Arch. Environ. Contam. Toxicol. 16, 461–466 (1987)

Archives of Environmental Contamination and Toxicology

© 1987 Springer-Verlag New York Inc.

Reproductive Toxicity in Female Mice of Dioxin-Contaminated Soils from a 2,4,5-Trichlorophenoxyacetic Acid Manufacturing Site

Thomas H. Umbreit, Elizabeth J. Hesse, and Michael A. Gallo

Department of Environmental and Community Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Abstract. Reproduction in female C57B/6 mice treated with contaminated soils from a 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing site and a metal scrap yard in Newark, New Jersey was studied. Soils contained a wide variety of contaminants, including halogenated dibenzodioxins and dibenzofurans, benzene, alkylbenzenes, chlorobenzenes, polyaromatic hydrocarbons, phenolics, phenoxy acids and other compounds. Acute and reproductive toxicity (primarily fewer live pups born and fewer pups surviving until weaning) was observed from the manufacturing site soil (soil A; with 2,050 μg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)/kg soil and 18 mg total dibenzodioxins and dibenzofurans/kg soil); the scrap yard soil (soil B; with 230 μg 2,3,7,8-TCDD/kg soil) had no observable effect. Soil recontaminated with 2,3,7,8-TCDD, or 2,3,7,8-TCDD in corn oil interfered with estrus cycling and completely prevented reproduction. If 2,3,7,8-TCDD treatment was terminated, estrus cycling resumed within four weeks, but reproduction was still unsuccessful for these mice. The results suggest that 1) the manufacturing site soil (soil A) is toxic but the signs of toxicity cannot be solely attributed to the 2,3,7,8-TCDD content; 2) even though the bioavailability of 2,3,7,8-TCDD from this soil is low (Umbreit *et al.* 1986), other reproductive toxins are bioavailable or the low doses of 2,3,7,8-TCDD that are bioavailable may be sufficient to be reproductive toxins; 3) 2,3,7,8-TCDD blocks estrus in mice and (as previously reported) interferes with reproduction; and 4) after cessation of dosing, the *in situ* concentrations of 2,3,7,8-TCDD that block estrus are decreased below an effective level within four weeks.

Address correspondence to: Dr. Michael A. Gallo, Dept. of Environmental and Community Medicine, University of Medicine and Dentistry of New Jersey, Robt. W. Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), produced as a contaminant in the manufacture of phenoxy chemicals, is the most toxic of a group of related chlorinated dioxins (Esposito *et al.* 1980). There is variation with species in the toxic effects seen with 2,3,7,8-TCDD but, in general, a "wasting syndrome" characterized by loss of body weight, accompanied by thymic atrophy, immunodeficiency, and liver weight increase (accompanied by hepatic cytochrome P-450 induction) occurs in intoxicated animals. 2,3,7,8-TCDD is a potent fetotoxin and teratogen (Courtney 1976) even at low doses, and the compound crosses the placenta and can be detected in the milk of treated animals (Nau and Bass 1981).

Human exposure to 2,3,7,8-TCDD has occurred in industrial accidents, during use of some phenoxy herbicides, and disposal of contaminated wastes. While considerable data have been obtained on the toxicity of pure 2,3,7,8-TCDD, little is known about the toxicity of materials contaminated by 2,3,7,8-TCDD in the environment (McConnell *et al.* 1984; Silkworth *et al.* 1982; Umbreit *et al.* 1986, 1987). Reported here are the results of a reproductive study in which female mice were treated with contaminated soil from a 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing site in Newark, New Jersey. Despite low bioavailability of 2,3,7,8-TCDD from these soils (Umbreit *et al.* 1986), reproductive effects which are well defined, such as cleft palate, were anticipated because of the extremely low levels of 2,3,7,8-TCDD required for

MAXUS1208035

these effects (Courtney 1976). However, the reproductive effects observed appear not to be solely attributable to 2,3,7,8-TCDD but also to other chemicals present in the soil samples.

