APPENDIX C. BIOAVAILABILITY OF DIOXIN

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C.1 Bioavailability Data

Umbreit et al. (1985, 1986a,b) conducted experiments in guinea pigs, administering 2,3,7,8-TCDD in corn oil, 2,3,7,8-TCDD added to chemically decontaminated soil, or soil from two industrial sites in Newark, New Jersey (a manufacturing site and a salvage site) contaminated with CDDs. 2,3,7,8-TCDD was the principal lower chlorinated isomer (dioxin or furan) present in the soil from the manufacturing site (for which a chemical analysis was presented). Soil from the manufacturing site was found to have 1,500 to 2,500 ppb 2,3,7,8-TCDD under soxhlet extraction; release under ambient temperature manual solvent extraction was much lower, reported as "$>$2.5 ppb." The soil from the salvage site was reported as approximately 180 ppb 2,3,7,8-TCDD under soxhlet extraction.

In this study, groups of two or four male and two or four female guinea pigs received single gavage doses of the test materials and were observed until death or sacrifice at 60 days. 2,3,7,8-TCDD in corn oil or in recontaminated soil (6 g/kg in both) proved highly toxic, without similar toxicity being observed in animals treated with up to twice this dose of 2,3,7,8-TCDD in the soil from the manufacturing site. The limited data on 2,3,7,8-TCDD levels in the liver showed much higher levels following administration of recontaminated soil versus contaminated soil from the manufacturing site.

Umbreit et al. (1986a) thus demonstrated that gavaged 2,3,7,8-TCDD containing soil from the manufacturing site was substantially less toxic than equivalent doses of 2,3,7,8-TCDD in corn oil. However, quantitative comparison of the effective doses in this study is difficult. Approaches to a quantitative comparison are outlined below.

(1) Guinea pigs receiving 12 ug/kg 2,3,7,8-TCDD in contaminated soil experienced no deaths, while five out of eight guinea pigs receiving 6 ug/kg 2,3,7,8-TCDD in corn oil died, with no groups tested having lower doses in corn oil. Other authors have provided data on the toxic effects of 2,3,7,8-TCDD in corn oil which could aid in the comparison.

McConnell et al. (1984) observed one out of six animals dying at 1 ug/kg and six out of six animals dying at 3 ug/kg. Silkworth et al. (1982) observed three out of six animals dying at 2.5 ug/kg and no deaths out of six at 0.5 ug/kg. Comparing these data directly with the Umbreit et al. results would suggest that the 2,3,7,8-TCDD in the Newark manufacturing site soil was less effective, by a factor of 10 or greater, in producing

toxicity than 2,3,7,8-TCDD in corn oil.

(2) Umbreit et al. reported a "slightly reduced" weight gain in guinea pigs receiving 6 ug/kg of 2,3,7,8-TCDD in Newark manufacturing site soil, and a "greater reduction" at the 12 ug/kg dose. No other signs of toxicity were noted in these groups. The animals receiving 6 ug/kg 2,3,7,8-TCDD in corn oil, in contrast, exhibited a marked loss of body weight and showed toxicity and mortality. Silkworth et al. (1982) also provided data on weights of guinea pigs receiving 2,3,7,8-TCDD in corn oil. Those receiving 2.5 ug/kg exhibited a marked reduction in weight gain among three out of six survivors, while those receiving 0.5 ug/kg showed a weight gain comparable to vehicle controls. Comparison of this weight data with that of Umbreit et al. suggests that the 2,3,7,8-TCDD in corn oil was more than 5 times but less than 25 times as potent as 2,3,7,8-TCDD in the Newark soil. This comparison assumes that the effect of the Newark manufacturing site soil on weight gain was due to 2,3,7,8-TCDD as opposed to other compounds in the soil. Numerous other dioxin and furan compounds and other chemicals have been identified in this soil (Umbreit et al., 1987a). It has not been established that 2,3,7,8-TCDD is the sole or prime source of toxicity in the soil.

(3) Umbreit et al. presented liver concentrations of 2,3,7,8-TCDD after death or sacrifice at 60 days following gavage. Much lower concentrations of 2,3,7,8-TCDD were found in the livers of animals receiving soil from the manufacturing site compared with those receiving the dose in corn oil. There are, however, two factors that limit the conclusions than can be drawn from this comparison.

