucifer yellow dye among cells in intercellular transfer assays (Boreiko *et al.*, 1986). This lack of interference in cell-to-cell communication suggests that TCDD is either not a tumor promoter, or that TCDD is a tumor promoter acting by a different mechanism than a classic promoter such as TPA.

Two of the major re-programming cellular effects caused by tumor promoters are increased mitosis in some cell subpopulations and increased terminal cellular differentiation in other cell populations (Willey J.C., *et al.*, 1984). For example, TCDD has been found to increase keratinization in skin (Knutson and Poland, 1980). Hyperplasia from TCDD exposure is seen *in vitro* in cultured human keratinocytes, in exposures to human epidermis, and in animal systems (Milstone and LaVigne, 1984; Gianotti, 1977; Poland and Knutson, 1982). Both properties of increased cellularity and maturation are descriptive of tumor promotion activity but are also manifest by complete carcinogens, which possess both initiation and promotion activities.

EVIDENCE OF TCDD ACTING ALONE AS A TOTAL CARCINOGEN

Sprague-Dawley rats were gavaged with average daily TCDD doses of 0, 1, 10, and 100 ng/kg/day (Kociba, *et al.*, 1978). The summary of the Kociba study is presented in **Fig. 5**. Increases in malignant tumors were observed in four different organs: hard palate (including nasal turbinate cancers), tongue, liver, and lung. This tumor activity is carcinogenic activity since TCDD caused malignant tumors in these organs (Kociba, 1984). Mechanistic activities of tumor initiation, promotion, and progression are all indicated by these rat malignant responses to TCDD oral exposure.

It is interesting to note that the *cancer* responses were positive at the 100 ng/kg/day dose and not at 10 ng/kg/day (**Fig.5**). The same pattern of response was seen (100 + and 10 -) in the Pitot tumor promotion experiment where TCDD was administered to rats subcutaneously (Pitot *et al.*, 1980). The observations that different routes of TCDD exposure show the same break in the *cancer* response curve (at the same dose level shows a null response), in radically different protocols, and in different rat strains, all suggest that this dose region may be one that observationally defines (not proves) an apparent cancer threshold for TCDD in the rat.

It is also interesting to note (in the same experiment): not only were tumors increased but also tumors were decreased by TCDD. These tumor decreases are shown in **Fig. 6** and **Fig. 7**. We note one exception to tumor incidence decreases, i.e. an increase in benign tumors of the adrenal gland cortex. However, decreases were observed at the same time in adrenal pheochromocytomas (**Fig. 7**). The tissues affected by TCDD-related cancer decreases are all endocrine organs, thereby strongly suggesting the mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is linked to these hormonal systems. This "protection effect" of TCDD has not been adequately discussed or factored into any complete hazard evaluation or risk assessment of TCDD as of yet. This dichotomous tumor pattern imposes an additional complexity when ascribing the "estimated" carcinogenicity hazard from TCDD exposure and how this relates to human carcinogenicity risk.

Later work on TCDD carcinogenicity has been reported by the NTP in Osborne-Mendel rats and B6C3F1 mice (U.S.NTP, 1982b). Hepatocellular carcinomas are the dominant cancer response in the Osborne-Mendel rats with females and males demonstrating carcinogenicity increases only at the highest dose tested (**Fig. 8**). A mostly benign response to TCDD was also observed in the follicular thyroid at the highest dose and in both sexes. It is notable that tongue, hard palate, and lung tumors did not appear increased in the Osborne-Mendel rat in this NTP study as in the above Kociba study in Sprague-Dawley rats (cf. **Fig. 5**). However, the liver repeated as being a cancerous site between the two studies as well as increases in an endocrine organ (thyroid), whereas decreases were observed in Sprague-Dawley rat endocrine organs (pituitary, pancreas, thyroid, uterus, mammae, and adrenals) with only benign tumors increased in the adrenal cortex Sprague-Dawley rat.