Methods

The manufacturing site, which produced 2,4,5-T, hexachlorophene and pentachlorophenol, ceased operations approximately ten years before the contamination with 2,3,7,8-TCDD and other dibenzodioxins and dibenzofurans was discovered by the New Jersey Department of Environmental protection (NJDEP). Soil from this site was designated "Soil A". Soil from a metal scrap yard where stills from the manufacturing site were broken down for scrap metal was designated "Soil B".

Soils were provided by New Jersey State Department of Environmental Protection and analysis showed 2,3,7,8-TCDD levels of approximately 2,050–2,500 ppb for soil A and approximately 180–230 ppb for soil B. Analyses for dioxin isomers have been published for soil A (Umbreit *et al.* 1986; summarized in Table 2) and other contaminants are presented in Table 1. Soil B has not been analyzed as extensively (Table 2). Soils were kept protected from light and sieved and dried before use.

Mice (strain C57B/6 males and females; Charles River Laboratories, Wilmington, Ma., initially 14 weeks old) were housed in plastic cages with chopped corn cob bedding (Anderson Mills, Maumee, Ohio) in groups of five until mating commenced. Mice were allowed unlimited access to Purina (St. Louis, MO) rodent chow and water. For mating, one female (treated with either 2,3,7,8-TCDD recontaminated soil, decontaminated soil, 2,3,7,8-TCDD-corn oil, or contaminated soil A or B) and one male (treated with decontaminated soil) were cohabitated until mating was successful (as determined by vaginal plug or a three gram weight gain in one week), whereupon the male was returned to group housing while the female remained until the litter was weaned. When mating was unsuccessful, the male was replaced with another who had successfully mated previously. Pups were counted and weighed at birth, and at days 7, 10, 14, 21, and 28. Data on the pups and their treatment and mating will be presented in a subsequent paper (a brief summary of these results is presented in Table 8). Reproductive indices examined included births, total number of pups born, live pups born, total pups/litter, live pups/litter, litters born dead, litters dying before weaning, and percentage of pups surviving to weaning. Estrus cycling in the parental generation was examined by daily vaginal smears after the tenth week of dosing. The second generation females were examined daily, starting two weeks before dosing. Statistical analysis was by ANOVA and Student's "t" test (SASS, Cary, NC).

Soils were suspended in 5% aqueous gum acacia (Sigma, St. Louis, Mo.), yielding a 10% soil suspension. All soils were administered by gavage and no volume exceeded 1 ml/mouse. In addition to the contaminated soils, female animals were administered pure 2,3,7,8-TCDD (99.9+%; Cambridge Isotopes, Cambridge, MA) in corn oil:acetone (9:1), decontaminated soil from which all hydrocarbons had been removed before use ("decontaminated soil"), and decontaminated soil to which 2,3,7,8-TCDD was added within one half-hour before use ("recontaminated soil"). The males were treated with decontaminated soil once weekly for six weeks before mating and throughout the experiment. Ten sexually mature female C57B/6 mice per treatment were dosed thrice weekly, starting two

Table 1. Analysis of Soil A from a 2,4,5-Trichlorophenoxyacetic acid manufacturing site in Newark, New Jersey[a]

