partial hybrid could be made that would be nearly all *D. melanogaster*, but would carry the *D. simulans per* gene. Such flies would be valuable for experiments on courtship song. Would such a *D. melanogaster* male fly, carrying *per*$^+$ DNA from *D. simulans*, sing with a *D. melanogaster* or *D. simulans* song rhythm? What types of songs would enhance the mating behavior of the corresponding partially hybrid females produced by transformation? These experiments will allow sophisticated molecular techniques to be applied toward answering more refined questions about the genetic control of song production and reception in *Drosophila*.

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Bioavailability of Dioxin in Soil from a 2,4,5-T Manufacturing Site

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Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) is a highly toxic contaminant produced in the manufacture of phenoxy herbicides. Despite its high TCDD content, soil from a contaminated area associated with a 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing site in Newark, New Jersey, did not induce acute toxicity when administered to guinea pigs (the most sensitive species) by gavage. Analysis of liver samples demonstrated low bioavailability of TCDD from this soil. A comparative analysis of soils showed that Soxhlet extraction was necessary for the determination of TCDD on Newark soil, whereas solvent extraction was sufficient for soil from Times Beach, Missouri. The difference in the bioavailability of TCDD from these soils is correlated with TCDD extractability and may be related to the different compositions of the soils.

DIOXIN (2,3,7,8-TETRACHLORODIbenzo-*p*-dioxin, TCDD) is one of the most toxic man-made compounds known. The effects of an acute dose vary with species and include liver and kidney damage, chloracne, reduction in weight, wasting, thymic atrophy, immunotoxicity, and death (TCDD syndrome). TCDD promotes liver tumors in rats and skin tumors in HRS/J hairless mice (*1*), and it is also an anti-initiator of benzo[*a*]pyrene skin tumor carcinogenesis in Sencar mice (*2*).

Although TCDD is not manufactured commercially, it is a contaminant in the manufacture of several chlorinated phenolic products including the herbicide 2,4,5-trichlorophenoxyocitic acid (2,4,5-T) and hexachlorophene. Humans have been exposed to TCDD in several industrial accidents (Nitro, West Virginia; Seveso, Italy) and through environmental contamination (Times Beach, Missouri; Newark, New Jersey; and Vietnam). Low levels of TCDD have been found in many populations around the world. Combustion and fly ash have been suggested as other possible sources of TCDD, and may be responsible for continued human exposure (*3*).

The toxicity of complex environmental mixtures contaminated with TCDD has received little study. Such materials, including soils, natural waters, foliage, fly ash, soots, and hazardous wastes may differ in toxicity by comparison with the pure compound because of the influence of the matrix on the bioavailability of TCDD. Adsorption of TCDD to the surface of matrix particles may alter the animal absorption of the compound. Poiger and Schlatter (*4*) found that rats treated by gavage with a mixture of TCDD and activated charcoal did not absorb the TCDD to any appreciable extent; TCDD from a soil-TCDD mixture was taken up into the liver to a lesser extent than pure TCDD, and this uptake could be decreased if the time of contact between the TCDD and soil was increased.

Studies on the toxicity of materials environmentally contaminated with TCDD have been reported. Heida and Olie (*5*) demonstrated the presence of polychlorinated dibenzodioxins and polychlorinated dibenzofurans in terrestrial and aquatic wildlife living in a contaminated refuse dump in the Netherlands. Other studies (*6, 7*) have demonstrated varying bioavailability of TCDD from fly ash. Silkworth *et al.* (*8*) showed substantial uptake and acute toxicity of soots from a polychlorinated biphenyl fire where TCDD and other chlorinated dioxins and dibenzofurans were present. McConnell *et al.* (*9*) reported acute toxicity, aryl hydrocarbon hydroxylase induction, and tissue accumulations of TCDD from soils from the Times Beach area of Missouri. On the basis of the positive control values reported by McConnell *et al.*, one can calculate a bioavailability of TCDD from these soils of ~85%. The results presented here, however, show very low acute toxicity resulting from the ingestion of soil from a heavily contaminated 2,4,5-T manufacturing site in Newark, New Jersey.

We examined two soils. One was from the vicinity of a Newark plant that manufactured several chlorinated phenol products,

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including 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T, and their esters. In about 1970 the plant stopped manufacturing 2,4,5-T. Over 10 years later, the site was found to be contaminated with dioxin during a survey conducted by the New Jersey Department of Environmental Protection. The second site was a salvage yard a few blocks away where the chemical stills from the 2,4,5-T manufacturing plant were broken down to recover the metal. The still bottoms were dumped on the site and became incorporated in the soil.

