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CARCINOGENICITY OF TCDD: Experimental, Mechanistic, and Epidemiologic Evidence[1]

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INTRODUCTION

In 1977 the International Agency for Research on Cancer (IARC) first reviewed the evidence of TCDD carcinogenicity in humans and other animals (1, 2). The group decided that TCDD was carcinogenic in animals but that carcinogenicity in humans could not yet be reliably evaluated. Epidemiological data from occupationally exposed workers have now established an association between exposure to TCDD and several human cancers (3–15), and we review these data in this chapter.

Recent findings in Seveso, Italy now link environmental exposure of a human population to dioxins with multiple-site cancers (3). Together with other accumulated and increasing evidence on human cancers (4–12), this research provides credible evidence that dioxin exposure is carcinogenic to humans. Although debates continue (13, 16, 17), studies of the effects of dioxins on humans (3–15) and experimental animals (18–20) and investigations of the mechanisms of cancer in mammals (19, 21–23) have provided convincing and increasing evidence that dioxin is a human carcinogen. In

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these cohort studies, exposure levels are significantly higher than typical background levels.

Evidence of dioxin's carcinogenicity was first discovered in experimental animals (24). Basing preventative strategies for potential carcinogens on long-term bioassay findings (24, 25) proved valuable and useful: Nearly 30 of the 110 agents or exposures presently associated with cancer in humans were first identified in long-term chemical carcinogenesis studies (24, 26–28).

General public concern over the hazards from dioxins and related compounds was amplified in July 1976 when an Italian chemical factory that produced trichlorophenol exploded and contaminated a highly populated area and the surrounding countryside with dioxins, primarily 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other reaction products (12, 29–32). Since then, other "accidents" or large-scale occupational and environmental exposures have occurred or been revealed (2, 33). Scientists first became aware of an adverse health effect of TCDD (occupational chloracne) in 1957 (34). Evidence linking TCDD with teratogenicity appeared in 1971 (35), and scientists reported cancer in animals from TCDD exposure in 1977 (36). Reports on the possible association between TCDD exposure and occupational cancer were published in the 1970s and 1980s (1, 37–40).

Since Van Miller et al (36) first discovered the carcinogenic effects of TCDD in animals, several studies have confirmed and extended their early observations (2, 41, 42). We review these later studies here. Although current reports list only one ongoing (and limited) experimental carcinogenesis study (43), considerable research on TCDD-induced carcinogenesis continues, focusing on mechanistic aspects. In addition, many studies exploring the mechanisms of carcinogenesis and other diseases have used TCDD as a model compound (19, 21–23, 44–46). Several of these investigations are discussed in this chapter.

Because TCDD acts like a potent and persistent environmental hormone and growth dysregulator, non-cancer endpoints such as reproductive, developmental, immunological, and neurological toxicity are being recognized as adverse outcomes following exposure to sufficient concentrations of dioxin and structural analogues. However, these are not considered in this chapter, which focuses on the relationships between dioxins and cancer.

EXPERIMENTAL STUDIES IN ANIMALS

Long-term carcinogenesis studies involving laboratory animals exposed to TCDD conclusively demonstrate that this chemical is a "complete" carcinogen, i.e. a chemical that causes cancer in laboratory animals that have not

been exposed to any other known chemical carcinogen (2, 20, 42). The uniqueness of carcinogenic responses in both sexes of various strains and species of laboratory animals exposed to low levels for varying durations supports this classification. In a series of studies beginning in 1977 with rats and ending in 1988 with the TCDD-resistant hamster (47), TCDD consistently induced cancers in a variety of organs and systems, frequently causing tumors not ordinarily observed or having low background incidence in control animals.

Seventeen long-term carcinogenesis studies reported between 1977 and 1988 (no such reports have been published since) have all positively demonstrated that TCDD is a multisite, multistrain, multispecies carcinogen in experimental animals of both sexes. TCDD induces cancers in organs and tissues remote from the site of exposure at dose levels well below the maximum tolerated dose. In fact, a noncarcinogenic exposure level could not be demonstrated, with 0.001 μg/kg per day as the lowest tested dose (2, 19, 20). TCDD also induced cancer in one site in the single study in the "TCDD-resistant" hamster (47). Thus, for practical purposes, TCDD can be considered a complete carcinogen (18, 42).

The 17 studies summarized in Table 1 include information on species, sex, dose, duration, and tumor site. Some of these studies are especially relevant to risk assessment; for example, female rat liver has been emphasized in studies of TCDD carcinogenicity, but the male rat thyroid gland is apparently a more vulnerable site (and one where TCDD has been associated with cancer and other diseases of the thyroid gland in humans). Detailed evaluations of these studies are available (18–20, 23, 41, 42). Despite limited data on tumor promotion by polychlorinated dibenzofurans and coplanar polychlorinated biphenyls, these compounds apparently act primarily as liver tumor promoters, with potencies dependent on their binding affinity to the Ah receptor.

Thus the reported studies show that TCDD is a transspecies (rat, mouse, and hamster), transstrain (Sprague-Dawley and Osborne-Mendel rats; B6C3F1, Swiss-Webster, and B6C mice), transsex, multisite, complete carcinogen. In rats, TCDD induces neoplasms in lung, oral and nasal cavities, thyroid and adrenal glands, and liver (36, 48, 49); in mice it causes neoplasms in the liver, subcutaneous tissue, thyroid gland, lung, and lymphopoietic system (lymphomas) (50–52); and in hamsters it produces squamous cell carcinomas of the facial skin (47). TCDD is considered the most potent of the identified chemical carcinogens because it can induce carcinogenic effects in laboratory animals with exposures as low as 0.001 μg per kilogram of body weight per day. In these carcinogenicity bioassays, TCDD caused tumors at the lowest concentration level tested.