Chemical	Parts per Million mg/kg
Chloromethanes	5.3
Chloroform	1.5
Carbon tetrachloride	3.8
Benzene and alkylbenzenes	39.2
Benzene	18.0
Styrene	17.0
Xylenes	2.5
Benzyl alcohol	0.3
Naphthalene	0.7
2-Methylnaphthalene	0.7
Phenolics	482.1
2-Chlorophenol	0.6
Phenol	8.9
Pentachlorophenol	290.0
2,4,6-Trichlorophenol	35.0
2,4,5-Trichlorophenol	58.0
Tetrachlorophenol	9.7
2,4-D octyl ether	17.0
2,4,5-T octyl ether	5.8
Tetrachlorodiphenyl thioether	6.5
2,4-Dichlorophenol	50.0
Chlorobenzene	39.4
1,4-Dichlorobenzene	0.5
Hexachlorobenzene	24.0
4-Chloroaniline	0.5
1,2,4-Trichlorobenzene	2.4
Tetrachlorobenzene	12.0
Polyaromatic hydrocarbons	128.6
Acenaphthene	0.2
Fluorene	0.3
Phenanthrene	17.0
Pyrene	16.0
Fluoranthrene	35.0
Chrysene	11.0
Acenaphthalene	0.3
Benzo(a)anthracene	10.0
Benzo(a)pyrene	5.5
Benzo(b)fluoranthracene	7.0
Benzo(k)fluoranthracene	7.0
Indeno(1,2,3-c,d)pyrene	9.2
Dibenz(a,h)anthracene	2.8
Benzo(g,h,i)perylene	7.3
Others	
Acetonitrile	9.2
Hexanes	1.8
Diethylphthalate	0.1
Dinitrobutylphthalate	0.8
Sulfur, elemental	6.6
DDT, DDE	0.9
2-Methyl-undecan-3-one	4.3
Methylene chloride	6,300[b]
unidentified	45.1

[a] Analysis by National Institute of Environmental Health Sciences, Research Triangle Park, NC
[b] Extraction solvent

MAXUS1208036

Table 2. Analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contaminated soils from Newark, New Jersey[a]

	Soil A (μg/kg)	Soil B (μg/kg)
Dioxins		
Tetrachloro	2,053	231
Pentachloro	115	ND[b]
Hexachloro	333	ND
Heptachloro	4,032	6
Octachloro	5,011	50
Furans		
Tetrachloro	233	ND
Pentachloro	97	21
Hexachloro	594	ND
Heptachloro	3,127	4
Octachloro	2,757	183

[a] Analysis by F. Hileman, Monsanto Corp., St. Louis, MO
[b] ND None detected

weeks before first mating and continuing through weaning of pups at 28 days post-partum. Female mice were dosed thrice weekly to ensure that blood levels were high throughout estrus cycling. Fifty females were used as negative controls receiving decontaminated soil; this number was selected to provide sufficient negative control females for mating with treated male mice in a second series of experiments (presented in a subsequent paper). Doses of 2,3,7,8-TCDD administered and total 2,3,7,8-TCDD received by the end of dosing are listed in Table 3. Doses were selected on the basis of maximizing the amount of soil given without unduely stressing the animals. All mice were weighed weekly, checked for signs of toxicity, and autopsied either when found dead or upon sacrifice at the termination of the experiment.

Results

Table 4 presents survival data for the maternal mice. Deaths in all groups occurring within the first two months were caused by fighting during mating. Subsequent deaths in the decontaminated soil group included deaths due to difficulties in parturition: one each in the third, fourth, and sixth month, and two in the fifth month of the experiment. One mouse in the decontaminated soil group developed a large well-defined mammary mass which was confirmed at autopsy. One mouse each in the recontaminated soil and the soil A groups died from difficulties in parturition during the sixth month of the experiment. Four mice treated with soil A died during the last 2 month period, by which time total cumulative TCDD doses were several times the reported LD_{50} and the cumulative doses of other contaminants were similarly high.

The deaths among the 2,3,7,8-TCDD-corn oil group after the fourth month occurred within one week of each other, and these mice had atrophied thymuses, and pale, enlarged and fatty livers; signs consistent with 2,3,7,8-TCDD intoxication. Mice treated with recontaminated soil that died from causes other than difficulty at delivery had toxic signs including loss of body fat, thymic atrophy, and hepatotoxicity (but the liver damage was not as severe as in the 2,3,7,8-TCDD in corn oil group). Mice from all other treatment groups did not show any signs of fatty liver.

