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A REVIEW OF THE TERATOGENICITY OF TCDD

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A REVIEW OF THE TERATOGENICITY OF TCDD

INTRODUCTION

During the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) a highly toxic impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), may be formed. TCDD was named as the culprit in cases of chloracne and chick edema disease in the 1940's and the 1950's. Attention was focused on the possible teratogenic effects of TCDD in the late 1960's when 2,4,5-T was used as a defoliant in certain areas of South Vietnam. Recent descriptions of the teratogenic effects of TCDD began when Courtney et al. (5) released a study using mice and rats treated with 2,4,5-T containing approximately 30 parts per million (ppm) of TCDD.

REVIEW

Using two strains of mice, Courtney et al. (5) reported an increase in the incidence of cleft palate in both the C57BL/6 and the AKR strain of mice when pregnant females were subjected to 2,4,5-T either subcutaneously or orally on days 6 through 14 or days 9 through 17 of gestation for the C57BL/6 strain and days 6 through 16 for the AKR strain. Only the C57BL/6 strain showed an increased incidence of cystic kidney. Doses varied from 21.5 mg to 113.0 mb 2,4,5-T per Kg of body weight with the 2,4,5-T containing approximately 30 ppm of TCDD.

Subsequently, other teratological studies using mice have shown that varying dose levels could affect prenatal development in mice.

Subsequently, other teratological studies using mice have shown that varying dose levels could affect prenatal development in mice. Specifically, the only teratogenic effects observed were an increased frequency of cleft palate and cystic kidneys, a special type of kidney abnormality. Other toxic signs were noted but cannot be referred to as teratogenic since they can also be demonstrated in the adult after treatment with TCDD (15).

Courtney and Moore (6), by using varying dose levels and mixtures of 2,4,5-T and TCDD, attempted to determine if the previously reported teratogenic results (Courtney et al. (5) were due to 2,4,5-T, TCDD or the combination of the two. Cleft palates were produced in all strains of mice tested using 2,4,5-T, TCDD and the combination with no potentiation of the teratogenic effect using the combination. Interestingly, the less pure grade of 2,4,5-T (Technical) produced less kidney anomalies than did the purest grade of 2,4,5-T (Analytical) while treatment with TCDD produced a marked increase in kidney anomalies. An additional finding of the study was that the C57Bl/6J strain of mice are the most susceptible to TCDD-induced effects.

A later study by Hart and Valerio (10) was able to show only a marginally significant increase in incidence of cleft palate and no causal relationship between other observed abnormalities and treatment with 2,4,5-T. In addition to using 2,4,5-T instead of pure TCDD, the investigators used the CD-1 strain of mice rather than the C57Bl/6J strain which is more sensitive to the teratogen.

Neubert and Dillman (14) performed an exhaustive study of the embryotoxic effects of both 2,4,5-T and TCDD in mice. In this study with NMRI mice only the appearance of cleft palate was considered as evidence of teratogenicity. It was found that both 2,4,5-T and TCDD produced cleft palate in mice, even with a single oral dose. The maximum teratogenic effect was seen with 2,4,5-T if the single oral dose was given on day 12 or 13 of gestation while with TCDD the maximal effect was obtained on day 11 of gestation. A potentiation of the effects of 2,4,5-T and TCDD was obtained when teratogenic doses of one were combined with threshold doses of the other. It was shown that TCDD was capable of producing cleft palate in this strain of mice at doses exceeding 2μg/kg which is well above that expected as a contamination of 2,4,5-T preparations. It was also suggested that other impurities than TCDD may be responsible for the teratogenic effects seen with preparations of 2,4,5-T.