A similar carcinogenicity response in the NTP study was observed in B6C3F1 mice (**Fig. 9**). That is, in the mouse just as in the rat, liver and thyroid tumors were also increased by TCDD exposure.

DISCUSSION

It is clear that repetitive TCDD exposure, after a subcarcinogenic initial exposure of a carcinogen, can bring about or promote tumors. This activity of tumor promotion seems to be manifest in Charles River rats and HRS/J

Fig. 6

KOCIBA [1978] GAVAGE CANCER INCIDENCE IN SPRAGUE-DAWLEY RATS

[EVIDENCE FOR DECREASED TUMOR RESPONSE FROM 2,3,7,8-TCDD]

ORGAN SITE / MALIGNANCY STATUS (M = MALIGNANT, B = BENIGN)		2,3,7,8-TCDD DOSE-RATE (NG/KG/DAY) 0 (PERCENT INCIDENCE)	1.0	10.0	100	RESULT
PITUITARY (M + B)	M	26/85 (30)	6/50* (12)	11/50 (22)	13/50 (26)	LOW DOSE DECREASE ONLY (?)
	F	49/86 (57)	18/50 (36)	13/50 (36)	14/49* (28)	HIGH DOSE DECREASE
PANCREAS ACINAR (M + B)	M	28/85 (33)	6/50* (12)	12/50 (24)	9/50* (18)	LOW & HIGH DOSE DECREASE
ISLET (M + B)	F	5/86 (5.8)	3/50 (6)	1/50 (2)	0/49 (0)	NO EFFECT
THYROID (M + B)	M	11/85 (13)	0/50* (0)	1/50* (2)	7/50 (14)	LOW & MID DOSE DECREASE
	F	18/86 (21)	3/50* (6)	2/50* (4)	6/49 (12)	LOW & MID DOSE DECREASE

NOTE: * INDICATES P-VALUE LESS THAN 0.05

Fig. 7

KOCIBA [1978] GAVAGE CANCER INCIDENCE IN SPRAGUE-DAWLEY RATS

[EVIDENCE FOR DECREASED TUMOR RESPONSE FROM 2,3,7,8-TCDD

ORGAN SITE/ MALIGNANCY STATUS (M = MALIGNANT, B = BENIGN)		2,3,7,8-TCDD DOSE-RATE (NG/KG/DAY) 0 (PERCENT INCIDENCE)	1.0	10	100	
		CONTINUATION OF KOCIBA STUDY				
UTERUS (B ONLY)	F	28/86 (33)	12/50 (24)	11/50 (22)	7/49* (14)	HIGH DOSE DECREASE
MAMMAE (B ONLY)	F	73/86 (85)	35/50 (70)	36/50 (72)	24/49* (49)	HIGH DOSE DECREASE
ADRENAL (B ONLY)		ADRENAL PHEOCHROMOCYTOMAS				
	M	28/85 (33)	6/50* (12)	10/50 (20)	4/50* (8)	LOW & HIGH DOSE DECREASE
	F	7/86 (8)	2/50 (4)	1/50 (2)	3/49 (6)	NO EFFECT
ADRENAL (B ONLY)		ADRENAL CORTEX				(EXCEPTION)
	M	0/85 (0)	0/50 (0)	2/50 (4)	5/50* (10)	HIGH DOSE INCREASE
	F	9/86 (10)	6/50 (12)	2/50 (4)	5/49 (10)	NO EFFECT

NOTE: * INDICATES P-VALUE LESS THAN 0.05.