Female mice showed slight weight gains during the first three weeks of the experiment, but failed to gain much weight thereafter. Females receiving 2,3,7,8-TCDD (2,3,7,8-TCDD-corn oil, from recontaminated soil, or from contaminated site soils) showed weight gains that were no different from those receiving decontaminated soil (Table 5).

After 25 weeks of dosing, surviving mice were sacrificed and autopsied. Mean body weights were similar in all groups. A comparison of ratios of organ to body weight showed significantly ($p < 0.05$) greater ratio of liver to body weight and significantly reduced ratio of thymus to body weight for mice treated with 2,3,7,8-TCDD-corn oil or recontaminated soil when compared to mice given decontaminated soil, soil A or soil B. No other significant differences from decontaminated soil were observed.

The reproductive indices (Table 6) of females treated with soil B or decontaminated soil were similar, except that the soil B treatment group had a much greater percentage of pups surviving until weaning. While decontaminated soil treatment resulted in an apparently low number of births, this is not unusual for this strain (Festing 1979). Soil A produced a similar number of litters, but fewer pups/litter and fewer live pups/litter than did decontaminated soil. Soil A treated mice did not produce any stillborn litters, but three litters died between birth and weaning. The positive control groups (2,3,7,8-TCDD-corn oil and recontaminated soil) produced only one litter between them, and all pups were born dead.

The lack of successful reproduction by the groups treated with the positive controls (2,3,7,8-TCDD-corn oil or recontaminated soil) caused us to examine estrus cycling, starting at the tenth week of dosing (Table 7). It was found that most of the mice in these two groups were not cycling. When this observation was made, seven of sixteen noncycling mice were removed from their original dosing materials and administered decontaminated soil (selection of mice was made using a random number table). Six of the seven 2,3,7,8-TCDD-treated mice placed on decontaminated soil resumed cycling within three weeks, while the group remaining on their original dosing materials con-

MAXUS1208037

Table 3. Oral doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and contaminated soils in female C57B/6 mice[a]

Material	Doses per week	Weeks dosed	Single dose μg/kg	Weekly dose μg/kg	Total dose μg/kg
TCDD in oil	3	25	3	9	225
Recontaminated soil	3	25	3	9	225
Soil A	3	25	9.6	28.8	720
Soil B	3	25	1.1	3.3	86.3
Decontaminated soil	3	25	0	0	0

[a] Nature of dosing materials: TCDD in oil = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in a corn oil:acetone (9:1) mixture. Recontaminated soil = decontaminated soil to which 2,3,7,8-TCDD in acetone was mixed within one-half hr before dosing. Soil A = manufacturing site soil. Soil B = metal scrap yard soil. Decontaminated soil = soil A after removal of chlorinated hydrocarbons. All soils were administered by gavage as 10% suspensions in gum acacia (5% aq). Dosages are in μg 2,3,7,8-TCDD/kg body weight

Table 4. Survival of female C57B/6 mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or contaminated soils[a]

Treatment	n	Deaths				
		# Dying during month			Total deaths	% Dead
		1–2	3–4	5–6		
TCDD in oil	10	3	1	3	7	70
Recontaminated soil	10	1	1	2	4	40
Soil A	10	1	0	4	5	50
Soil B	10	2	0	1	3	30
Decontaminated soil	50	4	3	5	12	24

[a] Nature of dosing materials: TCDD in oil = 2,3,7,8-tetrachlorodiebenzo-*p*-dioxin (2,3,7,8-TCDD) in a corn oil:acetone (9:1) mixture. Recontaminated soil = decontaminated soil to which 2,3,7,8-TCDD in acetone was mixed within one-half hr before dosing. Soil A = manufacturing site soil. Soil B = metal scrap yard soil. Decontaminated soil = soil A after removal of chlorinated hydrocarbons. All soils were administered by gavage as 10% suspensions in gum acacia (5% aq)

tinued noncycling. Four of the six mice that had resumed cycling were then mated, but only two became pregnant and no live pups were produced. Upon autopsy, mice that were switched to decontaminated soil had no noticeable gross differences from mice that had been treated with decontaminated soil from the outset of the experiment. The ratio of organ to body weight in these mice were not significantly different from the continuous decontaminated soil group.