Table 1 Summary of carcinogenicity experiments of TCDD[a]

Sex, Strain, & Species	Routes, Exposures, & Durations	Cancer Results	References
Male Sprague-Dawley rats 10/group	Feed: 0.001–1000 ppb, 78 weeks exposures, + 17 weeks observation	Total tumors increased in all groups but 0.001 ppb	36
M & F Sprague-Dawley rats 86/controls & 50/ exposure group	Feed: 21–2200 ppt (0.001–0.1 μg/kg/day), 2 years	M: tongue, nose, palate; F: Lung, liver, nose, palate	48
Male Swiss mice, 100 controls & 45/ exposure group	Gavage: 0.007–7.0 μg/kg/ week, 1 year exposure, life-span observation	Liver tumors 0.7 μg group; none in 0.007; higher doses died	50
M & F Osborne-Mendel rats, 75/control & 50/ exposure group	Gavage: 0.0014–0.071 μg/kg per day for 2 years	M: thyroid, liver?? adrenal gland; F: Liver, skin, adrenal gland.	49
M & F B6C3F1 mice, 75/control & 50/ exposure group	Gavage: M–0.0014–0.071 F–0.0057–0.29 μg/kg per day for 2 years	M: lung, liver; F: liver, thyroid gland, skin, lymphoma	49
Swiss-Webster mice 45/ control & 30/exposure group	Dermal: 0.001–0.005 mg/ application, 3 times/week for 2 years	F: skin fibrosarcoma; M: same??	51
M & F B6C3 mice 42–50/group	Gavage: 2.5–5.0 μg/kg per week for 52 weeks, observed until 78 weeks intraperitoneal inj: 1–30 μg/kg per week for 5 weeks, observed until 78 weeks	M: liver, F: liver	52
M & F B6C3 & B6C mice 89–106/group		All: lymphoma M & F B6C3: liver	
M Syrian Golden hamsters, 10–24/group	Intraperitoneal injection: 100 μg/kg, 2–6 times, one/4 weeks; subcutaneous injection: 50–100 μg/kg, 6 injections, one/4 weeks for 12–13 months	Both routes: facial skin, squamous cell carcinoma	47

[a] Notes: M = male; F = female; μg = micrograms; ?? = possibly

MECHANISMS OF ACTION

Despite the amount of research devoted to the mechanisms of TCCD-associated toxicological effects, including carcinogenesis, no complete step-by-step or even stage-by-stage model has yet been established. Proposed mechanisms of TCDD-induced carcinogenesis do not account for the many rare tumor types observed in exposed animals. The hypothesis that TCDD's effects are mediated through interaction with an intracellular binding protein termed the Ah receptor is gaining increasing acceptance (21, 44). This receptor system is very specific for dioxin-like compounds, and exists in low copy numbers in the cell, but binds dioxins with a very high affinity (53, 54). Binding of ligand (the dioxinlike compounds) activates the Ah-receptor; the receptor-ligand complex then binds to acceptor sites in the nucleus (at DNA) and probably alters expression of a subset of genes (55, 56).

In two-stage liver or skin carcinogenesis models, TCDD exhibits considerable tumor promotion activity. Here, however, we embrace the concept of "complete" carcinogenicity (42). [Note that such terms as initiation, promotion, progression, completeness, cocarcinogenicity, genotoxicity, and nongenotoxicity are operational terms useful in scientific discourse; but because they have not been accepted as mechanistic terms, their use often leads to misunderstanding (57).]

In the last few years, several studies have helped elucidate the mechanisms of TCDD carcinogenicity in experimental animals. For example, the evidence has recently become considerably stronger that TCDD does not damage DNA directly by forming DNA adducts. However, TCDD may alter the DNA–damaging potential of some endogenous compounds, including estrogens. Also, best-fit mathematical modeling of hepatic foci data indicates an increased mutation rate from TCDD exposure (CJ Portier, M Kohn, L Edler, A Kopp-Schneider, CD Sherman, et al, submitted). In addition, numerous studies report TCDD-mediated modifications of growth factor and cytokine pathways in experimental animals and cell systems. Some of the TCDD mediated alterations observed include changes in epidermal growth factor receptor, transforming growth factor α, estrogen receptor, glucocorticoid receptor, tumor necrosis factor α, interleukin 1β, plasminogen activator inhibitor 2, and gastrin. Because many of these pathways are involved in cell proliferation and differentiation, their disruption is a plausible mechanism of carcinogenic actions. The effects noted above are consistent with the generally accepted conclusion that in multistage models for chemical carcinogenesis TCDD acts as a tumor promoter but is virtually devoid of initiating activity. It is important to keep in mind that tumor promotion and tumor initiation are operational terms and they may involve fundamentally different mechanisms for each chemical or neoplastic endpoint. Further there

is no uniform mechanism for the many classes of chemicals which are tumor promoters in experimental systems.

The growing consensus among researchers is that most, if not all, of TCDD's biochemical and toxic effects require interaction with Ah receptors. However, it must be kept in mind that formation of the Ah receptor–TCDD complex is only the first of many steps involved in the production of biochemical, toxic, and carcinogenic effects. Although our understanding of subsequent steps is increasing, we still know little about certain components of the Ah receptor–mediated responses. Clearly, however, cell-specific factors other than the Ah receptor must be involved in determining tissue responses once TCDD binds that receptor.

Implications For Risk Assessment

Central to the risk assessment of TCDD and its structural analogues are (*a*) characterization of the shape of the dose-response curve for receptor-mediated events (23), (*b*) evaluation of the relevance of animal data in estimating human risks (22, 23, 25, 26), and (*c*) the health consequences of background exposures [1–10 pg toxic equivalents (TEQ)/kg per day] to dioxin and its structural analogues (59). Results from animal studies clearly demonstrate that there are different dose-response curves for different TCDD effects, which is consistent with the dogma for steroid receptor–mediated responses (21).

Assuming that all of TCDD's effects are receptor–mediated, the question arises: Do the effects of dioxin exhibit a threshold (or thresholds) of response? For some effects, like induction of CYP1A1 and CYP1A2 or decrease in hepatic plasma membrane epidermal growth factor receptor, a linear relationship between target tissue dose and effect was shown over a wide dose range (60–62). However, other responses, e.g. cell proliferation or development of preneoplastic foci, exhibit different dose-response relationships (63, 64). Because no single dose-response relationship is applicable to all Ah receptor–mediated events, it may be inappropriate to use a single marker for an Ah receptor–mediated event, e.g. enzyme induction, for estimating dioxin's risks for adverse health effects to humans.