First, the corn oil group experienced major toxicity and weight loss, particularly complete loss of body fat. These changes may have affected the partitioning of 2,3,7,8-TCDD within the body, leading to a higher concentration in the livers of the animals experiencing toxicity. Second, the animals gavaged with corn oil died early--half were dead by 26 days, while all of the guinea pigs treated with soil survived to 60 days (with the exception of one gavage death). The U.S. EPA (1985c) reported a half-life for 2,3,7,8-TCDD elimination of 30 $\pm$ 6 or 22 to 43 days from two studies in guinea pigs. Additionally, the U.S. EPA (1985c) stated that elimination in the guinea pig may follow zero-order kinetics. Differences in elimination due to differences in periods of survival are likely to have affected the relative quantities of 2,3,7,8-TCDD found in the livers of the test groups.

Perhaps a more appropriate comparison can be made with the four animals receiving 0.32 ug/kg of 2,3,7,8-TCDD in contaminated soil from the Newark salvage site. These animals experienced no reported toxic signs (weight data not presented) and survived the full 60-day experiment. Approximately 6% of the gavage dose was found in the liver of these animals, while only about 0.06% of the gavage dose was found in the livers of guinea pigs in the 12 ug/kg group receiving the Newark manufacturing site soil. This would suggest that the 2,3,7,8-TCDD in the manufacturing site soil was 100 times less bioavailable. However, given the different doses used and the fact that only a single pooled sample was analyzed for 2,3,7,8-TCDD in each group, caution must be used in interpreting this comparison.

The 2,3,7,8-TCDD in soil from the salvage site was substantially bioavailable, based on the single liver tissue analysis. Approximately 6% of the administered dose was recovered from the livers of these animals at 60 days. This can be compared with data on hamsters given 2,3,7,8-TCDD in corn oil by McConnell et al. (1984), where approximately 8% of the 2,3,7,8-TCDD could be recovered in the 1 ug/kg dose group among survivors at 30 days.

McConnell et al. (1984) treated Hartley guinea pigs (2.5 weeks old) with single gavage doses of either 2,3,7,8-TCDD or dioxin contaminated soil from two sites in Missouri. The 2,3,7,8-TCDD concentrations from the two sites were reported at 700 and 880 ppb respectively; total tetrachlorodibenzofurans (TCDF) concentrations in the soil were 40 to 80 ppb, and polychlorinated biphenyls (PCB) concentrations were 3 to 4 ppm. Taking into account the relative toxicities, the authors concluded that toxicity from the other compounds was likely to be small compared with that from 2,3,7,8-TCDD. Livers were analyzed for 2,3,7,8-TCDD at death or sacrifice at 30 days following treatment. Treatment deaths occurred between 5 and 21 days post-gavage.

Guinea pigs that died exhibited severe loss of body fat, markedly reduced thymus and testicle size, and adrenal hemorrhage. No adverse affects were noted in animals treated with decontaminated soil. For 2,3,7,8-TCDD in corn oil and for both contaminated soils, there were clear dose-responses in mortality. The calculated LD_{50} values for the two soil types were lower than the LD_{50} for 2,3,7,8-TCDD in corn oil by a factor of three to four.

There was a dose-response between the liver concentration of 2,3,7,8-TCDD and the gavage dose; the details of this relationship are complex. Animals dying during the experiment had liver concentrations a factor of 1.4 to 3.2 higher than animals in the same dose groups who survived 30 days. This observation makes quantification of the dose-response relationships difficult (all or most of the animals in the low-dose groups

survived the experiment, while all of the animals in the high-dose groups died). When the liver concentrations of 2,3,7,8-TCDD in animals dying early at the middle and high-dose groups are compared, there appears to be a greater-than-linear increase in liver concentration with dose for the Times Beach and Minker Stout soil groups, with a 3.3-fold increase in dose producing a 10- to 13-fold increase in liver concentration.

Liver concentrations of animals in the different dosing groups can best be compared among groups that experienced similar mortality.

(1) Animals in dose groups in which all animals died within 30 days: 2,3,7,8-TCDD in corn oil, approximately 20% of the administered dose was in the liver. For the soil-treated groups, 13% and 11% of the doses, respectively, were in the liver. Comparison of these data suggest that 2,3,7,8-TCDD was approximately twice as available through corn oil as through soil.

(2) Animals surviving the 30-day experiment (in groups where at least 4 out of 6 survived): For 2,3,7,8-TCDD in corn oil, 7.5% of the administered dose was in the liver. For soil-treated animals < 3.6, 1.3, < 4.2, and 2.0% of the doses, respectively, were in the liver. Comparison here would suggest that 2,3,7,8-TCDD was approximately four times as available through corn oil as through soil.