Discussion

Treatment of female C57B/6 mice, a 2,3,7,8-TCDD-responsive strain (Poland and Glover 1980), with 2,3,7,8-TCDD-corn oil produced significant maternal and reproductive toxicity when administered by gavage at 3 μg/kg thrice weekly for 25 weeks. The total cumulative dose (225 μg/kg) was approximately the reported LD_{50} for this strain of mouse. Less maternal toxicity was seen with recontaminated soil, but similar reproductive toxicity was observed. Metal scrap yard soil (soil B) was indistinguishable in effect from decontaminated soil. In contrast, manufacturing site soil (soil A), with the higher 2,3,7,8-TCDD content, produced toxicity to both the dams and the litters, but in contrast produced no apparent 2,3,7,8-TCDD-like syndrome. Soil A has been shown (Table 1, and Umbreit *et al.* 1986) to contain approximately fifty halogenated dibenzodioxins and dibenzofurans in the ppt (ng/kg) to ppb (μg/kg) range, and approximately 45 other major contaminants in the ppb to ppm (mg/kg) range. Soil B also contained other halogenated dibenzodioxins and contaminants.

Soil A-treated animals continued estrus cycling and were able to reproduce and sustain viable litters and no gross terata were observed. However, fewer pups were born live and still fewer survived until weaning. These results suggest that, since the bioavailability of TCDD from soil A is less than the positive controls (2,3,7,8-TCDD in recontaminated soil or in oil blocked estrus and reproduction), perhaps some other components of soil A are contributing to the observed toxicity. The neonatal deaths might be attributed to disruption of ovarian function, transplacental toxicity, and secretion of toxic agents in the milk. Milk analyses were not conducted because of sample size. Females treated with soil A received a cumulative dose equivalent to 189% of the TDLO (skin) of pentachlorophenol, 10% of the iv LD_{50} of fluoranthrene, 92% of the TDLO (skin) of chrysene, 31% of the iv LD_{50} of 2,4,5-trichlorophenol, and 5% of the subcutaneous TDLO of benzo(b)fluroanthracene (Lewis and Tatkan 1982). Logic dictates that all the xenobiotics present may have contributed to the neonatal tox-

MAXUS1208038

Table 5. Weight gains of non-pregnant females (survivors)

Treatment[a]	Initial	12 weeks	25 weeks
TCDD in oil	21.5 ± 1.0 (10)	25.0 ± 0.7 (7)	26.2 ± 1.3 (4)
Recontaminated soil	22.5 ± 1.4 (9)	25.5 ± 2.0 (9)	25.5 ± 1.4 (7)
Soil A	21.9 ± 1.4 (10)	25.9 ± 1.4 (8)	25.6 ± 1.4 (5)
Soil B	21.8 ± 1.4 (10)	25.8 ± 1.0 (6)	25.7 ± 2.0 (7)
Decontaminated soil	21.9 ± 1.2 (50)	26.5 ± 1.2 (29)	25.6 ± 1.5 (37)

[a] Nature of dosing materials: TCDD in oil = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in a corn oil:acetone (9:1) mixture. Recontaminated soil = decontaminated soil to which 2,3,7,8-TCDD in acetone was mixed within one-half hr before dosing. Soil A = manufacturing site soil. Soil B = metal scrap yard soil. Decontaminated soil = soil A after removal of chlorinated hydrocarbons. All soils were administered by gavage as 10% suspensions in gum acacia (5% aq). Mean ± standard deviation (number of animals included in mean)

Table 6. Reproductive indices for female C57B/6 mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and contaminated soil