In general, TCDD-associated biochemical and molecular responses such as cytochrome P-450 induction do not show any evidence for a threshold, although no unequivocal conclusions can be drawn until a possible mechanistic link is established between biochemical responses and toxic or carcinogenic effects. Coordinated biological responses such as TCDD-mediated cell proliferation and growth of preneoplastic lesions (foci of cellular alteration in liver) appear to be less sensitive endpoints. However, a high degree of interindividual variation complicates any evaluation of these responses: Some animals do not exhibit any increase in cell proliferation in response to chronic TCDD exposure (63, 63a).

Also controversial is whether the use of experimental animal models is appropriate to the goal of estimating human risks. Evidence increasingly suggests that biochemical responses of humans to TCDD exposure may be similar to those of experimental animals. However, awareness is also increasing that interindividual variation in human responses to dioxin are a complicating factor in risk assessment; some individuals appear to be more responsive than others to numerous environmental chemicals, including TCDD.

A mechanistic basis for interindividual variation is not known, complicating attempts to estimate human risks from experimental animal data. However, several studies on the effects of dioxins indicate that, for the most part, humans and experimental animals respond similarly with regard to biochemical and carcinogenic endpoints (22). Furthermore, all agents and exposure circumstances known to cause cancer in humans that have been adequately tested in animals are also carcinogenic in experimental animals (14, 28, 65–69), thus supporting the public health view of using results from laboratory animals for strengthening preventative strategies.

Much of the controversy surrounding the assessment of risk from TCDD exposure reflects the selection of quantitative mathematical methods: threshold vs linear multistage. Information gained recently about dioxin's mechanisms of action may permit the construction of biologically based models that will remove some of the uncertainty in current risk estimates. These recent approaches and these advances in our understanding (both for mechanisms of tumor formation, and for dose-response relationships relevant to the carcinogenic actions of dioxin) are presented in detail in other reviews (19, 21–23, 46, 58, 63). These are summarized briefly in the following sections.

TCDD-Mediated Mechanisms of Carcinogenesis

GENOTOXICITY Evidence is overwhelming that TCDD is not a direct genotoxic agent. Because "genotoxic" and "nongenotoxic" are controversial and often-misused terms it is prudent to state the primary criteria for nongenotoxicity here (70): TCDD is classified as a nongenotoxic agent because (*a*) it does not bind covalently to DNA (i.e. does not form DNA adducts), (*b*) the results of short-term tests for genotoxicity are negative, and (*c*) TCDD is a potent promoter and weak initiator in multistage models for chemical carcinogenesis. In a study (71) using accelerator mass spectrometry, DNA adducts were not detected in rodent tissue following exposure to TCDD. Accelerator mass spectrometry is extraordinarily sensitive and can detect one adduct in 10^{12} normal nucleotides. For comparative purposes, one adduct in 10^{6} normal nucleotides in rodent tissues associates with carcinogenic responses to benzo[a]pyrene, methylnitrosurea, or NNK in lung

and other tissues. Nonetheless, numerous studies have detected DNA adducts in normal tissues, which do not show any tumors or carcinogenic effects, whereas DNA adducts have not been discovered in organs exhibiting marked carcinogenic effects (72).

Results for TCDD are negative in the *Salmonella typhimurium* test, with a mixed-function, oxidase-activating system present or absent. Negative results were obtained using 13 different bacterial strains, and tests were performed in 9 laboratories (37, 70, 73–76). The National Toxicology Program concluded, after performing its battery of tests for genetic toxicity, that TCDD was nonmutagenic (77, 78). Additionally, several scientific panels have stated that false negatives for TCDD-induced genetic toxicity are highly unlikely (79). TCDD promotes the transformation of C3H 10T1/2 cells, but this response does not reflect an ability to damage DNA directly (80). There is no consistent evidence for increased frequencies of chromosomal aberrations in human populations exposed to TCDD either accidentally or occupationally (37, 70, 76).

Even though TCDD enhanced transformation of C3H 10T1/2 cells induced by N-methyl-N′-nitro-N-nitrosoguanine (MNNG) (80) and was mutagenic to mouse lymphoma cells (81), TCDD is not considered genotoxic (37, 70, 73). Importantly, neoplastic transformation of immortalized human keratinocytes was induced by TCDD at doses as low as 0.1 nM. This is the first evidence of neoplastic conversion of human cells exposed to TCDD (82). In four *Salmonella* tester strains exposed to the components of Agent Orange—2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and esters; TCDD—none exhibited any mutagenic activity (78). In a host-mediated in vivo–in vitro assay using peritoneal macrophages, TCDD transformed these cells in a dose-dependent manner (83); the TCDD cell-transforming potential was seven times that of tetrabromodibenzo-p-dioxin (TBrDD). Agent Orange per se has not been studied for genotoxicity. Both short-term in vitro genetic tests and long-term carcinogenicity bioassays using Agent Orange would be revealing, and provide important information about any interactive effects of the combination.

Although DNA binding is negative in genetic toxicity tests, recent reports have demonstrated that TCDD (50–100 μg/kg) induces single-strand breaks in Sprague-Dawley rats, presumably as a consequence of increased lipid peroxidation (84, 85). In another set of studies, human lymphocytes exposed to polychlorinated dibenzofurans (PCDFs) exhibited an increase in the frequency of sister chromatid exchanges when challenged with α-naphthoflavone (86, 87). PCDFs produce this effect by increasing rates of metabolic activation of α-naphthoflavone to DNA-reactive metabolites. These findings are consistent with the idea that TCDD's ability to induce

drug-metabolizing enzymes (CYP1A1 and 1A2) may lead to an increased formation rate of DNA reactive metabolites of some carcinogens, most notably the polycyclic aromatic hydrocarbons and aromatic amines. However, the opposite effect occurs in some cases: In vivo exposure to both CYP1A1 inducers and polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene actually leads to a decrease in DNA adducts in target tissue (88, 89) and, therefore, to a protective effect through enzyme induction. A reasonable conclusion is that TCDD exposure may increase the rate of DNA adduct formation for some carcinogens but decrease the rate for others. Predictions on DNA adduct concentrations in control and TCDD-treated animals should not be made without experimental data.

Finally, TCDD is designated a nongenotoxic carcinogen because it is a potent tumor promoter and weak initiator in two-stage models of liver (90–94) and skin (95). Penta- and hexachlorodibenzofuran were also found to be potent promoters in mouse skin (96). These findings are discussed in the following section, where we also discuss plausible mechanisms for tumor promoting activity of TCDD (19, 23).