The authors note that the differences in liver concentrations observed in the study may reflect varying partitioning of the 2,3,7,8-TCDD among internal organs, since dying animals suffered major loss of body weight and fat content. In addition, surviving animals would have had greater opportunity to metabolize and excrete 2,3,7,8-TCDD due to a longer lifetime.

Umbreit et al. (1986a) reported additional chemical analyses of the Times Beach soil. Soxhlet extraction of the Times Beach soil yielded a similar quantity of 2,3,7,8-TCDD to the solvent extraction reported by McConnell et al. (1984). This is in contrast to the Newark manufacturing site soil used in the Umbreit et al. (1987a) experiments, where only a small fraction of soxhlet-extractable 2,3,7,8-TCDD was extractable by the solvent extraction methodology used by McConnell et al. (1984).

McConnell et al. (1984) also reported an experiment in which groups of six Sprague-Dawley rats were given single gavage doses of 2,3,7,8-TCDD in corn oil or dioxin-contaminated soil from the Minker site. Induction of aryl hydrocarbon hydroxylase (AHH) in the rat livers was measured at sacrifice 6 days after dosing. Experimental doses ranged from 0.4 to 5.0 ug/kg 2,3,7,8-TCDD. Measured AHH induction was similar for groups receiving 2,3,7,8-TCDD in corn oil or receiving contaminated soil containing nearly equal doses of 2,3,7,8-TCDD. For example (based on the rate of formation of 3-

hydroxybenzo[a]pyrene), AHH activity was measured at 1,269 pmole min^{-1} mg^{-1} for the group receiving 5 ug/kg 2,3,7,8-TCDD in corn oil and at 1,230 pmole min^{-1} mg^{-1} for the group receiving 5.5 ug/kg 2,3,7,8-TCDD in contaminated soil. For the five dose groups, the AHH activity for the soil group ranged from 50% to 110% of the activity in the corn oil group.

The McConnell et al. (1984) rat data indicate that the bioavailability of 2,3,7,8-TCDD from the Minker site soil was at least 50% of that of equivalent doses of 2,3,7,8-TCDD in corn oil.

Lucier et al. (1986) provided additional information on the induction of hepatic enzymes in rats by the 2,3,7,8-TCDD contaminated soil from the Minker site tested by McConnell et al. (1984). AHH induction was similar for the groups of rats receiving 2,3,7,8-TCDD in corn oil and contaminated soil (within a factor of two) over a broader range of doses (0.015 ug/kg to 5 ug/kg) than reported by McConnell et al. (1984). In a second enzyme assay using the same animals, UDP glucuronyltransferase activity was found to be slightly higher in groups receiving 2,3,7,8-TCDD in corn oil than groups receiving equal doses in contaminated soil.

Liver concentrations of 2,3,7,8-TCDD for the rats were also reported. For the corn oil vehicle the liver concentrations were 40.8 $\pm$ 6.5 ppb at the 5 ug/kg dose and 7.6 $\pm$ 2.5 ppb at the 1 ug/kg dose. Assuming that the liver comprises 4.0% of body weight, the retention rates for the 5 and 1 ug/kg doses were 33% and 30%, respectively. In rats receiving 2,3,7,8-TCDD in contaminated soil, the 5.5 ug/kg group had liver concentrations of 20.3 $\pm$ 12.9 ppb, and the 1.1 ug/kg group had concentrations of 1.8 $\pm$ 0.3. Thus, retention rates for the 5.5 and 1.1 ug/kg groups are estimated at 14% and 7%, respectively. These data indicate that liver retention in the soil group was 20% to 40% of that in the corn oil vehicle groups.

Umbreit et al (1986b) report additional studies of mortality in guinea pigs treated with soil containing 2,3,7,8-TCDD from Newark (manufacturing site) and Missouri (Times Beach) previously tested by Umbreit et al (1985, 1986a) and McConnell et al. (1984), respectively. Guinea pigs received a single gavage dose of a soil suspension and were observed for 60 days. After autopsy, deaths were classified as whether or not they appeared to be due to TCDD toxicity. Substantial mortality (25% overall) from conditions not attributed to TCDD was observed across all groups.