We administered these soils to guinea pigs (single doses by gavage after light ether anaesthesia) as 10% suspensions in 5% gum acacia (*10*). Table 1 presents an analysis of the manufacturing site soil based on the use of two methods that vary greatly in the severity of the extraction procedure. Over 50 different dibenzodioxins and dibenzofurans were detected (*11*). The negative control was a decontaminated soil sample (*10*) from the manufacturing site (from which chlorinated hydrocarbons had been removed by solvent extraction). TCDD in a suspension of corn oil and acetone (9:1) and TCDD placed on decontaminated soil 1 hour before use (designated "recontaminated") were used as positive controls. Surviving animals were observed for 60 days, although TCDD effects usually appear 3 to 4 weeks after dosing.

Table 1. Summary of the results of the analysis of soil for chlorinated dibenzodioxins and dibenzofurans. Concentrations are in parts per billion. Abbreviations: TCDD, tetrachlorodibenzo-*p*-dioxins; 2,3,7,8-TCDD, TCDD chlorinated at the 2,3,7, and 8 positions; PnCDD, pentachlorodibenzo-*p*-dioxins; 2,3,7,8-PnCDD, all PnCDD isomers chlorinated at the 2,3,7, and 8 positions, regardless of the position of the fifth chlorine; HxCDD, hexachlorodibenzo-*p*-dioxins; 2,3,7,8-HxCDD, all HxCDD isomers chlorinated at the 2,3,7, and 8 positions, regardless of the positions of the other two chlorine atoms; HpCDD, heptachlorodibenzo-*p*-dioxins; OCDD, octachlorodibenzo-*p*-dioxins; TCDF, tetrachlorodibenzofurans; abbreviations for other furans are equivalent to the abbreviations for the dioxins; NR, not reported.

Component	Times Beach		Newark		
	Soxhlet*†	Solvent*†	Soxhlet‡	Soxhlet*†	Solvent*†
		Dibenzodioxins			
2,3,7,8-TCDD	950	770§	2280	2200	>2.5
Total other TCDD	NR	NR	347	NR	250
2,3,7,8-PnCDD	0	NR	22	NR	NR
Total other PnCDD	0	NR	80	65	7
2,3,7,8-HxCDD	0	NR	150	NR	NR
Total other HxCDD	0	NR	175	220	140
Total HpCDD	5	NR	2850	5000	620
OCDD	6	NR	8200	3600	1100
		Dibenzofurans			
2,3,7,8-TCDF	8	40–80§	167	135	30
Total other TCDF	NR	NR	272	NR	NR
2,3,7,8-PnCDF	0	NR	61	NR	NR
Total other PnCDF	0	NR	615	68	40
2,3,7,8-HxCDF	0	NR	81	NR	NR
Total other HxCDF	0	NR	3300	620	200
Total HpCDF	0	NR	4250	2400	390
OCDF	1	NR	1800	400	200

*Total number of isomers of each group. †Analysis by R. Haas, Triangle Laboratories, Inc., for Research Triangle Institute, Research Triangle Park, NC. ‡Analysis by C. Rappe, University of Umea, Umea, Sweden. §Values from McConnell *et al.* (*9*).

Table 2. Response of guinea pigs treated with TCDD-contaminated soil from a manufacturing site; ND, no deaths; G, gavage death, confirmed by autopsy. Times to death were measured after a single treatment with the materials indicated and described in the text.

Treatment	*n*	Sex*	Days to death	Autopsy summary†
Corn oil	8	m + f	ND‡	N
Decontaminated soil	8	m + f	ND‡	N
Contaminated soil§				
3 μg/kg	8	m + f	ND	N
6 μg/kg	8	m + f	ND‡	N
12 μg/kg	8	m + f	ND‡	N
Recontaminated soil	8	m	19, 20, 25, G	TS
(6 μg/kg)		f	15, 17, 18, ND	TS
TCDD in corn oil	8	m	5, 9, 21, ND	TS
(6 μg/kg)		f	26, 31, ND, ND	TS

*Four animals per sex. †TS, autopsy showed signs typical of TCDD-induced toxicity in animals that died; N, autopsy showed no signs consistent with TCDD-induced toxicity. ‡One death occurred, due to gavage error. §From the manufacturing site in Newark, NJ.