The scientific community generally accepts the idea that most, if not all, of TCDD's biochemical, toxicologic, and carcinogenic effects, including tumor promotion, are Ah receptor–dependent. TCDD provides an excellent case in which to evaluate risk-assessment with respect to receptor-mediated carcinogens. Whitlock (97) has reviewed the steps involved in Ah receptor–mediated induction of one cytochrome P-450 isozyme (CYP1A1). Moreover, mechanisms for TCDD-induced cancer are probably more complex involving interactions of several genes.

INITIATION-PROMOTION STUDIES In chemical carcinogenesis a number of discrete steps produce a genetically altered cell that is then clonally expanded and progresses to a tumor (70, 98–100). Briefly, the process involves damage to a specific site on DNA, a round of cell replication to fix that damage in the genome, clonal expansion of the genetically altered cells (tumor promotion), followed by additional genetic damage and rounds of cell replication (tumor progression); subsequent metastases generally represents the final stage.

Risk assessment models that recognize the multistage nature of chemical carcinogenesis focus on comparisons of the birth and death rates of genetically altered cells and normal cells (21, 98, 99, 101–3). Attempts to distinguish the discrete steps that result in cancer have investigated the roles of protooncogene activation and tumor suppressor gene inactivation. Cell proliferation is an essential component of chemical carcinogenesis; without cell replication, DNA damage would not be "fixed" in the genome, and clonal expansion of genetically altered cells would not occur (104–8). But

regardless of the crucial role played by cellular proliferation in the carcinogenesis process, current evidence does not indicate that cell-proliferation-inducing agents can be predicted to be carcinogenic (106, 109–11). Cell proliferation is essential for cancer development (and it is probably required at each stage in the process) but it is not sufficient for carcinogenesis.

Multistage models of carcinogenesis have been developed to identify the particular stage, or stages, in which carcinogens act to increase tumor incidence. There is a wealth of information on liver cancer initiation-promotion protocols in the scientific literature (112–14). These protocols frequently employ a single initiating dose of a chemical that damages DNA, followed by enhancement of cell replication (induced by either partial hepatectomy or cytotoxicity) to fix that damage in the genome (the initiation stage), and then chronic exposure to a chemical that produces clonal expansion of the genetically altered cells (the promotion stage). Chemicals can increase tumor incidence at either stage. Although these protocols do not elucidate initiation or promotion mechanisms, they have helped us to understand chemical carcinogenesis. Detailed descriptions of initiation-promotion protocols in liver and skin are available (112–19).

Biochemical Responses

The list of TCDD-induced effects in experimental animals and cell systems is expanding. TCDD may alter such normal cell-regulatory processes as cellular proliferation and differentiation, may change metabolic capacity, and affect hormonal pathways. In this section we discuss (*a*) the relevance of various biochemical responses to TCDD to the broader topic of TCDD-mediated cancer, (*b*) whether these biochemical responses are Ah receptor–mediated, (*c*) whether information is available on the role of transcriptional activation in each biochemical response, (*d*) the known dose-response relationships, and (*e*) whether biochemical responses in animal models are consistent with those in humans.

We do not attempt to evaluate all of the biochemical and molecular responses to TCDD in this section (23); instead we focus on those most relevant to carcinogenesis and/or those most studied. We evaluate biochemical responses involving cytochrome P-450 1A1 (CYP1A1), cytochrome P-450 1A2 (CYP1A2), and estrogen receptor (ER).

OTHER BIOCHEMICAL ENDPOINTS TCDD alters a number of pathways involved in regulation of cell differentiation and proliferation. The roles these alterations play in multistage carcinogenesis are not known, but the broad array of effects on hormone systems, growth factor pathways, cytokines, and signal transduction components is consistent with the notion that TCDD is a powerful growth dysregulator. This multiplicity of effects is also

consistent with findings that TCDD alters cancer risk at a large number of sites—findings that may indicate multiple mechanisms of carcinogenicity. Biochemical, molecular, and endocrine changes produced by TCDD involve epidermal growth factor receptor (EGFR), uridine diphosphoglucuronyltransferase (UDPGT) (19, 23, 61, 120), glucocorticoid receptor (121), tyrosine kinase (122), gastrin (123), interleukin 1β (124), plasminogen activator inhibitor 2 (124), tumor necrosis factor α (125), gonadotropin releasing hormone (126), testosterone (127), and luteinizing hormone (128). Though we cannot present further detail here, these changes—all dependent on the Ah receptor—are all potentially significant in carcinogenesis.

CYP1A1 AND CYP1A2 The most-studied biochemical response to TCDD is the induction of cytochrome P-450 isozymes (44, 53, 54), first reported in vivo and in vitro in 1973 (129–31). The hundreds of papers published on the subject since then have dealt with isozyme specificity, time-course, structure-activity relationships, molecular mechanisms of transcriptional activation of the CYP1A1 gene, identification of transcriptional activating factors, tissue and cell specificity, and dose-response relationships (e.g. 23, 132). Whitlock has reviewed the molecular mechanisms responsible for enzyme induction (53, 97).

No mechanism has been demonstrated through which CYP1A1 and CYP1A2 induction following dioxin exposure might cause cancer (or any other toxic endpoint). Considerable controversy surrounds this topic (133). Because CYP1A1 catalyzes the metabolic activation of many chemicals (such as PAHs) to DNA reactive metabolites, researchers have postulated that induction of CYP1A1 might increase the carcinogenic actions of certain chemicals, e.g. PAHs. In most cases, however, preinduction of CYP1A1 diminishes the carcinogenic potency of PAHs such as 3-methylcholanthrene, benzo[a]pyrene, and dimethylbenzanthracene, provided that exposure to the inducing agent is short term (88, 89, 134–36). This "protection" against carcinogenic activity is probably achieved through enhanced metabolism and accelerated detoxification and excretion of chemicals (and metabolites), such as aflatoxin, diethylnitrosamine, arylamines, and urethane (137–139), and occurs at numerous sites including the liver and lungs. For several reasons researchers believe enzyme induction is the mechanism responsible for the protective effect. First, treating mice that are deficient in the Ah receptor with inducers does not protect against PAH-mediated cancer (140). Second, the ability of inducing agents to protect against cancer is positively correlated with their potency as inducing agents (141, 142). Third, the inducing agent must be administered at least one day prior to treatment, which allows sufficient time for the inducer to produce elevated levels of CYP1A1 (143, 144). In summary, it appears that TCDD-mediated enzyme induction in-

creases the rate of detoxification of some carcinogens to a greater extent than it increases the rate of formation of DNA damaging metabolites.