The data for both the Newark and Missouri sites are similar in trend for the previous data on these sites; and clearly indicate the greater toxicity of the Newark soil for given equal administered doses of 2,3,7,8-TCDD. With larger groups of guinea pig studied, a toxicity-related death was observed in both the 5 and 10 mg/kg dose groups for

Newark soil while no deaths were observed in corresponding dose groups (6 and 12 mg/kg) with fewer animals in Umbreit et al. (1986a).

Comparing groups within this study, similar mortality (1 or 2 deaths in 10 to 16 animals) was seen in both the 5 and 10 ug/kg Newark groups and the 1 and 3 ug/kg Missouri groups. These results suggest that the toxicity of these materials differs by an order of magnitude or less. As noted above, the degree to which toxicity from these soils can be attributed to 2,3,7,8-TCDD in the presence of numerous other related toxic compounds is not known. 2,3,7,8-TCDD tissue concentrations were not reported in this work.

In another comparative study Umbreit et al. (1987b) compared the Newark manufacturing site and Times Beach soils in the induction of aryl hydrocarbon hydroxylase (AHH) in rats. While the use of only single dose levels prevents detailed analysis, the two soils proved quite similar in their ability to induce AHH. The explanation for the difference in this finding from those observed in the toxicity studies discussed above is not clear, but may relate to the presence of other toxic and/or AHH inducing compounds.

Umbreit et al. (1987a) report a reproductive toxicity study with soils from the Newark manufacturing site and salvage yard previously studied by Umbreit et al. (1986a). Female mice were treated thrice weekly with soil from these sites, with treatment continuing through fertilization to weaning of pups. The total doses of 2,3,7,8-TCDD received by the mice were 720 ug/kg in manufacturing site soil, and 86 ug/kg in salvage yard soil. A corn oil vehicle group and a recontaminated soil group received a total of 225 ug/kg.

Deaths in animals showing "classic signs" of TCDD toxicity were observed in the corn oil and recontaminated soil groups, and indicate appreciable bioavailability of 2,3,7,8-TCDD. Deaths were also observed in animals receiving manufacturing site soil but the authors did not observe "classic signs" of TCDD toxicity. Fewer live pups born and fewer pups surviving until weaning were observed in the manufacturing site soil group compared with those receiving decontaminated soil. TCDD completely blocked reproduction in the corn oil and recontaminated soil groups. The results of this study demonstrate acute and reproductive effects occurred in animals receiving manufacturing site soil. However, these effects were of a lesser magnitude than those seen in animals treated with 2,3,7,8-TCDD in corn oil at a dose three fold lower. The authors note the presence of substantial quantities of other toxic substances in the manufacturing site soil (chemical analyses presented). No toxic effects were noted in animals treated with salvage site soil, who received a much smaller 2,3,7,8-TCDD dose. The data does not allow a quantitative evaluation of the bioavailability of 2,3,7,8-TCDD.

Kaminski et al. (1985) and Silkworth et al. (1982) reported the results of a series of studies on the toxicity of soot containing dioxin and furan compounds from a fire involving transformer fluid containing PCBs. Hartley guinea pigs (500 to 600 g) received single oral doses of soot in an aqueous vehicle, a soxhlet extract of the soot in the same vehicle, or 2,3,7,8-TCDD in either an aqueous vehicle or corn oil.

The soot was reported to contain 2.8 to 2.9 ppm 2,3,7,8-TCDD and 124 to 273 ppm 2,3,7,8-TCDF. The total polychlorinated dibenzofuran content was estimated at 5,000 ppm. Animal weights and mortality were recorded for 42 days, at which point the survivors were sacrificed and LD_{50} values were calculated. Blood chemistry and a pathologic examination were performed at sacrifice.

Silkworth et al. (1982) noted that the LD_{50}s for contaminated soot and soot extract were similar at 410 and 327 equivalent ug/kg, indicating that the matrix had only a small effect on toxicity. If expressed in terms of the content of 2,3,7,8-TCDD, the LD_{50} from soot is 2.5 ug/kg, which is a factor of seven below the LD_{50} for 2,3,7,8-TCDD in an aqueous vehicle, suggesting that other compounds contributed to the toxicity of the soot and soot extract.

The authors stated that they adopted an aqueous vehicle in these experiments because it was nontoxic and provided a stable suspension of soot; they regarded this vehicle as more appropriate for modeling of human exposure conditions than an oil vehicle. The data from these experiments also demonstrate that use of an oil vehicle leads to substantially greater 2,3,7,8-TCDD toxicity than does an aqueous vehicle.