Fig. 8

U.S. NATIONAL TOXICOLOGY PROGRAM

[1982] GAVAGE STUDY IN THE RAT

RESULTS IN OSBORNE-MENDEL RATS (CARCINOMAS + ADENOMAS)

ORGAN SITE	2,3,7,8-TCDD DOSE-RATE (NG/KG/DAY) 0 (PERCENT INCIDENCE)	3.0	7.0	70	RESULT
LIVER					
M	0/124 (0)	0/50 (0)	0/50 (0)	4/50* (8) P = .006	INCREASE
F	5/124 (4)	1/49 (2	3/50 (6)	15/49* (31) P = 5 x 10^{-6}	INCREASE
THYROID - FOLLICULAR, MOSTLY ADENOMAS					
M	6/118 (5)	5/48 (10)	8/50* (16) P = .02	11/50* (22) P = .002	INCREASE
F	5/122 (4)	2/45 (4)	1/49 (2)	6/47* (13) P = .05	INCREASE

Fig. 9

U.S. NATIONAL TOXICOLOGY PROGRAM

[1982] GAVAGE STUDY IN THE MOUSE

RESULTS IN B6C3F1 MICE (CARCINOMAS + ADENOMAS)

ORGAN SITE	2,3,7,8-TCDD DOSE-RATE (NG/KG/DAY) 0 (PERCENT INCIDENCE)	3.0	7.0	70	
LIVER					
M	31/123 (25)	12/49 (24)	13/49 (27)	27/50* (44) P = 3 x 10^{-4}	INCREASE
F	4/123 (3)	6/50* (12) P = .03	6/48* (13) P = .03	11/47* (23) P = 2 x 10^{-4}	INCREASE
THYROID - FOLLICULAR BENIGN, ONLY					
M	0/114 (0)	3/48 (6)	0/48 (0)	0/49 (0)	NO EFFECT
F	0/136 (0)	3/50* (6) P = .02	1/47 (2)	5/46* (11) P = 8 x 10^{-4}	INCREASE

hairless mice (hr/hr), but not in some "normal" mice strains (Swiss-Webster or CD-1) which are presumed to be wild type at the hr locus. In the hairless mouse cross experiments mice which were heterozygous (hr/+) at the hr locus also did not show tumor promotion activity with TCDD suggesting the hr trait may be a necessary element for mouse skin tumorigenicity .

It appears, then, that tumor promotion can be observed in some test strains, and not in others. Furthermore, it is noted (Fig. 3) in Swiss-Webster mice that, although TCDD skin tumor promotion is not observed in DMBA treated mice, TCDD by itself caused significant increases in fibrosarcomas in females (p=0.007) and a borderline response in males (p=0.08). These results suggest that although TCDD did not promote or enhance tumors in this test system it can cause carcinogenesis. This points to a total carcinogen mechanism in this system for TCDD. In a single report TCDD showed tumor initiation activity in the mouse (Digiovanni, 1977). Consequently, we surmise from all the TCDD tumor promotion data that TCDD has tumor promotion characteristics only in certain strains tested in the two-stage carcinogenesis system.

The cancers observed by Kociba and his collaborators in Sprague-Dawley rats were tumors in liver and squamous-cell carcinomas of the lung, the later being an uncommon tumor type occurring spontaneously in the lung. Also, the tongue and hard palate responded with carcinomas both of which are direct-route sites and are rare tumors. The occurrence of tumors is revealing as to the carcinogenicity of TCDD: (1) it strengthens the causal relationship between chemical exposure and cancerous outcome because the outcome is improbable due to random chance, and, (2) it indicates the probable effect of TCDD is likely direct on these tissues to cause cancer rather than "promoting" cryptic initiation lesions [hypothetically caused by prior events] in these normally cancer-free tissues. Were this latter point not true, then it would be expected that the theoretical initiation lesions, if really present, might nonspecifically interact with other promotion agents, such as exist in the diet, to cause cancer. But, cancer is only rarely observed in the hard palate and tongue of the rat.

So it is reasoned that analogous to a complete cancer-causing agent in man, such as tobacco smoke which contains known initiation and promotion agents and is carcinogenic to the human tongue, hard palate, and lung, TCDD can also be carcinogenic to these same tissues in the Sprague-Dawley rat thereby indicating that TCDD is a complete carcinogenic agent, at least in the rat. It remains to be proven whether TCDD is also carcinogenic to these tissues in TCDD-exposed humans. Since no other known agent participated in the rare cancer formation in the Kociba TCDD rat study, then it is reasoned that TCDD is a *complete* carcinogen possessing both initiation and promotion properties, although the proportional degree of each is not yet known. The relative proportion of the initiation/promotion mechanisms need not be the same among compounds testing positive for cancer in test species and may not necessarily correlate with the same proportion of these mechanism types in man.