Using 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) separately and combined (TCDD content was less than 1 ppm) in varying dose levels, Bage, Cekanova and Larsson (1) found that high dosages were teratogenic in NMRI mice. The most common finding was isolated cleft palate followed by malformations of ribs and vertebra. Cystic kidneys, reported by Courtney et al. (5) after 2,4,5-T treatment, were not observed. As stated by the investigators, the dose resulting in teratogenic effects was extremely high as compared with the dose a pregnant woman could normally be exposed to. They also noted that the results of their study and other studies did not substantiate any special risk to the human embryo from the regular use of phenoxy herbicides.

The findings of Moore et at. (13) supported the observations made by Courtney and Moore (6) of the teratogenic effects of TCDD on mice of the C57Bl/6 strain. Cleft palate was reported with an incidence of 55.4 percent while kidney anomalies were found with an incidence of 95.1 percent when the dose was 3µg TCDD/kg given on gestation days 10 through 13. With a dose level of 1µg/kg on the same days, the incidence levels dropped to zero and 58.9 percent for cleft palate and kidney anomalies, respectively.

Starting with Courtney et al. (5) the rat has also been used to test the teratogenicity of TCDD. The only teratogenic evidence seen was that of cystic kidneys. These investigators failed to say what strain of rats were used or to describe the renal anomalies other than as cystic kidneys. The 2,4,5-T used in the treatment contained approximately 30 ppm of TCDD.

The results of a study by Emerson et al. (8) failed to substantiate the finding of Courtney et al. (5). Examination of fetuses failed to reveal any serious teratogenic effects when the dams were given daily doses of 2,4,5-T containing approximately 27 ppm of TCDD on days 6 through 15 of gestation. In this study Sprague-Dawley rats were used as experimental animals.

Sparschu, Dunn and Rowe (17) gave TCDD orally to pregnant female Sprague-Dawley rats on days 6 through 15 of gestation. No gross teratogenic effects were noted in the fetuses but both maternal and fetal toxicity was associated with TCDD at high doses (8.0µg/kg/day).

Using a CD strain of rats, Courtney and Moore (6) found that neither 2,4,5-T nor TCDD produced cleft palate in rat fetuses when given on days 6 through 15 of gestation. However, it was found that 2,4,5-T produced only a minimal response in kidney malformation while TCDD produced a 34 percent incidence of malformation.

Since many of the conclusions concerning the teratogenicity of TCDD were drawn from results of studies using TCDD contaminated 2,4,5-T, Emerson et al. (9) attempted to evaluate the teratogenic effects of a routine production lot of 2,4,5-T containing 0.5 ppm TCDD. Groups of Sprague-Dawley female rats were dosed once daily on days 6 through 15 of pregnancy with 1,3,6,12 or 24 mg 2,4,5-T/kg. Examination of all fetuses following Caesarean section on day 20 of gestation revealed no teratogenic effects. Therefore, in this experiment, 2,4,5-T was not teratogenic to rats nor was TCDD at the concentration of 0.5 ppm.

Khera, Huston and McKinley (11), using 2,4,5-T herbicide containing less than 0.5 ppm TCDD, found no apparent teratogenic effects using 25 mg 2,4,5-T/kg/day on Wistar rats during days 6 through 15 of gestation. Using a dose level of 50 mg/kg/day, the significance of the teratogenic effects observed was inconclusive.

To determine if TCDD actually caused the teratogenic effects attributed to 2,4,5-T by Courtney et al. (5), Sparschu, Dunn and Rowe (18) subjected Sprague-Dawley rats to orally administered TCDD on days 6 through 15 of gestation at levels of 0, 0.03, 0.125, 0.5, 2.0 and 8.0 µg/kg/day. Cleft palate and cystic kidneys were not observed in this study although both maternal toxicity and embryotoxicity were

evident at the higher doses. It is believed, however, that the TCDD present as a contaminant in the 2,4,5-T tested earlier may have accounted for the reported observations of teratogenic effects which were attributed to 2,4,5-T.

Following the procedures used by Emerson et al. (9), but increasing the dose levels to 50 and 100 mg 2,4,5-T/kg/day, Sparschu et al. (19) showed no teratogenic effects with commercial-grade 2,4,5-T containing 0.5 ppm TCDD. The higher dose showed severe maternal toxicity and some fetal toxicity.