Comparison of mortality and weight loss in groups of female guinea pigs receiving 500 ug/kg of soot or the equivalent amount of soot extract suggests that the extract may be somewhat more toxic; however, all six animals died in the 1,000 ug/kg soot group, while four out of five died in the 500 ug/kg extract group. Taken together, these data indicate that the soxhlet extract of soot in an aqueous vehicle was between one and two times as toxic as the soot itself. It is likely that a larger difference in toxicity would have been observed if the soot extract was in an oil vehicle.

Van den Berg et al. (1983) fed small groups of male Wistar rats fly ash from a municipal incinerator (pretreated with HCl) containing dioxins and furans, a soxhlet extract of the fly ash, or a purified extract of the ash that was obtained using column chromatography. 2,3,7,8-TCDD was present as 3.3% of the TCDD isomer group in the fly ash extract. (The authors did not specify whether this reference was to crude or purified extract.) 2,3,7,8-TCDF was present as 17.9% of the tetra-CDF isomer group in the extract. The rats were fed 2 g/d fly ash mixed with diet or the residual from 2 mL/d extract after the extract was mixed with diet and the solvent was evaporated. The animals

were exposed to the treated diet for 19 days, and then sacrificed, and the liver tissue was analyzed for the presence of dioxins and furans.

Approximately 1% of the 2,3,7,8-TCDD dose from fly ash was retained in the liver, and approximately 4% of the dose of this isomer from fly ash extract was so retained. The corresponding percentages for 2,3,7,8-TCDF are 0.3 and 1.0. Data on the retention of isomer groups in adipose tissue were presented for the extract-treated groups but not for the fly ash-treated group. The concentrations of the various isomers in adipose tissue are comparable to, or less than, the concentrations in liver tissue.

The U.S. EPA (1985b) reported a half-life for elimination of 2,3,7,8-TCDD in the rat of 20 days at high dose. If a similar half-life is assumed in this experiment, the quantities of 2,3,7,8-TCDD in the animals at the end of the 19-day feeding experiment would be significantly less than the absorbed dose, but still of the same order of magnitude. However, the recovery percentages in this study are low for both the fly ash and fly ash extract groups in comparison with other studies in which 2,3,7,8-TCDD was administered to rats. Fries and Marrow (1975) fed rats diets containing 7 or 20 ppb of 2,3,7,8-TCDD for a period of up to 42 days. After 14 days of feeding, the rat livers contained an average of 32% of the cumulative administered dose; at 28 days, 21% of the dose; and at 42 days, 18% of the dose. Thus, in the van den Berg et al. (1983) study, the liver retention of 2,3,7,8-TCDD for the fly ash extract group is a factor of five to eight below what could be anticipated for the Fries and Marrow (1975) data, and the liver retention in the van den Berg et al. (1983) group fed soot is a factor of 20 to 30 lower than that seen by Fries and Marrow (1975). Data from Kociba et al. (1976), Rose et al. (1976), and Kociba et al. (1978) lead to similar conclusions to those from the Fries and Marrow (1975) data regarding the fraction of cumulative 2,3,7,8-TCDD dose retained in the rat liver.

An explanation of the low level of recovery for the animals receiving the soxhlet extract of soot is not apparent. It is possible that the presence of multiple compounds affected absorption or metabolism in the rats fed soot and soot extract.

A second approach to the van den Berg et al. (1983) data is to compare the ratios of liver concentrations for dioxins in fly-ash-treated animals to the concentrations in extract-treated animals. These ratios, based on measurements in small numbers of animals, indicate a substantial bioavailability of dioxin and furan compounds from the tested fly ash. This availability varied among the different isomers with the value of 0.3 for 2,3,7,8-TCDD, indicating that this isomer was three times as available from fly ash extract as from fly ash.

Van den Berg et al. (1985) fed fly ash (pre-treated with HCl) to Wistar rats, guinea pigs, and Syrian golden hamsters. Fly ash was mixed with standard laboratory diet at

2.5% by weight, and animals were allowed to eat ad libitum. The amount of fly ash consumed by each group of five rodents was determined by the authors. For each species there were three groups of animals each fed fly ash for approximately 32 days (group I), 60 days (group II), or 94 days (group III). Concentrations of dioxin and furan isomer groups in the food were presented, and include 1.4 ng/g TCDD compounds and 2.1 ng/g TCDF compounds.