Index	Treatment of females[a]					
	Decont	TCDD	Recont	Soil A	Soil B	Decont after TCDD
Number of mating trials	16	13	13	14	13	4
Litters born	6	0	1	7	7	2
Total pups born	55	0	6	46	62	8
Pups/litter	9.1 ± .75	0	6	6.4 ± 2.3*	8.9 ± 1.8	4
Litters born dead	0	0	1	0	0	2
Total live pups born	51	0	0	33	49	0
Live pups/litter	8.5 ± 1.0	0	0	4.1 ± 3.6*	7.0 ± 3.4	0
Litters dying before weaning	1	NA	NA	3	1	NA
Survival to weaning (%)	71	0	0	39	94	0

[a] Mated to males treated with decontaminated soil. * = statistically different from decontaminated soil ($p < 0.05$). decont = decontaminated soil. recont = recontaminated soil. soil A = manufacturing site soil. soil B = metal scrap yard soil. TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in corn oil
Decont after TCDD = animals were placed on decontaminated soil after 12 weeks of dosing with 2,3,7,8-TCDD. Soils were administered by gavage as 10% suspensions in gum acacia (5% aq). 2,3,7,8-TCDD and recontaminated soil were not subjected to statistical analysis because of low numbers, but are clearly different from decontaminated soil

icity, but it is not possible to identify a solitary active agent. Mice did not receive significant portions of the LD_{50} of other contaminants present in the soil. These results suggest that interactions of the components of this complex mixture, rather than any one component alone, caused the reproductive toxicity.

Allen *et al.* (1979) reported that 2,3,7,8-TCDD affected menstrual cycling in monkeys. Our results show a similar effect on the estrus cycle in C57B/6 mice. This was first recorded after 10 weeks of dosing (after a total dose of 90 μg/kg), but had not been examined previously. Recovery of estrus cycling was rapid upon cessation of 2,3,7,8-TCDD dosing, despite a total cumulative dose at that time of 126 μg 2,3,7,8-TCDD/kg (in another experiment C57B/6 mice, treated with 30 μg/kg 2,3,7,8-TCDD/wk by gavage, stopped cycling after 3 weeks). However, while return of cycling was sufficient for occurrence of pregnancies, it was insufficient for birth of live pups. These findings suggest that 2,3,7,8-TCDD may be directly affecting ovarian function as well as hormonal status. Further studies on these effects are in progress. The return to normal cycling coincides with the normalization of relative liver weight, suggesting that the apparent anti-estrogenic effects of 2,3,7,8-TCDD may be related to estrogen metabolism. However, recent evidence indicates that 2,3,7,8-TCDD has a direct effect on estrogen receptor regulation as seen in receptor binding assays (Romkes *et al.* 1986) and blockage by TCDD of the estradiol uterotrophic effect (Gallo *et al.* 1986).

The soil from the Newark 2,4,5-T manufacturing site has reproductive toxicity that cannot be attributed solely to 2,3,7,8-TCDD; but neonatal toxicity occurred that may be associated with 2,3,7,8-TCDD and other contaminants either as fetotoxins

MAXUS1208039

Table 7. Estrus cycling of C57B/6 mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or contaminated soil for ten weeks

Treatment[a]	Total dose of TCDD μg/kg	Number examined	Number not cycling	% not cycling
TCDD in oil	90	8	7	87.5
Recontaminated soil	90	10	9	90
Soil A	288	9	3	33.3
Soil B	33	8	1	12.5
Decontaminated soil	0	45	9	20

[a] Nature of dosing materials: TCDD in oil: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in a corn oil:acetone (9:1) mixture. Recontaminated soil was decontaminated soil to which 2,3,7,8-TCDD in acetone was mixed within one-half hr before dosing. Soil A: manufacturing site soil. Soil B: the metal scrap yard soil. Decontaminated soil was soil A after removal of chlorinated hydrocarbons. All soils were administered by gavage as 10% suspensions in gum acacia (5% aq). Estrus cycling was assessed by vaginal cytology

