Sensitive Non-Carcinogenic Effects of TCDD in Animals

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Exposure to TCDD and related chemicals has led to a plethora of effects in multiple species, tissues, and stages of development. Responses range from relatively simple biochemical alterations through overtly toxic responses, including lethality. The spectrum of effects shows some species variability, but many effects are seen in multiple wildlife, domestic, and laboratory species, ranging from fish through birds and mammals (1). The same responses can be generated regardless of the route of exposure, although the administered dose may vary. The target tissue concentration appears to be the appropriate dose metric for reversible responses and for developmental effects (2,3). However, it is not yet clear whether some form of the area under the concentration-response curve may be appropriate for irreversible effects such as oxidative damage, porphyrin accumulation, and cancer.

Many of the effects often attributed to TCDD are associated with relatively "high" doses (1). This includes lethality, wasting, lymphoid and gonadal atrophy, and chloracne, one response which occurs in humans which is unequivocally associated with exposure to TCDD and related chemicals. Hepatotoxicity, including fatty infiltration, hyperplasia, and porphyrin accumulation, require relatively high doses. Neurotoxicity in adult animals also appears to be a relatively insensitive response. Cardiotoxicity, while occurring at low doses in chickens, appears not to be a sensitive target in mammals. Hyperplastic and metaplastic changes in various non-human primate tissues also require relatively high doses. Changes in multiple hormonal systems are seen, including effects on estrogens, androgens, glucocorticoids, thyroid hormones, insulin, gastrin, etc. In some cases, the level of the hormone is altered, either by decreased synthesis or by enhanced catabolism. In other situations, the number of receptors is altered, in a manner which is tissue, sex, and age-dependant. In still other situations, the plasma transport of the hormone is altered. Changes in growth factors and their receptors, such as retinoic acid, EGF/TGFα, and TGFβ have also been noted. Until recently, induction of oxidative stress appeared to require high doses. However, the recent report demonstrating elevated measures of oxidative damage in brain of adult mice following 90 days of exposure to 0.45 ng TCDD/kg/day (4) suggests that further investigation of this response following chronic

exposure to low doses is warranted.

The most sensitive effects observed in multiple species appear to be developmental, including effects on the developing immune, nervous, and reproductive systems, alterations in the adult immune system, and biochemical alterations. Adverse developmental effects, including growth retardation, lymphoid atrophy, hemorrhage, edema, and fetolethality, have been seen in many species studied at doses below where any overt toxicity is seen in the mother. Frank terata are relatively rare, except in the mouse where cleft palate and hydronephrosis are diagnostic of exposure to dioxin and related compounds. These responses have been shown to involve alterations in both proliferation and differentiation of specific cell types in the developing embryo/fetus. Altered dentition has been seen in both rats and mice.

Among the most important observations of the past several years is the demonstration of functional developmental toxicity in multiple species by several laboratories. Prenatal exposure to rats during organogenesis results in multiple effects on the offspring, many not obvious until puberty or even later. Some of the key findings have been altered mating behavior (5) and decreased sperm count in the male pups (6,7), and changes in the external genitalia of the female pups, including cleft phallus and a persistent vaginal thread (8). Premature reproductive senescence has also been seen in the female offspring. The decreased sperm count, as well as premature eye opening, could be observed following a dose as low as 50 ng/kg on GD15 (9); the persistent vaginal thread was noted following exposure of 200 ng/kg to the dam (10). Similar effects on both male and female offspring were seen in hamsters exposed to 2 ug/kg on GD 12 (7). Mice appeared to be less sensitive to these developmental effects (11) than were developing rats or hamsters. In addition to effects on the developing reproductive system, prenatal exposure to rats and hamsters has been shown to permanently alter the set point for core body temperature (12,13). Other effects on the nervous system have been indicated by hearing deficits in offspring of pregnant rats exposed on GD19 to 300 ng/kg (14). Persistent immunotoxicity, as measured by suppression of delayed type hypersensitivity, has been recently shown to occur in rat pups following a single exposure of the dam on GD14 to 100 ng/kg (15).

Adverse effects on non-human primates have occurred following low-dose, chronic treatment. Exposure of Rhesus monkeys to TCDD in the diet (25 ppt), resulting in a daily dose of ~0.8 ng/kg/day, led to fetal loss, primarily due to spontaneous abortions (16). A dose of ~0.15 ng/kg/day (5 ppt in the diet) resulted in deficits in object learning in the young monkeys (17). Four years of exposure to the higher dose (~0.8ng/kg/day) lowered the ratio of helper to suppressor T cells and altered macrophage function, although there was no clinical evidence of immunodeficiency (18). However, exposure to both 5 and 25 ppt TCDD in the diet for four years was associated with an increase in endometriosis in the adult females seven to ten years later (19). Exposure of young marmosets to ~0.2 ng/kg/day altered the T cell subsets (20); however, this was not associated with any obvious functional deficit (21). In contrast, the mass mortalities associated with distemper virus in marine mammals have also been shown to be due to the immunosuppressive effects of TCDD and related chemicals. Exposure of harbor seals to 1-5 ng/kg/day in their diet led to a suppression of delayed type hypersensitivity and of the

antibody response (22). Suppression of the primary antibody response in mice can be detected at an acute dose as low as 100 ng/kg (23), and following subchronic exposure to 1.5 ng/kg/day (24). Enhanced mortality due to influenza virus was seen in mice treated one week earlier with 10 ng TCDD/kg (25).

Biochemical effects on cytokine expression and metabolizing enzymes occur at similar doses to those which cause some of the reproductive, immunological, and developmental effects mentioned above. For example, daily doses of ~0.3 ng/kg/day are associated with increased expression of IL-1β (26). This dose results in a similar body burden to a single dose of 10 ng/kg. Induction of CYP1A1 activity occurs following subchronic exposure of mice to 0.15 ng/kg/day (27). A similar dose was associated with induction of CYP1A1 mRNA in rats (28). Increases in CYP1A2 mRNA was seen at ~0.3 ng/kg/day in mice (26). Down regulation of the EGF receptor in rats occurs at a similar dose (28). Increased oxidative damage has been seen in mice at 0.45 ng/kg/day (4). Whether or not these responses are actually adverse, remains to be determined. However, effects on the immune system, learning, and the developing reproductive system of multiple animals occur at similar doses, resulting in body burdens which are in the range of current human exposures (29).

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