Although the mechanism linking CYP1A1 induction and cancer is not understood, many CYP1A1 inducers are themselves carcinogenic in long-term bioassay regimens; thus, the protective effect of inducing agents appears limited to relatively short-term exposure. For example, benzo[a]pyrene, 3-methylcholanthrene, and TCDD are CYP1A1 inducers and multisite carcinogens (145–50).

The relationship between CYP1A2 induction and the carcinogenic actions of other compounds is less clear than that between carcinogenic actions and induction of CYP1A1. CYP1A2 catalyzes the formation of catechol estrogens from 17β-estradiol (91). The catechol estrogens are considered possible toxic metabolites because they may lead to increased free-radical damage to cellular macromolecules such as DNA (151, 152). This involvement of estrogens may explain why TCDD is a hepatocarcinogen in female but not male rats and why ovariectomy protects against the hepatocarcinogenic actions of TCDD. Also consistent with the hepatocarcinogenicity data is the observation that CYP1A2 is induced in liver but not in extrahepatic organs [with the possible exception of the nasal mucosa (153), which is a carcinogenic target site in rats]. In contrast, CYP1A1 induction occurs in virtually every tissue of the body, a finding consistent with the observation that the Ah receptor is found in a wide variety of cell types.

A number of studies have reported dose-response relationships for TCDD's effects on CYP1A1 and CYP1A2 (23, 60, 91, 129, 131, 153–58); these studies include both single and chronic exposure schedules, time-course evaluations, and species comparisons. Dose-response relationships have been evaluated through quantitation of CYP1A1- and CYP1A2-dependent enzyme activities, Northern blot analyses of mRNA levels, and quantitation of CYP1A1 and CYP1A2 protein by radioimmunoassay and immunolocalization in tissue sections. All of these methods have yielded consistent results.

The single dose ED_{50} for CYP1A1 or CYP1A2 induction is approximately 0.5–1.5 μg TCDD/kg in both rats and mice. In a chronic exposure regimen, the ED_{50} is in the range of 10–25 ng/kg per day (60). The limit of detection for enzyme induction varies depending on the method used for quantitation—i.e. P-450-dependent enzyme activities, mRNA, or protein. Vanden Heuvel et al (147, 148) showed that TCDD-mediated increases in CYP1A1 mRNA were detectable following a single dose of 0.1 ng/kg, which produces a concentration of TCDD in the liver equivalent to a chronic dose of 2–5 pg/kg per day.

Evaluations of various data sets on TCDD-mediated dose-response relationships have revealed important information. The shape of the dose-re-

sponse curves for receptor-mediated events may be analyzed using the Hill equation (159). A Hill coefficient of 1 suggests a linear relationship between exposure and dose throughout the experimental dose range and would predict a proportional relationship between concentration of TCDD in the target tissue and biological response at all dose levels. This relationship would imply that the response has no practical threshold, or no no-effect level. Hill coefficients >1 would indicate sublinearity in dose response, whereas a Hill coefficient of <1 would indicate supralinearity for response in the low-dose region. Analysis of both single-exposure and chronic-exposure data for CYP1A1 and CYP1A2 induction in rat or mouse liver indicates a Hill coefficient of slightly greater than 1 for CYP1A1 and slightly less than 1 for CYP1A2 (62, 64). Although these analyses involve extrapolation beyond the range of the experimental data, they are consistent with the hypothesis that a practical threshold does not exist for TCDD-mediated induction of CYP1A1 and CYP1A2.

Reports on immunological detection of induced CYP1A1 and CYP1A2 in liver sections obtained from rats exposed chronically to TCDD suggest heterogeneity of hepatocyte response to TCDD (60, 160). For example, relatively low doses of TCDD (1 ng/kg per day) appear to maximally induce some cells around the centrilobular region of the liver. Increasing doses of TCDD concomitantly increase the number of cells responding rather than the amount of induction in responding cells. These data, which document cell differences in sensitivity to induction, complicate evaluation of dose response relationships. For example, some hepatocytes appear to be induced maximally by low doses of TCDD, whereas others exhibit no detectable P-450-induction response to these same doses. As mentioned above, no mechanism linking P-450 induction and cancer has been established. Evaluation of P-450 induction and TCDD-mediated cell proliferation by immunocytochemical methods in rat liver reveals that cells that express CYP1A1 and CYP1A2 are different from those exhibiting TCDD-mediated increases in DNA replication (21, 46, 63).

Placentas from Taiwanese women who had been exposed to rice oil contaminated with PCDFs have markedly elevated levels of CYP1A1 (161, 162). Comparison of these data with induction data in rat liver suggests that humans are at least as sensitive as rats to the enzyme-inducing actions of TCDD and its structural analogues (163). Consistent with this hypothesis is the observation that the in vitro EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is approximately 1.5 nM in either rodent or human lymphocytes (164). However, binding of TCDD to the Ah receptor occurs with a higher affinity in rat than in human cellular preparations (165, 166). This difference may be related to the greater lability of the human receptor during tissue preparation and cell fractionation procedures

(167). In any event, it does appear that human cells contain a fully functional Ah receptor (168, 169), as evidenced by significant CYP1A1 induction in tissues from exposed humans—a response that occurs with similar sensitivity in experimental animals.

ESTROGEN RECEPTOR Interactions of TCDD and estrogens are critical to some carcinogenic responses to TCDD (170, 171). Although the precise mechanisms of those interactions have not yet been established, recent data indicate that TCDD effects on the estrogen receptor (ER) and on estrogen metabolism are involved. The mechanisms for TCDD-estrogen interactions appear to be tissue specific. Of particular interest is the finding that TCDD increases liver tumor incidence in female rats while at the same time decreases tumor incidence in organs such as the mammary gland, uterus, and pituitary gland (2, 18, 20, 48); these decreases may simply be due to decreases in body weight often seen in TCDD-exposed rodents (172, 173). Therefore, TCDD-estrogen interactions are examined separately for liver and other endocrine organs.