Thompson, Emerson and Sparschu (20), again using commercially produced 2,4,5-T, treated Sprague-Dawley rats orally with doses of 1, 3, 6, 12, 24 or 50 mg/kg/day on days 6 through 15 of gestation. A dose of 100 mg/kg/day of 2,4,5-T was given to rats on days 6 through 10 of gestation. Rats treated with up to 50 mg/kg/day did not reveal teratogenic effects. The 100 mg/kg/day dose produced maternal toxicity and death and some fetuses which showed toxic effects, but no teratogenic effects.

Khera and Ruddick (12) reported no teratogenic effects by treating female Wistar rats orally with 0.125-16 µg/kg/day of TCDD during days 6 through 15 of gestation. However, postnatal survival of the pups was severely decreased with increased prenatal doses. The investigators reasoned that this was due to embryonic damage induced by the TCDD.

Another test animal used to study the teratogenic effects of TCDD was the golden Syrian hamster. Collins and Williams (4) administered 2,4,5-T containing varying amounts of TCDD to groups of hamsters on days

6 through 10 of gestation. Fetotoxicity and abnormalities such as the absence of eyelid and delayed head ossification increased with TCDD content in the 2,4,5-T. Cleft palates and cystic kidneys were not observed in hamsters.

Emerson et al. (9) also evaluated the teratogenic effects of a routine production lot of 2,4,5-T containing 0.5 ppm TCDD using groups of female New Zealand White rabbits. Each group received 0, 10, 20 or 40 mg 2,4,5-T/kg daily on days 6 through 18 of pregnancy. No anatomical malformations were observed and it was concluded that under the conditions of this experiment, 2,4,5-T was not teratogenic to rabbits.

Thompson, Emerson and Sparschu (20) also used groups of New Zealand White rabbits to study the teratogenic effects of 2,4,5-T. Visceral and skeletal examinations did not reveal teratogenic effects in any of the groups of rabbits given daily oral doses of 10, 20 or 40 mg 2,4,5-T/kg on days 6 through 18 of gestation.

Sheep were used by Birns and Balls (2) to test the teratogenicity of 2,4,5-T containing 1 ppm of TCDD. Teratogenic effects were not induced in any of the lambs from ewes fed as much as 113 mg 2,4,5-T/kg body weight for various periods during gestation.

Dougherty, Coulston and Golberg (7) subjected mature female Rhesus monkeys (Macaca mulatta) to 2,4,5-T containing less than 0.05 ppm TCDD. Concentrations of 0.05, 1.0 or 10.0 mg 2,4,5-T/kg body weight were given to the test groups from day 22 through 28 of pregnancy. Detailed examination of the live infants and one stillborn fetus revealed no gross developmental abnormality in any of the groups. Therefore, within

the conditions of this experiment, there was no evidence that 2,4,5-T containing less than 0.05 ppm TCDD is teratogenic in the Rhesus monkey.

Neubert et al. (15), in reviewing the data on the teratogenic effects obtained using pure TCDD, noted that doses as low as 1-10 µg/kg could produce malformations of certain types. The two major types of malformations were cleft palate, reported only in mice, and kidney abnormalities, observed in both mice and rats. No mention was made of using pure TCDD in teratological experiments performed with any other species, even those that are highly sensitive to TCDD. The investigators found that potentiation can occur after treatment with a single dose of a combination of two or more known teratogenes. This was the case with the TCDD-2,3,5-T combination, but not when nonteratogenic doses of 2,4,5-T are used with less than 10-20 ppm TCDD.

