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12. These values are closer to the Venera findings than those used in (*8*); the reference longitude (see text) was chosen such that the values apply to the mean radius of 6050 km (*4*).
13. The analysis of the Mariner 5 radio tracking data [J. D. Anderson and L. Efron, *Bull. Amer. Astron. Soc.* **1**, 231 (1969)] yielded an upper bound on $[C - (A + B)/2]/M_{a0}^2$ of 10^{-5}, which is difficult to reconcile with a value of $(B - A)/C$ of the order of 10^{-4}. Aside from (remote) possibilities, such as Venus not spinning about its axis of maximum moment of inertia, the discrepancy may be explained by errors in the values used for κ and $\sigma(\phi)$ or by an unrealistic bound for the tracking data, or by a combination.
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15. The earth-spacecraft radio tracking data will also be sensitive to variations in the gravity potential, but only to the harmonics of relatively low degree and order. For these, the tracking data can serve to check the results from the direct observations of the surface at two frequencies, and perhaps to calibrate $\kappa(0,\lambda)$.
16. Research at the Haystack Observatory is supported by NSF grant GP-25865 and NASA grant NGR 22-174-003, contract NAS 9-7830.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin: A Potent Inducer of δ-Aminolevulinic Acid Synthetase

Abstract. *2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, a toxic contaminant frequently formed during the synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid, was shown to be a potent inducer of hepatic δ-aminolevulinic acid synthetase in the chick embryo. As little as 4.66×10^{-12} mole of the contaminant per egg produces a significant increase in the activity of the enzyme. Induction of the enzyme is related to the dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and, in contrast to that produced with other drugs, is prolonged in time, with 70 percent of the maximum induced activity present 5 days after a single dose. This contaminant is implicated as the likely causative agent in an outbreak of porphyria cutanea tarda in workers in a factory where 2,4,5-trichlorophenoxyacetic acid was being synthesized.*

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an unwanted contaminant formed during the synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (*1*) (Fig. 1). This contaminant, TCDD, is perhaps the most potent small-molecule toxin known; the oral LD_{50} (mean lethal dose) in guinea pigs is 1 μg per kilogram of body weight (3×10^{-9} mole/kg) (*2*). The widespread use of 2,4,5-T as a defoliant in Vietnam (*1*, *2*) and the discovery of the teratogenic potency of TCDD (*3*) have caused concern about the potential public health hazard created by contamination of the environment with TCDD. The chemistry of the toxin has been extensively investigated, but little is known about its biological actions (*1*, *4*).

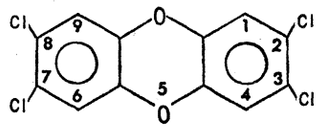


Fig. 1. Structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

In 1964, Bleiberg *et al.* (*5*) reported an outbreak of occupationally related acne and porphyria cutanea tarda (PCT) among workers in a factory where 2,4,5-T was being produced. The acne was shown to be directly attributable to TCDD (*6*). Porphyria cutanea tarda is an acquired defect in hepatic porphyrin metabolism characterized by uroporphyrinuria, photosensitivity as manifested by blisters, and mechanical fragility of the skin (*7*). The etiology of PCT in these factory workers is unclear, but upon reinvestigation of the factory in 1969, we discovered that the PCT had disappeared in all workers following the institution of procedures to reduce TCDD contamination (*8*). Hepatic porphyria can be produced experimentally by a number of drugs, all of which have the ability to stimulate the activity of the initial enzyme in heme synthesis, δ-aminolevulinic acid synthetase (ALA synthetase) (*9*, *10*). Stimulation of this enzyme is thought to represent induction, that is, enhanced protein synthesis (*9*).

We report here that TCDD is an inducer of ALA synthetase, and is at least three orders of magnitude more potent than any other compound known to produce experimental porphyria.

The chick embryo was chosen as the experimental animal because (i) it is highly sensitive to the toxic effects of TCDD (*11*), (ii) induction of ALA synthetase in the liver is well characterized in the chick embryo (*12*), and (iii) the egg is a closed system, which reduces the risk of laboratory contamination.