The authors presented calculated recovery percentages for the cumulative dose of specific isomers in the rodent liver. For 2,3,7,8-TCDD in guinea pigs, 3.7%, 0.9%, and 1.4% of the administered dose was recovered in the liver in groups I, II, and III, respectively. The 32-day (group I) recovery percentage is somewhat higher than seen in the lower dose groups receiving 2,3,7,8-TCDD contaminated soil in McConnell et al. (1984). The value in hamsters was approximately 2% (only reported for group II), and analytical problems prevented this determination in rats. No other TCDD compounds were quantified. Similarly, for 2,3,7,8-TCDF, guinea pigs showed retention of 4.7%, 2.2%, 2.5% of the administered dose in groups I, II, and III, respectively. For both 2,3,7,8-TCDD and 2,3,7,8-TCDF the recovery percentages in guinea pigs at 32 days were approximately a factor of 4 to 15 higher than that observed in the van den Berg et al. (1983) study in rats.

Other TCDD compounds that were present showed comparable or somewhat lower retention, averaging 1% to 2% over the animals groups. No TCDD or TCDF compounds were detected in hamster liver or analyzed for in rat liver. Higher chlorinated congeners most typically showed retention in the range of 2% to 5% in rat liver and 1% to 3% in guinea pig liver, with the exception of 2,3,4,7,8-PeCDF (9.8%, 8.3%, and 11.3% in the hamster groups). Few other compounds were found in hamster liver, but 2,3,4,7,8-PeCDF was found with a recovery of 5% to 8% and 2,3,4,7,8-HxCDD was found at 3% to 7%.

As with other experiments in which the retention of dioxins in the liver has been determined, these percentages place a lower bound on the bioavailability of the dioxins but, because not all dioxin is localized in the liver, do not permit bioavailability to be estimated without knowledge of the elimination of the administered dose over time and the quantity of dioxins in the remainder of the organism. No positive control group receiving 2,3,7,8-TCDD was included for comparison.

Poiger and Schlatter (1980) conducted several experiments in Sprague-Dawley rats (180 to 220 g) in which liver concentrations of tritium label from 2,3,7,8-TCDD were determined using various doses and vehicles. All experiments consisted of a single gastric intubation of 2,3,7,8-TCDD-containing material, followed by animal sacrifice at

predetermined times. The doses used were well below the LD_{50} in the rat (the maximum dose applied was 5 ug/kg), and no deaths or toxic effects were reported.

In a preliminary experiment, rats were treated with 14.7 ng/rat 2,3,7,8-TCDD in ethanol. The results indicate substantial localization of 2,3,7,8-TCDD in the rat liver, with a decrease of a factor of two in the fraction of the dose in the liver between 1 and 4 days. Poiger and Schlatter (1980) conducted all further studies with sacrifice at 24 hours to maximize the recovery of 2,3,7,8-TCDD from the liver.

In a second experiment, the authors administered 2,3,7,8-TCDD doses in ethanol ranging from 15 to 1,070 ng/rat to groups of six rats. They found a graded increase in percentage retained in the liver from 37% $\pm$ 1% at the 15 ng dose to 51% $\pm$ 4% at 280 ng. At the high-dose point, the percentage may have fallen (42% $\pm$ 10% at 1,070 ng).

In a further experiment, 2,3,7,8-TCDD was administered at low dose in a series of vehicles. These data demonstrate that administration of 2,3,7,8-TCDD in soil reduced the retention of the dose in the liver to 66%, or 44% of the retention seen with 2,3,7,8-TCDD
in ethanol. The lower value, 44%, was obtained for soil that was aged for 8 days at 30-40 °C following addition of 2,3,7,8-TCDD. This observation is consistent with the findings of other studies reported here that 2,3,7,8-TCDD from environmental soil (naturally aged) was generally less available than 2,3,7,8-TCDD freshly added to clean samples of these soils. The aqueous suspension of 2,3,7,8-TCDD in activated carbon showed little evidence of bioavailability; this is supported by the authors' measurements showing that 2,3,7,8-TCDD was only slightly extractable from the activated carbon matrix by various solvents. In contrast, 58% to 70% of 2,3,7,8-TCDD could be recovered from soil samples by washing with hexane/acetone (4:1 v/v).

Poiger and Schlatter (1980) also presented results from several skin application experiments with TCDD-containing materials using rats and rabbits (not reviewed here).