Liver contains a fully functional ER, which possesses characteristics similar to those identified for ER in mammary gland and uterus (174–76). For example, liver exhibits high affinity binding for 17β-estradiol and other potent estrogens, liver ER-binding is specific for estrogens, the ligand receptor complex interacts reversibly with DNA, and this interaction leads to transcriptional activation of estrogen-responsive genes. Synthesis of hepatic ER, unlike ER in other target tissues, is under pituitary control (177). A single dose of TCDD to rats decreases binding capacity of the hepatic ER, and this effect correlates with a decrease in ER protein (178–82). TCDD also decreases rat hepatic ER in chronic exposure experiments, with a threefold decrease evident following a dose of 100 ng/kg per day for 30 weeks (93).

TCDD decreases hepatic ER binding in C57Bl6 mice, but a much higher dose is needed to produce this effect in congenic mice deficient in the high affinity Ah receptor. TCDD-mediated decreases in ER are thus dependent on the Ah receptor (183). Dose-response studies in mice demonstrate that the single dose ED_{50} is approximately 0.7 μg TCDD/kg, which is similar to the ED_{50} for other biochemical endpoints such as CYP1A1 induction, loss of hepatic plasma membrane EGF receptor, and induction of UDPGT. The observation that TCDD decreases hepatic ER is in apparent contradiction to the finding that TCDD increases hepatocyte proliferation since ER is thought to produce mitogenic signals. However, quantitation of ER in control and TCDD-treated rats was done using preparations from liver homogenates. Immunolocalization studies are needed for more careful evaluation of the relationship of ER concentrations to cell proliferation in normal and pre-neoplastic cells.

In addition to effects on hepatic ER, TCDD may influence estrogen action

in another way. CYP1A2 efficiently catalyzes the conversion of estrogens to catechol estrogens in liver (91, 184). CYP1A2 is not found in extrahepatic tissues, with the possible exception of the nasal cavity, so catechol estrogen would be expected to form only in liver. Researchers postulate that catechol estrogens possess macromolecule-damaging properties as a consequence of free radical generation (151, 152). Therefore, TCDD may increase the DNA-damaging capacity of catechol estrogens through enhanced metabolic activation in liver as a function of CYP1A2 induction. This effect may, in part, explain the carcinogenic actions of TCDD in female rat liver and is consistent with the knowledge that ovariectomy protects against the hepatocarcinogenic actions of TCDD (48, 92, 93). Importantly, cancer is more than a two-stage process, and the stage-specific actions of TCDD in multistage cancer models are not known, although TCDD-mediated cell proliferation and possible indirect genotoxic effects may be critical at more than one stage. Vickers and Lucier (185) have proposed a hypothetical mechanistic scheme for TCDD-mediated liver cancer.

Chronic TCDD exposure decreases tumor incidences in pituitary, mammary gland, and uterus, which may reflect TCDD's effects on ER, estrogen metabolism, and/or body weight. As discussed earlier, TCDD decreases uterine ER concentrations in cytosolic and nuclear fractions of rats and mice, and these changes are associated with diminished estrogen action in in vivo as well as in vitro studies. TCDD also increases estrogen metabolism, presumably as a consequence of CYP1A2 in liver and UDPGT induction in liver and extrahepatic tissues (186). Likewise, addition of TCDD to a breast cancer cell line (MCF-7) results in increased estrogen degradation (187). However, only small effects are seen on serum 17β-estradiol levels following administration of TCDD to either rats or mice (188). Therefore, effects on serum estradiol are considerably less sensitive than effects on the uterine receptor. This comparison has led investigators to conclude that the antiestrogenic actions of dioxins are primarily caused by effects on ER levels in reproductive tract tissues. Final evaluation on the role of estrogen metabolism awaits data on concentrations of estrogens in responsive cells of control and TCDD-treated rats, which may be different than serum estradiol levels. Clearly TCDD possesses antiestrogenic properties that are probably important for decreasing the incidence of tumors in some reproductive tract and endocrine organs. Although numerous studies have documented that the estrogen receptor is found in virtually every tissue of the body, the effects of TCDD on human estrogen receptors have not been studied in vivo.

EPIDEMIOLOGICAL STUDIES IN HUMANS

For practical and public health purposes, the weight of experimental data, bolstered by mechanistic insights, indicate that TCDD should be considered

Table 2 Summary of epidemiological evidence of TCDD carcinogenicity

Location	Target sites	SMR (C.I.)[a]	References
Germany	Cancer mortality	201 (122–315; 90% CI); 20+ years after exposure	5
Germany	Cancer mortality	187–182[b]; 20+ years employment 161–187[b]; employment before 1955 142–178[b]; group with highest exposure	7
	Total	124–139 (100–152)–(110–175)[b]	
	Lung[c]	167 (109–244)	
	Hematopoietic[c]	265 (121–503)	
	Breast	265 (98–409)[d]	
USA	Cancer mortality	115 (102–130); total cohort 146 (121–176); >1 year & >20 year latency	6
	Connective and soft tissues	922 (190–2695); >1 year & >20 year latency	
	Respiratory	142 (103–192); >1 year & >20 year latency	
	Lung	137 (98–198)[d]	
Seveso, Italy	Hepatobililary	333 (130–810); female Zone B	3
	Hematopoietic	210 (100–430); male Zone B	
	Lymphoreticulosarcoma	570 (170–1900); male Zone B	
	Multiple myeloma	530 (120–2260); female Zone B	
	Myeloid leukemia	370 (90–1570)[d]; female Zone B	
	Non-Hodgkin's lymphoma	200 (120–360); male & female > 5 years	
	Connective and soft tissues	280 (100–730); male Zone R	
	Soft tissue sarcoma	350 (120–1040); male & female > 5 years	

[a] Standard mortality ratio: observed cases/expected cases times 100 (95% confidence interval).
[b] First SMR of cohort versus national West Germany, second SMR of cohort versus worker cohort from gas supply company.
[c] SMR compared to gas workers
[d] CI includes 100, and thus $p > 0.05$; however, at 90% CI, each of these would probably exhibit $p < 0.05$.

as a probable carcinogen in humans. Epidemiological studies have substantiated this conclusion by showing significant associations between relatively high exposures to dioxins and increases in cancers of the respiratory system (particularly lung), connective and soft-tissue sarcomas, hematopoietic system (lymphomas, myeloma, lymphoreticulosarcoma), liver, and in total cancers (Table 2). Workers exposed to chlorophenoxy herbicides, which

may contain dioxins, experience higher risks of cancers of the testicle, thyroid gland, and other endocrine glands.