Halogenated dibenzo-*p*-dioxins were dissolved in *p*-dioxane. Fertile chicken eggs, 15 to 20 days of gestation, were injected with 25 μl of the chemical solution or of solvent alone, through a small hole punched into the shell over the air sac. After the appropriate time interval, the animals were killed, and the activity of hepatic ALA synthetase was assayed (*13*). The TCDD produced a dose-related increase in ALA synthetase activity (Fig. 2). Even at the lowest dose tested, 4.66×10^{-12} mole per egg (1.5 ng), there was significant ($P < .05$) doubling of enzyme activity. Enzyme activity increased more than 35-fold at the highest dose tested, 1.55×10^{-9} mole per egg (0.5 μg). The in vitro addition of TCDD to a reaction mixture containing control liver did not increase enzyme activity. The stimulation of ALA synthetase in

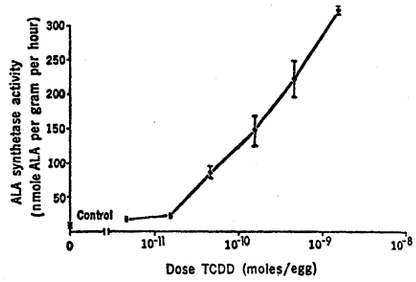
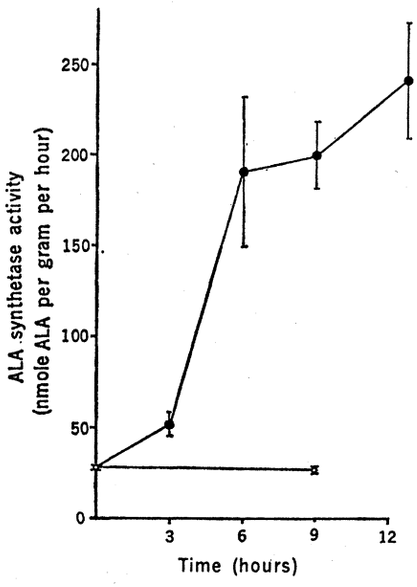


Fig. 2. The induction by TCDD of ALA synthetase: dose-response relationship. Chicken eggs of 17 days' gestation were injected with 25 μl of solvent (control), or of solvent containing various doses of TCDD. The embryos were killed 48 hours later and assayed for hepatic ALA synthetase activity (*13*). The points represent the mean ± standard error of three or four groups of pooled livers.

Fig. 3. Time course of induction of ALA synthetase. Chicken eggs of 20 days' gestation were injected with 25 μl of solvent containing 4.66×10^{-10} mole of TCDD (●) or of solvent alone (○). At the indicated intervals, the embryos were killed, and their livers were assayed for ALA synthetase activity (*13*). Each point represents the mean ± standard error of four groups of pooled livers.



vivo can be prevented by the simultaneous administration of actinomycin D (30 μg) or cycloheximide (10 μg), at doses which did not kill the embryos. Hence, the stimulation of ALA synthetase produced by TCDD and other drugs probably represents increased synthesis of the enzyme, and not activation or decreased degradation (*14*).

The time course of enzyme induction (Fig. 3) has an initial lag phase, probably representing drug absorption and synthesis of messenger RNA, and then a rapid rise in enzyme activity reaching near maximum levels by 6 hours. In contrast to other porphyrigenic compounds that have been tested in this system (for example, allylisopropylacetamide and diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate), which have transient effects on ALA synthetase, the induction by TCDD was prolonged. Embryos (15 days old) given 4.66×10^{-10} mole of TCDD per egg (150 ng) still had 70 percent of their maximum induced enzyme activity after 5 days. The prolonged duration of induction is a reflection of the long biological half-life of TCDD (*15*).

Studies with other halogenated dibenzo-*p*-dioxins suggest that the halogen atoms must occupy at least three of the 2,3,7, and 8 positions on the ring, in order to induce ALA synthetase. The 2,3,7-trichloro- and 2,3,7-tribromo-isomers are potent inducers, while 2,3-dichloro-, 2,7-dichloro-, 2,8-dichloro-, 1,3,6,8-tetrachloro-, and 1,2,3,4,-tetrachloro-isomers all fail to induce at doses up to 2.5 μg per egg. Of the limited number of halogenated dibenzo-*p*-dioxins which have been tested for lethality and the ability to produce acne (*16*), those which are toxic at low doses also induce ALA synthetase.

Induction of ALA synthetase by TCDD is the first specific biochemical action identified for this toxic compound. The relationship between enzyme induction and the delayed hepatic necrosis presumably responsible for the lethality of TCDD is not known. However, it seems most likely that the outbreak of PCT in workers in the factory producing 2,4,5-T is attributable to induction of hepatic ALA synthetase by TCDD. While other chlorinated compounds present in the factory were found to stimulate ALA synthetase activity in our system, TCDD is by far the most potent inducer, and the reversal of the porphyria occurred when TCDD contamination was reduced. We suggest routinely monitoring the urinary porphyrins in workers in factories producing 2,4,5-T to assess their exposure to TCDD.

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