Bonaccorsi et al. (1984) reported the results of a study of gut absorption of 2,3,7,8-TCDD from soil taken from the Seveso, Italy accident site. Soil containing 81 $\pm$ 8 ppb 2,3,7,8-TCDD from the "highly contaminated" area in Seveso was administered to albino male rabbits (2.6 $\pm$ 0.3 kg) in daily gavage doses for seven days. Additional samples of clean soil were spiked with 2,3,7,8-TCDD in the laboratory to yield 10 and 40 ppb contamination levels and were administered to rabbits following the same protocol. For comparison, rabbits were also treated with 2,3,7,8-TCDD in solution in acetone-vegetable oil (1:6) or alcohol-water (1:1). Rabbits were sacrificed on the day after treatment stopped and liver concentrations of 2,3,7,8-TCDD were measured. The authors did not remark on the presence or absence of toxicity in the treated rabbits. EPA (1985a)

reports values for the single dose LD_{50} of 2,3,7,8-TCDD in rabbits of 115 and 275 ug/kg. The total doses received by the rabbits in this study were approximately 54, 107, and 215 ug/kg over seven days. Based on this comparison, there is a likelihood that toxic effects occurred in the Bonaccorsi work, and as noted above, toxicity has the potential to affect the tissue concentrations of 2,3,7,8-TCDD. For this reason, the most appropriate comparisons among these data are between groups showing similar liver concentrations of 2,3,7,8-TCDD, which may then be inferred to have experienced similar toxic effects.

That this method of comparison is desirable can also be seen from the Bonaccorsi et al. (1984) data, where both solvent vehicle groups and the spiked soil groups show an increase of the fraction of the dose in the liver at the higher administered doses. However, it should be mentioned that use of two different solvent vehicles complicates interpretation. Similar liver concentrations of 2,3,7,8-TCDD were seen in the 40 ug/d solvent vehicle and 80 ug/d Seveso soil groups. Comparing the percentage of liver retention in these two groups indicates absorption from Seveso soil was 40% of that from the solvent vehicle. Using the same approach, comparison of the 80 ug/d solvent vehicle and 160 ug/d Seveso soil groups indicates that absorption from the soil was 41% of that from the solvent.

The same approach can be used to compare absorption from the solvent vehicle and from the spiked soil. In this case the 40 ug/d solvent vehicle group had the liver concentrations closest to either the 40 or 80 ug/d spiked soil groups. Comparison of the percentage of dose in the liver indicates absorption from spiked soil is 68-73% of that from the solvent vehicle. Bonaccorsi et al. (1984) conducted work with either aged or non-aged spiked soil but do not present data to allow a comparison of these groups.

Shu et al. (1987, as cited by Leung and Paustenbach, 1987) studied 2,3,7,8-TCDD from the Missouri site tested by McConnell et al. (1984). Their paper reports an oral bioavailability of approximately 43% in the rat dosed with environmentally contaminated soil from Times Beach, Missouri. This figure did not change significantly over a 500-fold dose range of 2 to 1450 ng 2,3,7,8-TCDD per kg of body weight for soil contaminated with approximately 2, 30 or 60 ppb of 2,3,7,8-TCDD. The data from this study is not now available to the Exposure Assessment Group of EPA for review.

C.2 Summary of Bioavailability

Table C-1

summarizes data that are pertinent to the bioavailability of 2,3,7,8-TCDD from environmental matrices. Studies of bioavailability, which examined soil samples, soot, and fly ash, have utilized three methodologies: measuring acute toxicity, retention of 2,3,7,8-TCDD in the liver, and induction of hepatic enzymes.

Among the five samples of soil from contaminated sites that have been tested, three have shown substantial bioavailability, e.g., 25% to 50%, when compared with 2,3,7,8-TCDD in corn oil gavage. A fourth soil sample was compared with 2,3,7,8-TCDD administered in a solvent vehicle, and fell in this range. The fifth soil, tested by Umbreit et al. (1986a,b; 1987a,b) showed bioavailability substantially less than the other soils tested. While difficult to gauge quantitatively, dioxin from this fifth soil may be an order of magnitude less available than from the other soils.

Additionally, three samples of soil spiked with 2,3,7,8-TCDD have been tested for bioavailability, including one sample in which the 2,3,7,8-TCDD was incubated with soil at

an elevated temperature. The 2,3,7,8-TCDD added to these soil samples proved to be highly available (e.g., 40% to 70%).