DISCUSSION

The Food and Drug Administration expects three studies to be completed in order for a drug to be acceptable for use (16). These three tests are: (1) study of fertility and general reproductive performance; (2) teratological study; and (3) perinatal and postnatal study. The United Kingdom's Committee on Safety of Drugs differs from the FDA in that it will accept a teratology study alone, but the drug must be administered from day one of gestation until just before term rather than during the period of embryogenesis as accepted by the FDA. All of the articles reviewed above reported teratogenic studies with the addition of postnatal studies being reported by Khera, Houston and McKinley (11), Khera and Ruddick (12), and Moore et al. (13).

According to Robson (16), when a teratology study is done, the drug should be given during the period of organogenesis, which would be about days 6 through 15 for mice and rats, and days 6 through 18 for rabbits. With the exception of Bage, Cekanova and Larsson (1) (days 6 through 14 for mice) and Moore et al. (13) (days 10 through 13 for mice) all investigators administered the test doses over the prescribed time period. In addition, Moore et al. (13), Neubert and Dillman (14) and Neubert et al. (15) tested single doses during the period of organogenesis.

All the investigators in the reviewed articles have satisfied the FDA recommendation of two dose levels. With the exceptions of references 1, 2, 10, 13 and 19 the reports also satisfied the British recommendation of at least three dose levels. Some teams such as Neubert and Dillman (14) employed as many as 20 dose levels.

It has been recommended (16) that offspring be removed by Caesarean section one or two days before term. For the most part all offspring were collected in this manner. Binns and Balls (2) allowed the ewes to go to term (160 days) as did Courtney and Moore (6), Thompson, Emerson and Sparschu (2), and Khera and Ruddick (12) with rats. Dougherty, Coulston and Golberg (7) also allowed the monkeys to go to term (164 days).

With few exceptions, fetuses have been examined for both visceral anomalies and skeletal abnormalities. For some reason Binns and Balls (2) did not report what examinations were made, but only that the offspring were normal after birth. An examination for skeletal abnormalities was not mentioned by Courtney and Moore (6), Moore et al. (13), and

Neubert et al. (15). For this reason these four reports may be considered somewhat incomplete.

To properly test a drug for tetratogenic effects at least two species of test animals should be used (16). Many criteria are to be considered when selecting test animals to be used in teratological investigations. According to Clegg (3) some of these criteria are availability, ease of maintenance, economics, gestation period, estrus cycle, fertility in captivity, litter size, etc. All criteria are probably best met by the hamster, mouse, rabbit and rat. Extrapolation of the experimental data to man would necessitate the selection of test animals whose embryonic development and metabolic parameters are similar to those in man. Nonhuman primates may be the animals of choice when metabolic data on the drug is not known.

Only four of the investigating teams reviewed used two species of test animals (references 5, 6, 9 and 20). The rat was selected by all four, the rabbit by two, and the mouse by two. One team of investigators used sheep (2) which is not one of the recommended species, but was probably readily available. Collins and Williams (4) selected hamsters while Dougherty, Coulston and Golberg (7) used the Rhesus monkey which would seem to be an animal of choice if one were trying to extrapolate the results of the teratological investigations to man. Strangely enough, the highly TCDD-sensitive guinea pig was never selected as a test animal. It would be interesting to compare the high toxicity seen in the adult of this species with any possible teratogenic effects on the fetus and with data obtained from other animals such as rats and mice.

Very few teratological studies have been done with TCDD alone as the agent. Usually TCDD is studied in combination with another agent such as 2,4,5-T. Many of the early studies were done with very high concentrations of TCDD. Indeed, one such study was completed before the concentration of the TCDD in the 2,4,5-T was even known (5). Later investigations also used TCDD in combination with 2,4,5-T, but at a much lower and more realistic concentration (7). In addition, in an attempt to more closely approach the embryonic development and metabolic parameters found in man, Rhesus monkeys have been employed as test animals.

The highly controversial question as to the potential hazard to man resulting from exposure to 2,4,5-T containing even a minimal amount of TCDD still remains to be settled. However, it appears from these studies that the teratogenic effects of TCDD in 2,4,5-T will not be seen at concentrations below approximately 10 ppm.

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