Tumor promotion is not a new concept. Inception of this etiology took place in the 1920's by investigators who suggested a noncarcinogenic effect such as wound healing could evoke cancer in some cases, called traumatic cancer (Deelman, 1924, 1927). Later, the seminal work of Peyton Rous showed that certain chemical treatments could "seed" apparently normal tissue with persistent cells, in which later in time could be stimulated or enhanced by other noncarcinnogenic chemicals (or even by mechanical stimulus) to form tumors in the seeded tissues (Rous and Kidd, 1939; MacKensie and Rous, 1941). These authors referred to the latter stimulus which caused cell growth leading to tumors as "extraneous encouragement agents", and it was later defined as promoting agents (Friedwald and Rous, 1944). In this work the term initiating agents was also given to the irreversible carcinogenic action of chemicals which would start the process. With such concepts of stages of effects in sequence started the conceptualization of the commonly accepted initiation/promotion hypothesis of carcinogenesis which still is considered a valuable working model today.

Other investigators pursued the initiation/promotion concept referring to it as "specific cellular reaction"/developing factor (Mottram, 1945), and initiation process/promotion process (Berenblum and Shubik, 1949). These studies, and other genetic studies, provided support for the two stage hypothesis (Glinos, *et al.*, 1951). The operative concept was also supported by the evolving somatic mutation hypothesis (Berenblum and Shubik, 1949): cancer was determined to arise from a somatic mutation of a few cells [there being variation among these few mutated cells as to the type of lesion (Shubik, 1950)]. Some of these cells, in time, could be developed by a number of diverse agents to unscheduled cellular growth which proceeded in greater abundance [presumably] from mutated cells to hyperplastic regions, tumors, and finally in some cases to cancers which disseminated throughout the body. These developing agents, which by all authors were referred to as noncarcinogens, enhance the chances of cancer in initiated tissues and were referred to as tumor promoters.

This concept of tumor promotion has been studied by a number of investigators (Boutwell, 1964; Van Duuren, 1969; Berenblum, 1969; Shubik, 1984; Pelling and Slaga, 1985; Schulte-Herman, 1985) since the foundation works discussed above. The concept of tumor promotion in our opinion may be generally defined as:

> *Promotion is a reversible set of cellular processes which can cause unscheduled and/or mis-controlled cellular growth allowing for relative enrichment of initiated cells compared to the normal field of cells in which these initiated cells reside. Promotion leads to expanded clones, as well as, allows for reversible qualitative changes in the normal cellular differentiation process. Cancer can result from this promotion process if further changes in genetic expression [progression] are imposed on the exposed field of cells.*

For other discussion of tumor promotion definition refer to U.S. EPA, 1987.

It is instructive to note that (1) tumor promotion does not take place in the absence of initiation, i.e. initiation precedes promotion which in turn precedes progression into malignancy, and (2) tumor promoters are not carcinogens. We reason that TCDD produces cancers (malignancy) alone without any known prior tissue initiation which indicates TCDD is a complete carcinogen needing no assistance in causing cancer. If TCDD were <u>just</u> a tumor promoter as has been suggested (Shu *et al.*, 1987), then all the responding tissues (liver, lung, hard palate, tongue, skin, and thyroid) would of necessity have already been initiated at the time of TCDD exposure. We view this alternative as unlikely since the responses occur in a number of species/strains, and in a number of organs, including palate and tongue which are rare sites. These occurrences are not indicative of promoters studied to date which usually affect one organ, and only if that tissue has been initiated previously. We know of no published example where just a promoter exposure alone caused a dose-response of malignant tumors at a rare site. Lastly, even though it may be argued that the tongue and hard palate may have extraordinarily high TCDD exposures, this does not necessarily account for the rare carcinogenic response of these tissues any more than other carcinogens which are similarly administered by gavage. We conclude there must have been some specificity for the tongue and hard palate in the Sprague Dawley rat in the Kociba study. However, since this response was from a single study, it will be necessary to replicate these results in these tissues in order to increase certainty of the hard palate, tongue, squamous lung cancer responses.