In one study, soot from a transformer fire containing dioxins and furans proved similarly toxic to a soxhlet extract of the soot in an aqueous vehicle. However, the soot extract may have proved more toxic if delivered in corn oil, as was 2,3,7,8-TCDD in the soil studies. The availability of 2,3,7,8-TCDD and other dioxins and furans from incinerator fly ash have been addressed by van den Berg et al. (1983, 1985) in extended feeding studies. In these studies, liver retention of 2,3,7,8-TCDD from either fly ash or fly ash extract proved low, with availability from fly ash being approximately 25% of that from the extract.

The individual studies reviewed have a variety of limitations, as discussed in the preceding text. A notable limitation was that some experiments were conducted using highly toxic doses of 2,3,7,8-TCDD, so that determination of bioavailability was complicated by wasting and early death of the test animals. It should also be noted that, while the relative retention of 2,3,7,8-TCDD in the liver can serve as an appropriate indication of differences in bioavailability between samples, the percentage of dose found in the liver only places a lower bound on absorption. This is particularly relevant to experiments where animals have been maintained for many weeks after dosing and an undetermined quantity of 2,3,7,8-TCDD has been excreted.

Finally, toxicity data for mixtures for which both toxicity and bioavailability of individual compounds may vary are difficult to interpret quantitatively in terms of bioavailability.

As presented in U.S. EPA (1985c), Rose et al. (1976) determined gut absorption of 2,3,7,8-TCDD in a 1:25 mixture of acetone to corn oil (by volume) in the rat. In both single dose and multiple dose experiments, measured absorption was approximately 85%. Assuming that absorption from pure corn oil is similar to that from this mixture, and assuming that absorption in other species for which data are not available is similar, the 85% factor can be applied to the data presented here to obtain an approximate range for typical 2,3,7,8-TCDD absorption from soil. Using this factor, the estimated relative bioavailability of 2,3,7,8-TCDD from soil is 25% to 50% and, when compared with corn oil, provides an estimate of gut absorption of 20% to 40% of ingested 2,3,7,8-TCDD in soil. This estimate is comparable with the 20% to 26% absorption from 2,3,7,8-TCDD treated soil from the work of Poiger and Schlatter (1980).

Recognizing these limitations, the weight of evidence indicates that 2,3,7,8-TCDD is often highly available from environmental materials. However, in one tested soil sample the compound was substantially less bioavailable. While the data are too sparse to allow

a prediction as to whether a particular environmental sample will prove more or less bioavailable, one important suggestion has emerged. In the two samples that have proved least bioavailable (the Umbreit et al. (1986a) manufacturing site soil sample, and 2,3,7,8-TCDD on activated carbon tested by Poiger and Schlatter (1980)) the 2,3,7,8-TCDD was largely resistant to solvent extraction. This was not the case for more bioavailable materials.

Further research, using short-term experiments in which animals are handled under identical conditions and are fed dioxins in different media, is needed for an improved comparison of absorption between different environmental samples. Acutely toxic doses should be avoided to ensure that tissue concentrations are directly interpretable. Experiments studying both tissue retention and enzyme induction should prove valuable for this research. Whole-body levels of 2,3,7,8-TCDD need to be related to liver concentrations, and the effects of metabolism need to be addressed. The vehicle of administration has been shown to affect acute 2,3,7,8-TCDD toxicity, and vehicle effects need to be considered in designing experiments.

C.3 Distribution

Ryan et al. (1985) examined the distribution of 2,3,7,8-TCDD in two humans at autopsy. On a weight basis, there were 6 ppt of TCDD in fat, 2 ppt in liver and below levels of detection in kidney and muscle. They reported that on a per lipid basis the levels were similar between tissues. It is important to note that one of these subjects suffered from a fatty liver syndrome, possibly resulting in higher levels in the liver than might normally be found in healthy individuals.

Poiger and Schlatter (1986) estimated that about 90% of the total body burden of 2,3,7,8-TCDD was sequestered in fat. Levels of 2,3,7,8-TCDD averaging 5-10 ppt have been reported for background populations in St. Louis, MO, by Graham et al. (1986), and in Atlanta, GA, and Utah by Patterson et al. (1986). These data are consistent with the lipid bioconcentrations assumptions made in calculations of daily intakes (*vidae supra*).

Patterson et al. (1987) developed a high resolution gas chromatographic/high resolution mass spectrometric analysis for 2,3,7,8-TCDD in human serum. A high correlation was reported between adipose tissue and serum concentrations when adjusted for total lipid content. The reader is referred to other documents (U.S/ EPA, 1993; Schlatter, 1991; Schecter, 1991) for more details on the distribution and elimination.

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