Table 2 presents dosage, time to death, and autopsy results for guinea pigs treated with soil from the 2,4,5-T manufacturing site (*12*). Positive controls produced death with typical signs of TCDD toxicity in over half the animals treated. These signs included thymic atrophy, absence of body fat, and loss of ~40% of body weight (a typical "wasting" syndrome). Survivors did not show these characteristics but did not gain as much weight as the negative controls. The results are consistent with a reported LD_{50} (dosage lethal to 50% of tested animals) of less than 0.5 μg/kg and an LD_{100} (dosage lethal to 100% of the tested animals) of ~10 μg/kg.

Animals treated with decontaminated soil or corn oil showed no signs of toxicity (Table 2); the "TCDD syndrome" was absent. All these animals survived 60 days. Animals treated with soil from the manufacturing site, at any dose given, showed no signs of toxicity or of the TCDD syndrome. Weight gain was slightly reduced in guinea pigs receiving an equivalent of 6 μg of TCDD per kilogram of body weight, and a greater reduction was observed in guinea pigs that received an equivalent of 12 μg/kg, but these weight effects were not as severe as those seen in animals treated with the positive control (either those dying or those surviving). All guinea pigs treated with site soil survived 60 days (there were occasional deaths due to gavage error). These results indicate some toxicity, but the lethal dose is clearly greater than 12 μg/kg.

Table 3 presents data from a separate experiment in which guinea pigs were treated with soil from the metal salvage site. Although fewer animals were tested (the same amount of soil was used together with a lower TCDD dose because of the lower TCDD content of this soil), the results were similar to those for the 2,4,5-T manufacturing site: neither contaminated-site soil nor negative control (decontaminated) soil caused any signs of toxicity or of TCDD syndrome, whereas the positive control showed the typical characteristics of TCDD-induced deaths.

Ratios of organ to body weight were calculated for all autopsied animals. The results showed enlargement of liver and heart in TCDD-treated guinea pigs dying with signs of TCDD toxicity as compared to negative controls or animals treated with contaminated-site soils. Ratios of organ to body weight for animals surviving TCDD treatment did not show clear differences

Table 3. Response of guinea pigs treated with TCDD-contaminated soil from a metal salvage yard. Times to death were measured after treatment with a single dose of the indicated materials as described in the text and in Table 2. Notes are the same as in Table 2, except * = two animals per sex.

Treatment	*n*	Sex*	Days to death	Autopsy summary
Decontaminated soil	4	m + f	ND	N
Contaminated soil (320 ng/kg)	4	m + f	ND	N
TCDD in corn oil (6 μg/kg)	4	m	21, ND	TS
		f	4, 8	TS

from those of negative controls, but results from survivors are equivocal because of low numbers. Animals treated with contaminated soil showed no differences in their organ weights relative to animals given negative controls. Thymus weight was recorded when possible, but animals that died after TCDD treatment lacked grossly observable thymus for comparison.

Table 4 presents data on the TCDD content of composite liver samples from test animals (*11*). The TCDD on recontaminated soil was highly available to the animals, but only small amounts of TCDD were found in the livers of guinea pigs given soil from either the 2,4,5-T manufacturing site or the metal salvage yard.

TCDD found in the 2,4,5-T manufacturing site soil in Newark is slightly bioavailable (less than 0.5% by tissue analysis) and relatively nontoxic to guinea pigs. These results are in marked contrast to those for soil from the Times Beach area (*9*) and from the metal salvage yard in Newark (bioavailability of 21.3%), and suggest that generalizations on bioavailability may be premature. Public health risks may vary between sites as a function of the contaminants present and the bioavailability from the matrix.

There are several possible explanations for the observed differences. First, desorption and gastrointestinal uptake of other compounds in the soil may have inhibited the intestinal absorption of TCDD or competed with TCDD at the receptor level. We addressed this possibility in a preliminary experiment by dosing guinea pigs with the contaminated soil and then 24 hours later with 6 μg of TCDD per kilogram of body weight in corn oil:acetone (9:1). All animals so treated died with signs of TCDD toxicity within 19 days, whereas animals treated with decontaminated soil before TCDD died within 23 days. These results indicate that other compounds present in the contaminated soil did not alter TCDD toxicity.

Second, the nature of the soil and matrix material may alter the bioavailability of the dioxin. There is a strong possibility because the manufacturing site was developed on a clean-fill levy along a bulkhead on the Passaic River. The fill for this site contained asphalt and concrete as well as coarse sand-soil fill. On the basis of the work of Poiger and Schlatter (*4*), we believe that the carbonaceous materials in the asphalt and tar could enhance binding to the matrix and therefore inhibit bioavailability.