Several epidemiological studies have been conducted on the adverse health effects associated with exposures, through manufacturing or application, to phenoxyherbicides and chlorophenols contaminated with dioxins. Like animal carcinogenicity data, most epidemiology studies have focused on occupational or accidental exposures to TCDD. The greater accuracy of body burden exposure assessments after measurements of TCDD levels in the serum (rather than milk and adipose tissue), which became possible in the late 1980s (189), has created a broader basis for and enhanced the validity of epidemiology studies. Observed health effects can now be related to actual tissue levels rather than estimated concentrations, and the question of dose-response relationships can at least be partially addressed.

Three recent studies of various exposure levels provide evidence for increased cancer in chemical workers with high occupational exposure to TCDD (Table 2). Fingerhut et al (6) reported a cohort study on over 5000 male chemical workers in the U.S.. This cancer mortality study showed a significant increase in overall cancer, expressed in standard mortality ratios (SMR: observed deaths/expected deaths $\times$ 100). Because of the typically long latency period (time since first exposure) for the development of chemically induced cancers, a subcohort was identified with a 20-year latency and at least 1 year of exposure. In this subcohort, the cancer mortality rate showed an excess of 46% (SMR 146, higher than the SMR 115 in the overall cohort). In addition to overall cancer, increased cancer at specific sites (soft tissue sarcoma and respiratory tract tumors) was detected in the long latency and high exposure subcohort.

Two other epidemiological studies involve chemical workers in Germany, each with smaller cohorts. Manz et al (7) investigated the cancer mortality among 1184 men and 399 women employed between 1952 and 1984 in a chemical plant that produced herbicides contaminated with TCDD. After an outbreak of chloracne in 1954, the company (Boehringer, Hamburg) changed the production process to reduce TCDD contamination. Cancer mortality was increased in the cohort overall, but more significantly among men employed 20 or more years and among men who began employment before 1955 (high TCDD exposure and long latency subcohort). As in the NIOSH study (6), the SMR data indicated increases in malignant neoplasms in overall sites (SMR 142), as well as for lung and hematopoietic systems.

Only 7% of the women worked in locations with expected high exposure, and no increased risk of death from cancer was observed, with the important exception of a significant increase in breast cancer mortality (SMR 215, based on nine deaths). Interestingly, TCDD does not appear to induce tumors of the mammary gland in rodents.

Zober et al (5) reported an increase in overall cancer mortality (SMR 117) in a cohort of 247 workers who had been exposed to TCDD in a 1953 accident in a chemical plant (BASF). A subcohort was selected based on the development of chloracne and, therefore, suspected high exposure. The authors reported a twofold excess in cancer mortality in this subcohort.

These studies all show an increase in overall cancer mortality after exposure to TCDD, especially after high exposure and long latency, which might indicate a dose-response relationship for the development of cancer. Table 2 summarizes the SMRs of the studies overall and in the high exposure subcohorts. Dose-response estimates in epidemiological studies pose several difficulties: (*a*) exposure to TCDD usually occurs as a part of complex mixtures, and background exposure to dioxins makes it difficult to determine control cancer rates; and (*b*) although more information is available on concentrations in human tissue (189, 190), little is known about the impact of background exposure on the occupancy of the Ah-receptor. Few epidemiological studies have actually quantitated dioxin exposure.

There is still controversy on whether or not dioxins cause cancer in humans, and especially about their carcinogenic potency. In particular, there is a possibility that soft tissue sarcoma and malignant lymphoma reported earlier in epidemiologic studies may be caused by chemicals other than dioxin (16). Nonetheless, the experimental, epidemiological, and mechanistic findings, taken together, lead to a strong association between exposure to TCDD and cancer in humans.

Another problem is the lack of site consistent specificity for the carcinogenic action of TCDD in the epidemiological studies. However, dioxin is a multisite carcinogen in rodents, and the apparent lack of site specificity in humans is, therefore, consistent with the observations from studies using laboratory animals. In most cases, the tumors observed in humans exposed to TCDD and related chemicals have also been induced in experimental animals. Other epidemiological studies not yet published and/or currently being conducted should help to clarify some uncertainties and refine or identify other specific risks resulting from dioxin exposures in terms of cancers and other adverse health effects, such as reproductive toxicity, neurotoxicity (e.g. 191, 192), and other diseases, including endometriosis.

Bertazzi et al (3) reported on a ten-year follow-up of the Seveso accident (1976–1986) that shows increased cancer risks among the various exposed cohorts. Significantly increased incidences of cancers were reported for various organs including: liver; the hematopoietic system (non-Hodgkin's lymphoma, lymphoreticulosarcoma, multiple myeloma, myeloid leukemia); and connective and other soft tissues (Table 2). Only in rare instances would significant indications of environmentally caused cancers be expected only ten years after initial exposure. Also, the results stem from a single, high

explosion-exposure, followed by intensive clean-up, although one might consider these exposures "continuous" due to environmental and biological persistence. These findings are, therefore, particularly important, and even higher rates of site-specific cancers may be anticipated. These results (17), together with the increased cancers reported in dioxin-exposed cohorts from other independent studies, clearly establish that TCDD and other dioxins are carcinogenic to humans exposed to relatively high levels from occupational or accidental exposures.

SUMMARY AND CONCLUSIONS

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and its structural analogues, polychlorinated aromatics (e.g. chlorinated dibenzodioxins), dibenzofurans, and polychlorinated biphenyls, are ubiquitous environmental pollutants that produce a broad spectrum of biochemical and toxic effects in animals and humans (54, 132, 192). Dioxins are produced inadvertently during manufacture of chlorinated phenols and phenoxy herbicides, chlorine bleaching of paper pulp, and combustion of chlorine-containing waste (1, 2, 37, 59).