Table 8. Summary of birth and growth of second generation of C57B/6 mice

	Treatment of female parent[a]				
	Decont	TCDD	Recont	Soil A	Soil B
Live pups born	51	0	0	33	49
Birth weight per pup	1.37 ± 0.19	0	0	1.30 ± 0.13	1.56 ± 0.41
% born female	52%	—	—	51%	43%
Average weight gain to weaning	766%	—	—	992%	748%

[a] Nature of dosing materials: TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in a corn oil:acetone (9:1) mixture. Recont: recontaminated soil, which was decontaminated soil to which 2,3,7,8-TCDD in acetone was mixed within one-half hr before dosing. Soil A: the manufacturing site soil. Soil B: the metal scrap yard soil. Decont: decontaminated soil, which was soil A after removal of chlorinated hydrocarbons. All soils were administered by gavage as 10% suspensions in gum acacia (5% aq)

or toxic products in the milk. An alternative interpretation, based on our experiments of replacing 2,3,7,8-TCDD control treatment with decontaminated soil treatment, is that the low level of dioxin absorbed from the contaminated soil may be affecting ovarian function or hormonal status.

Acknowledgment. This research was supported by the New Jersey Department of Environmental Protection (Contract #C-29786) and the U.S. Environmental Protection Agency cooperative agreement #CR812114-01-1.

References

Allen JR, Barsotti DA, Lambrecht LK, Van Miller JP (1979) Reproductive effects of halogenated hydrocarbons on nonhuman primates. Ann NY Acad Sci 320:419–425

Courtney KD (1976) Mouse teratology studies with chlorodibenzo-*p*-dioxins. Bull Environ Contam Toxicol 16:674–681

Esposito MP, Tieman TO, Dryden FE (1980) Dioxins. Industrial Environmental Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Cincinnati, Ohio, pp 1–351

Festing MFW (1979) Inbred strains in biomedical research. Oxford UP, NY, p 182

Gallo MA, Hesse EJ, Macdonald GJ, Umbreit TH (1986) Interactive effects of estradiol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic cytochrome P-450 and mouse uterus. Toxicol Letters 32:123–132

Lewis RJ Sr, Tatkan RL (1982) Registry of toxic effects of chemical substances. US Dept Health Human Services, Public Health Service, National Institute of Occupational Safety and Health, Washington, DC

Lucier GW, Rumbaugh RC, McCoy Z, Hass R, Harvan D, Albro P (1986) Ingestion of soil contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters hepatic enzyme activities in rats. Fund Appl Toxicol 6:364–371

McConnell EE, Lucier GW, Rumbaugh RC, Albro PW, Harvan DJ, Hass RJ, Harris MW (1984) Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science 223:1077–1079

Nau H, Bass R (1981) Transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to the mouse embryo and fetus. Toxicology 20:299–308

Poland A, Glover E (1980) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: segregation of toxicity with Ah locus. Molecular Pharmacol 17:86–94

Romkes M, Piskorska-Pliszczynska J, Safe S (1986) Effects of 2,3,7,8-TCDD on the estrogen receptor in immature Long Evans rats. Toxicologist 6:42

Silkworth J, McMartin D, DeCaprio A, Rej R, O'Keefe P, Kaminsky L (1982) Acute toxicity in guinea pigs and rabbits of soot from a polychlorinated biphenyl-containing transformer fire. Toxicol Appl Pharmacol 65:425–439

Umbreit TH, Hesse EJ, Gallo MA (1986) Bioavailability of dioxin in soil from a 2,4,5-T manufacturing site. Science 232:497–499

———, ———, ——— (1987) Observations on the reproductive toxicity in mice of dioxin contaminated soils from a 2,4,5-trichlorophenoxyacetic acid manufacturing site. II. Effects on males and the second generation. in prep

Manuscript received October 31, 1986 and in revised form December 31, 1986.

MAXUS1208040