A control often done in earlier work was to test whether a chemical could promote itself (e.g. Boutwell, 1964). This control started the process by a subcarcinogenic dose of the suspected carcinogen. If no other treatment is given, no tumors result; but if repetitive isoquantal doses of the same test chemical are applied, tumors <u>do</u> result if the chemical is a carcinogen. This is interpreted to mean that processes of initiation and promotion occur, i.e. a total carcinogen can promote itself. We view TCDD as having promotion activity, but this does not preclude that TCDD is a total carcinogen capable of promoting it's own initiation process. It should not be a surprise, then, that at the right dose-rate total carcinogens will act as tumor promoters in tumor promoter assays. We view TCDD acting this way in the tumor promotion assays reviewed here (Pitot *et al.*, 1980; Poland and Glover, 1982).

It remains then to address what kind of carcinogen is TCDD. The present genotoxicity information on TCDD is mostly negative although positives do occur (U.S. EPA, 1985). The *mostly* negative genotoxicity results suggest that TCDD is not generally positive by current methodology, and the true genotoxicity may not be known since the appropriate end-point may not have tested yet. Some authors have taken the position that TCDD must act upon the genetic material [but by an unknown mechanism] (Giri, 1986). The classical mutation mechanism whereby covalent-binding mutagens alter the DNA content may not be operating in the case of TCDD. TCDD may alter the genetic informational flow by a here-to-fore undescribed carcinogenic mechanism.

Due to the extended residence times of TCDD in the body [$(t_{1/2})_e$ = 1-4 mo. in rodents and 6-10 yrs.in man], TCDD could interact with functional chromatin, cell generation after cell generation, so as to alter genetic expression by *persistent* phenotypic changes. TCDD likely interacts by noncovalent binding in the rat since DNA measured covalent binding ≤ 1 TCDD molecule/10^{11} nucleotides (Poland and Glover, 1979). However, assuming 2 x 10^9 base pairs/cell, the estimated number (maximum) of hits in the rat are:

$$\frac{\text{1 molecule TCDD}}{10^{11}\ \text{nucleotides}} \times \frac{4\times10^{9}\ \text{nucleotides}}{\text{one eukaryotic cell}} = \frac{\text{1 molecule of TCDD}}{\text{25 cells}}$$

This hit frequency of TCDD covalent linkage to DNA is very low in rats being less than one hit per cell and 4-6 orders of magnitude less than most chemical rat carcinogens (Poland and Knutson, 1982a). However, in long term human TCDD exposure over 50% of fecal label has been reported to be TCDD-metabolites (Wendling *et.al.*, 1988), thereby not ruling out the possibility of tumor initiators being generated [over long periods] in man.

The relationship of TCDD cellular actions is known to be related to a specific cytosolic receptor protein Ah, which binds tightly to TCDD, K_d = 0.3 x 10^{-9} M^{-1}. The receptor-ligand complex is thought to initiate and promulgate many of the pleiotropic cellular effects of TCDD including wasting of some tissues and hyperplasia in others (Poland and Knutson, 1982). One of the most characteristic TCDD-induced biochemical events is a rapid increase in P-450 enzymes. This event leads to an important observation: pretreatment of animals with TCDD can abrogate subsequent cancer responses from known carcinogens (DiGiovanni *et al.*, 1980). Also interesting, and perhaps related, is the TCDD-related suppression of tumors in the pituitary, pancreas, thyroid, uterus, mammae, and adrenals. Such tumor incidence suppressions are likely to be related to TCDD → hormonal influences in these tissues. Neither of these negative carcinogenic influences caused by TCDD have been factored into the hazard evaluation of TCDD in any risk assessment to date.