Third, differences in the application of the TCDD to the soil and in the residence time of TCDD on the soil may affect bioavailability. At the Newark manufacturing site, TCDD accumulated in small amounts, from a generally aqueous medium, over long periods of time. The site was open to the environment; solvent materials may have enhanced the percolation into the soil and the binding. The Times Beach site was contaminated by direct application to a sandy loam soil of still bottoms or still bottoms mixed with waste oil (*9*). The absence of carbonaceous material and the presence of oil may account for the higher bioavailability of TCDD from the Times Beach soil.

The comparative extractability (Table 1) indicates that both Soxhlet and solvent extraction should be used when one is categorizing exposure potential. These data strongly support the hypothesis that the differences in bioavailability between Times Beach and Newark soils are a function of soil binding. TCDD is weakly sorbed onto Times Beach soil and is easily extracted; TCDD is tightly sorbed onto the Newark manufacturing soil and is very difficult to extract. Hence, matrix binding is better determined by both solvent and Soxhlet extractions, and, in the cases under discussion, bioavailability is directly correlated with binding to the matrix.

Table 4. Results of an analysis of composite liver samples from TCDD-treated guinea pigs; *n*, number of animals of each sex contributing to the composite. All animals received a single dose, and livers were taken at 60 days after dosing for all animals except the group treated with recontaminated soil. These animals were autopsied at death and the livers were frozen. Each composite was analyzed only once because the amount of material was limited (ppt= parts per trillion).

Treatment of animal	*n*	Sex	TCDD (ppt)
Recontaminated soil	2	m	18,000
(6 μg/kg)	4	f	
Contaminated soil			
Manufacturing site	3	m	90
soil (12 μg/kg)	1	f	
Metal yard soil	2	m	230
(320 ng/kg)	2	f	
Decontaminated soil	2	m	0
	3	f	

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10. Soils were sampled by the New Jersey Department of Environmental Protection and stored in sealed glass jars inside seven layers of plastic inside metal containers. Soils were mechanically homogenized and randomly subdivided. Before use, soils were sifted to remove large particles and were analyzed by ETC, Inc., Edison, NJ. The method used is described in "Determination of 2,3,7,8-TCDD in Soil and Sediment" (Environmental Protection Agency Region VII Protocol, Washington, DC, May 1983); it requires (i) radiolabeled internal standard; (ii) extraction with a mixture of sodium sulfate, methanol, and hexane; (iii) silica and alumina column chromatography, and (iv) gas chromatography–mass spectral analysis on fused silica capillary columns. Decontaminated soil consisted of the soil from the manufacturing site that had been chemically stripped during analysis. R. Haas and Research Triangle Institute [selected by the National Institute of Environmental Health Sciences as a reference laboratory for the analysis of both these soils and those of McConnell *et al.* (*9*)] analyzed the Newark soil, using two extraction procedures: (i) C. Rappe's Soxhlet extraction for 72 hours (*11*) and (ii) the solvent extraction procedure of McConnell *et al.* (*9*).
11. Isomer-specific analysis of the manufacturing site soil was done in the laboratory of C. Rappe, University of Umea, Sweden. A 48-hour Soxhlet extraction was carried out, with additional sample cleanup via chromatography on an SP2330 column [C. Rappe *et al.*, in *Chlorinated Dioxins and Furans in the Total Environment*, C. Choudhary *et al.*, Eds. (Butterworth, London, 1983), pp. 99–124]. This analysis revealed the presence of over 50 different chlorinated dibenzodioxins and dibenzofurans (totaling approximately 25 ppm) in the manufacturing site soil. ETC, Inc., determined the TCDD level of the contaminated soil from the manufacturing site to be between 1500 and 2500 ppb and that in the soil from the metal scrapyard to be ~180 ppb. Approximately the same TCDD doses were used in our experiment and in the experiments of McConnell *et al.* (*9*). Manufacturing site soil was characterized by the New Jersey Department of Environmental Protection as a medium dense, black, coarse- to fine-grained sand fill with some medium to fine gravel, and with traces of silt, organic material, and cinders. Soil from the metal scrap yard was similar.
12. Animals weighed 250 to 280 g at the time of dosing, were housed in groups, had free access to standard lab diet and water, and were given fresh greens once a week. The amount of soil received by individual animals varied with the desired TCDD dose, and the amount of suspension varied between 4.1 and 15 ml. Weight (recorded twice weekly), time of death, and general signs of toxicity were observed. Any animal that died in the course of the experiment was autopsied as soon as possible. All remaining animals were necropsied 60 days after dosing. Tissues were weighed and sections preserved for histopathology or TCDD analysis.

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