TCDD, the prototype compound of these polychlorinated hydrocarbons, is one of the most toxic chemicals tested in laboratory animals and is the most potent multisite carcinogen (18–20, 41, 42, 48, 49). Because of the acute toxicity, high carcinogenic potential, and ubiquitous presence as trace contaminants in food, water, and soil (and therefore continuous human exposure) (59, 190, 193), TCDD and related compounds are of great public health concern. Considerable controversy still centers on how best to regulate TCDD and its cogeners (22, 133). A central issue in risk assessment is whether humans are a species resistant or sensitive to the effects of dioxins. There are wide differences among species to the effects of dioxins (30, 31, 194), but it is unclear whether carcinogenic responses to TCDD exposure would vary as much as acute toxicities. Risk assessment is also hampered by uncertainty about dose-response relationships, especially following chronic low-dose exposure to dioxins (23).

The hallmark of dioxin toxicity is a significant tissue- and species-specificity. Although we do not completely understand the mechanism(s) of action, most if not all known effects of TCDD seem to involve an initial interaction with an intracellular binding protein, termed the Ah-receptor (44, 53) (or dioxin-receptor).

Assuming that binding to the receptor results in a biological response, the extent of response should relate to some function of receptor occupancy. The magnitude of response and the shape of the dose-response curve are dictated by amounts and activities of receptor status in the particular tissue (as well as status of other proteins necessary for the

response). Various target genes and responses may have different dose-response curves even within the same tissue, which means they vary in their sensitivity to dioxin exposure. Also, if a certain number of receptor molecules must be occupied to result in a measurable biological response, there may be a practical threshold for dioxin exposure, which means there might not be a measurable effect below a certain exposure or tissue concentration. Thus, to more accurately assess quantitative risk for humans, we need additional information on background exposure and resulting receptor occupancy.

Dose-response relationships of dioxin-induced effects are described in various in vivo and in vitro systems. Even assuming that all effects are mediated through the Ah-receptor, the shape of dose-response curves varies for different endpoints or in different target tissues. The possibility of linearity in the low dose range (nonthreshold behavior) cannot be rejected on the basis of the assumption that the effect of dioxin is receptor mediated.

Another conclusion is that humans are very similar to rodents in their sensitivity of response to biochemical effects. Several systems are available to make animal-to-human comparisons and to identify specific biomarkers for dioxin-induced responses. Because of the great variability in the toxic response in tissues and species, we need more information on the shape of dose-response curves of dioxin-mediated effects after long-term low dose exposure, as well as their relevance to toxicity and carcinogenicity. Dose-response relationships of biological markers need to be better characterized in animal models and compared, when possible, to dose-response relationships in humans. Much research has focused on defining the shape of dose-response curves for multiple Ah receptor–mediated events in animal models. Together with information on animal-to-human comparison, individual variation, human background exposure, and receptor occupancy, these results will be integrated into a more biologically (or mechanistically) based risk assessment for humans.

In light of the collective experimental, epidemiological, and mechanistic evidence of TCDD carcinogenicity (Table 3), the prudent course of action would be to minimize to the greatest extent possible all potential exposures to this and other dioxins and phenoxyacid herbicides. To the extent that sound agricultural policy and practice makes feasible, exposures should be eliminated.

VISIONARY AFTERWORD

Lorenzo Tomatis concluded his introductory remarks to an article in an *IARC Scientific Publication* on cancer causes with these comments (195):

Table 3 Conclusions of carcinogenesis and epidemiological studies

1. TCDD causes cancer in animals and in humans:
   In animals:
   - •in multiple species and animals strains: mice (Swiss, B6C3F1, B6C), rats (Sprague-Dawley, Osborne-Mendel), hamsters (Syrian Golden);
   - •in both sexes;
   - •in multiple organs and tissues: nose, palate, tongue, adrenal glands, skin, respiratory system, liver, thyroid gland;
   - •over a range of exposure concentrations;
   - •by multiple routes: feed, gavage, dermal, injection;
   - •after various durations of exposure: 5, 16, 78, and 104 weeks; life span

   In humans:
   - •after high occupational and accidental exposure;
   - •in both sexes;
   - •in multiple organs and tissues: respiratory system, thyroid gland (?), connective and soft tissues, hematopoietic system, liver

2. Evidence for complete carcinogenic activity of TCDD includes:
   - •results from whole-animal studies;
   - •induction of uncommonly occurring and typically nonpromotable site-specific cancers;
   - •multiple-site carcinogenicity;
   - •irreversibility of neoplasia;
   - •neoplastic transformation of human cell lines;
   - •modelling of hepatic focal lesion data

3. Suggested mechanism(s) of carcinogenesis, TCDD is a(n):
   - •dysregulator of cellular differentiation and/or cell division;
   - •a promoter or enhancer of initiated cells;
   - •Ah receptor–mediated tumor promoter;
   - •classical "non-genotoxic" complete carcinogen;
   - •weak initiator;
   - •Ah receptor–mediated cytochrome P-450 inducer

4. Exposure of animals and humans to TCDD and other dioxin & dioxin & dioxin-like compounds also indicates:
   In animals:
   - •TCDD induces phase I and phase II drug metabolizing enzymes;
   - •TCDD is extremely toxic—immunotoxic, fetotoxic and teratogenic, impairs reproductive performance, induces endometriosis, carcinogenic at very low exposures

   In humans:
   - •all humans have dioxin body burdens;
   - •TCDD and related compounds are potent cytochrome P-450 inducers;
   - •TCDD's biological half-life is ~7 years;
   - •TCDD and related compounds are chloracnegenic and associated with cancer and other diseases

   In experimental models:
   - •TCDD-induced Ah receptor–mediated effects exhibit different dose-response relationships for various responses in various tissues

The notion of multidisciplinarity is penetrating even the most impervious strongholds of pure science. It would be most beneficial if it were clear to all scientists that, not only are the various areas of research not mutually exclusive or incompatible, but, on the contrary, cross-fertilization may ensure greater possibilities of success. Thus (*i*) basic and applied research, studies on the mechanisms of carcinogenesis and research on etiology and prevention are neither opposite nor separate areas of scientific activity; they must be, and indeed are, closely interrelated; and (*ii*) the assumption that only basic research is 'true' science and all other approaches to the primary prevention of cancer are not, is not justified and may reflect a certain degree of intellectual snobbery.

There is a clear continuity between studies of the mechanisms of disease, epidemiological and laboratory-based investigations on etiology, and implementation of primary prevention. To consider basic and applied research as separate and competing areas is a grave error which can only serve the purpose of preventing scientists from forming a common front in spending the available resources rationally and efficiently and perhaps obtaining more of them.

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