Evidence is continuing to build from a number of laboratories that TCDD affects the adrenals and thymus (Greenlee *et al.*, 1985), thyroid (Rozman *et al.*, 1984; Henry and Gasiewicz, 1987; Romkes, *et al.*, 1987), estradiol-(Umbriet *et al.*, 1988) glucocorticoid and cholesterol-producing systems, as well as epidermal growth factor cellular activity (cf. Greenlee *et al.*, 1987, and references therein). It has been suggested that (1) there is a

relationship of the TCDD Ah receptor protein function to the steroid and thyroid receptor protein functions, and (2) the relationship may be due (in part) to sequence homology among these receptors (Evans, 1988). There are no cross-bindings of these ligands among the other systems'receptors. The sharing of a common motif among the essential receptor proteins indicates a superfamily of receptors which coordinate major cellular systems (Green and Chambon, 1986). TCDD may cause its pleiotropic effects by perturbing the superfamily gene expression by interaction with each of the various hormone-receptor reaction sequelae at their respective functional sites in chromatin.

Given the hormonal relationships of TCDD to cancer (at least in animal test systems), we postulate that TCDD is a hormonal carcinogen. TCDD may be unique in its close association with so many essential organismic control systems, and might be expected to act in a hormonal-like fashion. Moreover, the tight hormone-like binding to the Ah receptor may relate to the supreme cancer potency estimate for TCDD. As a comparative example, estimated cancer potency [to humans, units are in $(mg/kg/day)^{-1}$] based on rodent positive tests are: TCDD (156,000), aflatoxin B_1 (2,900), ethylene dibromide (41), benz[α]pyrene (11.5), bis-(2-chloroethyl)-ether (1.1), DDT/DDE (0.34), vinyl chloride (0.017), and methylene chloride (0.008). Part of this extreme potency is no doubt due to the accumulation of this metabolically stable compound. Lastly, the carcinogenicity effects of TCDD in hormonal organs are also expected to be influenced by the interplay hormonally among the interactive target systems, i.e. direct TCDD effects in one target affect a second target [in part] by disturbance of homeostasis between the targets.

We conclude: the bioassay data designate TCDD as a total carcinogen, which can participate in tumor promotion assays so as to complete the carcinogenicity action started by other carcinogens. TCDD is unique in its supreme cancer potency and close functional relationships with a number of key hormonal regulation systems. Inference to other hormonal actions suggests that TCDD may also mechanistically show a threshold, which would suggest that the traditional assumption made with all carcinogens that there is a finite positive chance of cancer from even at one molecule of exposure (some 15 orders of magnitude lower than total body burden of TCDD) may not be the correct assumption to make in the case of TCDD. The existence of a threshold has not yet been demonstrated and further work on TCDD dosimetry needs to be done to properly assess the carcinogenic hazard.

We think the uniqueness of this compound offers an excellent investigative tool for hormonal carcinogenicity mechanisms. The derivation of risk from human exposure, using quantitative modeling should take into account the qualitative biological concepts discussed here: complete carcinogenicity, presumptive nongenotoxicity or mutation, probable dose-rate limitation at low doses, and hormonal systems interactions.

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16. ABSTRACT

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has a high estimated cancer potency in animals which has been reasoned to imply that TCDD might be carcinogenic to man. The animal cancer data show that TCDD can act in a solitary manner causing tumors without the participation of other known factors. However, there exist animal cancer data indicating that TCDD can act as a tumor-promoting compound. This analysis examines which type of carcinogen and which mechanism best characterize TCDD cancer activity. It is suggested that TCDD acts by a hormonal mechanism to cause cancer in a solitary manner, at low doses, in two species, and in a number of different organs, including rare sites. These observations *in toto* characterize TCDD as a complete carcinogen, which by definition encompasses both initiation and promotion carcinogenic activities.

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